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REUNIÓN DE SOCIEDADES DE BIOCIENCIAS 2021

LXVI REUNIÓN ANUAL DE LA
SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXIX REUNIÓN ANUAL DE LA
SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

LIII REUNIÓN ANUAL DE LA
ASOCIACIÓN ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL (AAFE)

XI REUNIÓN ANUAL DE LA
ASOCIACIÓN ARGENTINA DE NANOMEDICINAS
(NANOMED-AR)

17-20 de noviembre de 2021

EDITORES RESPONSABLES
Dr. Alejandro Curino
Dra. Mariana Maccioni
Dra. Paula Schaiquevich
Dra. Hebe Duran

ANNUAL MEETING OF BIOSCIENCE SOCIETIES 2021

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RESPONSIBLE EDITORS
Dr. Alejandro Curino
Dra. Mariana Maccioni
Dra. Paula Schaiquevich
Dra. Hebe Duran

LA TAPA

Daniela Kantor. Médanos, 2018

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Daniela Kantor nació el 23 de marzo de 1970. Es diseñadora gráfica (FADU-UBA), pintora, dibujante, historietista e ilustradora. Autora de la novela gráfica Mujer Primeriza (Ed. Burlesque, 2014), Aprendiza (2019) y Naturalella (con guión de Arekasadaro, 2017) publicada en Dis-Tinta (Ed. Sudamericana, coordinado por Liniers y Martin Pérez). Con guión de Alejandro Farias dibujó Las moradas de Santa Teresa de Jesús en historietas (Ed. Loco rabia + CCEBA Centro Cultural de España en Buenos Aires) y Marilyn (Tren en movimiento, 2019). Es miembro de la revista de historietas “El Tripero” fundada en 1993 junto al grupo de alumnos de Alberto Breccia. En el ámbito de la enseñanza es Jefa de Trabajos Prácticos en la materia Ilustración inicial, y docente en Ilustración Editorial en la Facultad de Arquitectura, Diseño y Urbanismo FADU/ UBA. Dicta talleres sobre pintura e ilustración (C C Recoleta, 2019/ Quinta Trabucco, 2020/ taller particular junto a Daniel Roldan, 2019). Es maestra de niños y niñas en Dibujo e Historieta en Escuelas primarias, talleres (Filbita, Festival de literatura de Buenos Aires, 2018-9/ CCK, 2018/ taller propio desde 2014). Estudió Dibujo de Historieta con Alberto Breccia, Técnicas de Acuarela y Pastel con Carlos Nine, charlas sobre Historieta con José Muñoz, Curso de Color con Carlos Gorriarena, Clínica de Pintura con Mariano Sapia y Tulio de Sagastizábal, y Sumi- e en el Centro Okinawense. Trabaja para editoriales y revistas con ilustraciones e historietas (Ed. Troquel, Abran Cancha, Ed. Norma, Unicef, Barcelona, Crisis, Suplemento Ñ/ Clarín, Borges en la Biblioteca Nacional- Lectores de Borges). Fue invitada a la Feria del libro de los Universitarios de UNAM para presentar el libro “Palabra de ilustrador”, y en 2019 ganó la Beca UBA Internacional en el marco de un programa de intercambio docente con la Universidad Regiomontana, Monterrey, México.

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Mensaje de Bienvenida de los Presidentes

Discurso del Dr. Alejandro C. Curino, Presidente SAIC.

Estimados integrantes de la comunidad de investigación biomédica.

Es para mí un enorme placer darles la bienvenida a nuestra LXVI Reunión Anual que este año se realiza en forma conjunta con la Sociedad Argentina de Inmunología (SAI), la Asociación Argentina de Farmacología Experimental (AAFE) y la Asociación Argentina de Nanomedicinas (NANOMED).

Como es tradición y como creo que corresponde, quisiera comenzar estas palabras realizando los agradecimientos pertinentes. Las actividades realizadas por la Sociedad Argentina de Investigación Clínica (SAIC) a lo largo del año y la organización de un congreso como este, serían imposibles sin un verdadero trabajo en equipo. En este sentido quiero destacar y agradecer el trabajo de la Comisión y del Consejo Directivo. No puedo dejar de manifestar mi reconocimiento a algunas personas en particular.

Al Dr. Alejandro Urtreger que ha desarrollado una enorme tarea de organización a lo largo del año en general y para nuestra reunión anual en particular.

A las Dras. Mariana Maccioni, Hebe Duran y Paula Schaiquevich, presidentas respectivamente de SAI, Nanomed y AAFE, por la cálida relación y el buen trato con los que trabajamos en la organización de esta Reunión Conjunta. Vaya para ellas y sus respectivas comisiones directivas todo mi agradecimiento.

A los integrantes del grupo G2 y a nuestra secretaria Ivana por todo el trabajo realizado y por soportar con paciencia y buen humor mis cientos de llamados, emails y mensajes.

A la Dra. Cristina Carrillo, presidenta 2020 de la SAIC, por haberme dado lugar para participar ampliamente en todas las actividades de la SAIC durante su mandato, lo que me permitió adquirir experiencia en la difícil organización de la Sociedad en un año tan atípico. Esta experiencia fue una ayuda invalorable para la organización de la reunión anual de este año en la que, contra todo pronóstico, también tuvimos que trabajar con las muchas limitaciones impuestas por la pandemia.

Al Dr. Daniel Alonso, actual vicepresidente de SAIC, por ser una fuente permanente de consulta, intercambio de opiniones y consejos.

Con Cristina y Daniel hemos coincidido en que SAIC debe tener funciones que van más allá de la organización de la reunión anual. Pensamos que nuestra Sociedad debe comprometerse con los temas del país que le atañen y en los que pensamos que puede realizar un aporte útil e importante. En este sentido, comenzando con la presidencia de Cristina y continuando este año, hemos hecho público documentos que tuvieron como único objetivo brindarle a la sociedad una visión científica sobre los temas relacionados con la vacunación y el manejo de la pandemia ante la sorprendente difusión de información incorrecta que en muchos casos inducía en la población temor y resistencia a la vacunación. Este año la SAIC se ha incorporado al “Foro de Sociedades Científicas, Organizaciones de la Sociedad Civil y Universidades” y en conjunto con las muchas organizaciones que constituyen este foro, también hemos emitido varios documentos en el mismo sentido y con el mismo objetivo. Creemos que la enorme difusión de estos documentos, así como la alta repercusión que tuvieron, demuestra que es un aporte necesario e importante que SAIC puede realizar.

La SAIC fue creada en 1960 por el Dr. Alfredo Lanari en conjunto con otros prestigiosos investigadores, con el objetivo principal de contribuir al desarrollo de la investigación básica y aplicada y a la difusión del conocimiento científico en el ámbito de la biomedicina. En un principio, su contenido científico era principalmente fisiológico y clínico, de allí el nombre de Sociedad Argentina de Investigación Clínica. Sin embargo, con el paso del tiempo y con el desarrollo del conocimiento científico fue incorporando diversas temáticas incluyendo los mecanismos celulares, moleculares y genómicos que contribuyen al desarrollo de las diversas patologías. Por esta razón, la mayoría de los integrantes de SAIC actualmente investigamos en lo que podríamos

denominar investigación biomédica básica. Pensamos que, sin descuidar esta importante orientación en la que tanto se ha destacado la comunidad científica de nuestros socios, también debemos hacer un esfuerzo para tender un puente entre estas investigaciones y su aplicación en el ámbito clínico. En este sentido, el año pasado se instituyó el premio Prof. Dr. Horacio A. Repetto en el área de enfermedades infecciosas pediátricas. Este año hemos mantenido la organización de este premio tratando de incentivar la participación de investigadores clínicos en este área y hemos establecido un convenio de colaboración recíproca con la Sociedad Argentina de Pediatría. Debo agradecer en este punto la colaboración de las Dras. Cristina Ibarra y María Marta Amaral. También hemos iniciado conversaciones con la Sociedad Argentina de Patología para establecer un convenio similar. Como se puede observar en el programa de esta reunión anual, muchas conferencias y simposios fueron organizados teniendo presente este objetivo, como por ejemplo la conferencia inaugural "Immune checkpoint blockade in cancer therapy: new insights into therapeutic mechanisms" a cargo del premio nobel PhD James P. Allison y los simposios "Translational research" y "Translational research in oncology: preclinical, clinical and healthcare approaches" organizados con la colaboración de la Dra. Laura Bover y el Dr. Daniel Alonso, respectivamente.

Sin duda la pandemia nos ha causado mucho dolor y sufrimiento pero también ha tenido un costado favorable para la ciencia. En primer lugar ha demostrado algo que ya sabíamos y que la historia nos demuestra pero que una parte de la sociedad muchas veces parece olvidar y es que las soluciones a los grandes problemas de la humanidad no pueden prescindir de los conocimientos científicos.

Mucho se repite que el desarrollo social y económico de un país depende del desarrollo científico y tecnológico del mismo. Sin embargo, a veces tengo la sensación que muchos investigadores de nuestro país no tienen la íntima convicción que esto sea verdad. Siempre me sorprendió, cuando en años anteriores el gobierno de turno decidió incrementar el número de investigadores y becarios del CONICET y fomentar el retorno de los que estaban fuera del país, escuchar a mucho colegas decir que ese incremento era demasiado, que era "un lujo" que un país como el nuestro no podía darse. Esto es más sorprendente si pensamos que muchos de los investigadores hemos tenido la oportunidad de trabajar en otros países y hemos visto que los mismos tienen una mayor cantidad de investigadores en relación a la población económicamente activa (PEA) y que sin duda de esto y de la inversión en ciencia y tecnología depende el desarrollo que han alcanzado. Es oportuno citar aquí las palabras de uno de nuestros premios Nobel, el Dr. Bernardo Houssay: "La disyuntiva es clara, o bien se cultiva la ciencia y la investigación y el país es próspero y adelanta, o bien no se la practica debidamente y el país se estanca y retrocede. Los países ricos lo son porque dedican dinero al desarrollo científico-tecnológico y los países pobres lo siguen siendo si no lo hacen. La ciencia no es cara, cara es la ignorancia"

Si no estamos convencidos nosotros mismos de la relevancia de lo que hacemos para el desarrollo de nuestro país, difícilmente podremos convencer al resto de la sociedad y a la dirigencia política de la importancia de apoyar e invertir en ciencia y tecnología.

Es en este sentido que creo que la pandemia ha sido positiva, pues la sociedad ha visto cómo numerosos grupos de investigación de áreas como la biomedicina y las ciencias sociales de nuestro país rápidamente aplicaron sus conocimientos al tratamiento y control de la Covid-19 con notables resultados.

También se ha demostrado que los investigadores, y como decía más arriba las sociedades científicas, tienen un importantísimo rol en la difusión del conocimiento científico hacia el resto de la sociedad, especialmente cuando surgen informaciones falaces y anticientíficas como las que lamentablemente han circulado durante el transcurso de la pandemia.

Creo que esta es la senda en la que, como comunidad científica, debemos perseverar y en este sentido deseo destacar enfáticamente, porque según mi parecer no ha sido suficientemente difundida y valorada en toda su importancia, la reciente sanción por unanimidad en ambas cámaras del Poder Legislativo (el 24 de febrero del 2021 fue votada en el Senado) de la "Ley de Financiamiento del Sistema Nacional de Ciencia, Tecnología e Innovación" que había sido presentada originalmente en el año 2015. Dicha ley plantea incrementar el presupuesto para el área desde el 0.28% del PBI actual hasta el 1% del PBI en el 2032. Cabe destacar que la ley también indica que si un año el PBI disminuye, no se puede destinar menos presupuesto que el año anterior.

La alternancia histórica de dos modelos económicos contrapuestos, uno de desarrollo industrial y tecnológico con exportación de productos con valor agregado y otro de producción y exportación de productos primarios, ha hecho que el apoyo al sector científico y tecnológico en nuestro país tenga avances en el primer caso y retrocesos en el segundo. La aprobación de esta ley y su votación por unanimidad en ambas cámaras parecería indicar que el apoyo a la ciencia será de ahora en más una política de estado. Sin embargo, también es cierto que en el pasado reciente leyes votadas con amplios consensos luego no fueron aplicadas o fueron derogadas. Dependerá en parte de que la comunidad científica y los organismos que nos representan, como es el caso de nuestra querida SAIC, hagan escuchar su voz fuerte y clara para exigir el total cumplimiento de esta ley.

Las autoridades de las cuatro sociedades científicas que organizan esta reunión anual hemos esperado hasta el último momento para ver si podíamos realizarla aunque sea en forma parcialmente presencial, lo que finalmente no fue posible. No obstante hemos hecho un enorme esfuerzo y hemos dado lo mejor de nosotros para vencer las dificultades y limitaciones que nos impuso la pandemia. Esperamos que a pesar de estas dificultades hayamos logrado organizar una reunión que les sea de provecho y especialmente que contribuya a la formación de nuestros jóvenes investigadores.

¡Ojalá que sea de su agrado y la disfruten!

Discurso de la Dra. Mariana Maccioni, Presidenta SAI.

Estimadas y estimados miembros de las sociedades hoy reunidas, colegas, amigas y amigos:

En nombre de la Comisión Directiva de la Sociedad Argentina de Inmunología les doy la bienvenida a nuestra Reunión Anual número 69, que se realizará en conjunto con la Sociedad Argentina de Investigación Clínica, la Asociación Argentina de Farmacología Experimental y la Sociedad Argentina de Nanomedicinas. Ha sido muy grato compartir con las autoridades de estas sociedades el desafío de organizar este evento virtual, en un clima de cordialidad, en donde las desaveniencias se resolvieron con afabilidad y comprensión. Mis más sinceros agradecimientos a todos ellos.

Si bien sabemos que la virtualidad no reemplaza en absoluto el encuentro presencial, el abrazo entre colegas tan añorado, esperamos que en estos tres días y medio interactuemos, se promuevan nuevas colaboraciones y se generen discusiones enriquecedoras en un entorno amigable.

La organización de este Congreso se llevó a cabo en un año teñido por la desazón de una pandemia que recién comienza a apaciguarse y en el que la Ciencia en general y la Inmunología en particular consolidaron su rol protagónico inesperado, erigiéndose como la dosis de esperanza requerida para atravesarlo. Un año que nos encontró con la experiencia acumulada durante el 2020 en cuanto al uso y la potencialidad de las plataformas virtuales, a las cuales nos propusimos sacarles el máximo provecho. Un año en el que los científicos y científicas continuamos alejados de nuestras actividades habituales, procurando atender los problemas inmediatos que nuestras respectivas comunidades demandaban y en el que tuvimos que aprender a comunicar y a divulgar novedades científicas sumamente cambiantes. Un año en el que la aparición de las vacunas puso a la Inmunología bajo la lupa de toda la sociedad.

Justamente, por este contexto, en donde la salud y la ciencia fueron prioridad, el Gobierno Nacional declaró el año 2021 como el año de homenaje al premio Nobel de Medicina César Milstein, no solamente porque su legado configuró un hito en la historia de la medicina, influyendo en la inmunología, la oncología, la biotecnología y la industria, sino porque César Milstein representa el modelo de científico apasionado por el conocimiento básico, pero comprometido con la realidad, capaz de vislumbrar que la curiosidad, y por ende el quehacer científico, son una fuente de desarrollo, riqueza y soberanía para los pueblos.

En sus propias palabras “la ciencia y la investigación básica son como una pieza de cristal, hermosa, hecha por un gran artista, pero de cristal. En cualquier momento, por un mal movimiento, a veces queriendo hacerlo y a veces sin darse cuenta, esa pieza de cristal se rompe y se pierden años y años de trabajo y de preparación…”. Una frase pronunciada hace más de 30 años que tiene más vigencia que nunca.

Es por ello, que celebramos la sanción de la tan anhelada Ley de Financiamiento del Sistema Nacional de Ciencia, Tecnología e Innovación, en la que se declara de interés nacional el establecer un “incremento progresivo y sostenido del presupuesto nacional” destinado al área “por su capacidad estratégica para el desarrollo económico, social y ambiental”. Esperamos sinceramente que su implementación no esté atada a los vaivenes ideológicos de turno y que se transforme realmente en una política de Estado a largo plazo. Uno de los desafíos que esta norma propone es la de lograr la federalización del sistema científico, una deuda pendiente que implica “la producción, difusión y apropiación del conocimiento científico y tecnológico en todo el territorio nacional, priorizando las zonas geográficas de menor desarrollo relativo”. Los que trabajamos y vivimos en el interior sabemos de las enormes dificultades e inequidades existentes que se magnifican cuanto más lejos de Buenos Aires nos encontremos, desaprovechando así un enorme potencial de desarrollo para nuestro país. Muchas veces, a igualdad de talento y esfuerzo, los resultados son significativamente más pobres en el interior, ya que los procedimientos burocráticos se complejizan, incrementando costos y tiempos. Si realmente se entiende el potencial estratégico del desarrollo de una ciencia de calidad en el interior del país para el progreso nacional, se requiere de políticas públicas, nacionales y también provinciales coordinadas que, por citar sólo un ejemplo, alivianen el impacto de los gastos de envío y transporte que deben afrontarse con subsidios de montos similares otorgados a investigadores alojados a

lo largo y ancho de nuestro país. Además, a pesar de los esfuerzos que se realizan, siguen existiendo fallas en el flujo comunicacional que invisibilizan el trabajo, en muchos casos altamente promisorio, de los grupos de investigación del interior. Mucho se ha avanzado, pero aún nos queda un largo camino por recorrer, para resolver un problema que tiene tantos años como la ciencia argentina misma.

La pandemia también abrió nuevas oportunidades de participación y permitió que muchos de nuestros miembros, como así también nuestra recientemente creada Comisión Covid-19, a cuyos integrantes agradezco profundamente, se convirtieran en actores cruciales de esta coyuntura histórica, asesorando a la Subsecretaria de Estrategias Sanitarias del Ministerio de Salud de la Nación, a la Dirección Nacional de Epidemiología e Información Estratégica, y a la Defensoría del Público de Servicios de Comunicación Audiovisual. Agradecemos a los encargados de las diferentes reparticiones nacionales su confianza en nuestra Sociedad y esperamos que este nuevo vínculo con otras reparticiones públicas, no asociadas tradicionalmente a la ciencia, se consolide y robustezca en el futuro.

Desde la Comisión Directiva trabajamos intensamente para estar a la altura de las circunstancias, estableciendo un cronograma de actividades virtuales mensuales que tuvieron como objetivo la formación y actualización permanente de nuestros socios, no sólo en los temas que clásicamente han sido el foco de estudio de nuestra Sociedad, sino que exploramos otras temáticas, como por ejemplo, la enseñanza de la Inmunología en el grado y posgrado. Además, promovimos la visualización de las actividades que nuestros socios y socias más jóvenes realizaron para combatir la información falsa y para generar confianza en la vacunación. Por otra parte, haciéndonos eco de una demanda de nuestros inmunólogos clínicos, tratamos de motivar su participación en nuestra Sociedad, generando actividades de su interés particular. Quisiera agradecer especialmente a la Comisión Clínica, que ha desempeñado su rol con suma responsabilidad atendiendo más de 50 consultas durante el año.

Entendiendo la importancia de la divulgación y comunicación de la ciencia en este momento histórico, generamos un Concurso Nacional de MiniVideos #LasVacunasfuncionan en conjunto con la Academia Nacional de Ciencias, para niños de edad escolar de nivel inicial a secundario, que nos sorprendió gratamente por la cantidad y calidad de los trabajos y la alta participación a nivel nacional. Uds podrán disfrutar de algunos los trabajos representativos ya que serán transmitidos en el escaso tiempo libre que tenemos en el Congreso. Agradezco el compromiso y dedicación de la Comisión de Docencia que participó activamente en este desafío.

Una motivación para continuar con nuestro trabajo diario es la continua solicitud de nuevas membresías y reincorporaciones que este año ascienden a 36, no sólo de miembros adherentes, sino también de miembros titulares. Además, aun en este contexto desfavorable, hemos recibido más de 150 pósters y dos presentaciones a premios Satz. Nuestro comité científico, a quien también quisiera reconocer por su compromiso y dedicación, ha trabajado arduamente para aprovechar los beneficios de la virtualidad y proponer un programa científico de calidad.

Para finalizar, me gustaría agradecer a la Comisión Directiva, con quien trabajamos arduamente en un entorno de cordialidad que hizo amenos nuestros encuentros virtuales. Particularmente, a nuestro vicepresidente, Dr. Emilio Malchiodi, quien siempre apoyó con una actitud positiva nuestras propuestas e ideas. A la Dra Silvia Correa, nuestra Secretaria, por su presencia, su apoyo incondicional y su capacidad de organización. A Mercedes Fuertes, nuestra tesorera por su compromiso y disposición. A CONICET y al MINCyT que nos apoyó financieramente. Vuelvo agradecer a todas y todos los integrantes de nuestras comisiones ad-hoc, incluyendo a los que se dedicaron al manejo de nuestras redes sociales. Les doy también mi agradecimiento al grupo G2 y a todos los y las participantes, coordinadores, y evaluadores.

Espero que disfruten al máximo este programa que hemos desarrollado con mucho entusiasmo y anhelo que prontamente podamos volver a encontrarnos en la tan añorada reunión presencial.

Noviembre, 2021.

Discurso de la Dra. Paula Schaiquevich, Presidenta AAFE.

Queridos colegas

Es un honor para mí darles la bienvenida a la LIII reunión anual de la Asociación Argentina de Farmacología Experimental (AAFE) que se realiza de manera conjunta este año con la Sociedad Argentina de Investigación Clínica, Sociedad de Inmunología y la Sociedad Argentina de Nanomedicinas.

En primer lugar, agradezco el trabajo, dedicación y compromiso de los miembros de las comisiones directivas pertenecientes a las sociedades científicas biomédicas que participamos de este evento, para llevar adelante la reunión anual que hoy nos convoca en tan importante evento académico-científico.

Una vez más, debemos desarrollar la presente reunión de manera virtual como es de público conocimiento como consecuencia de la pandemia de COVID-19. A pesar de la distancia física, nos encontraremos cercanos, compartiendo días con alta intensidad de contenidos académicos abordando una diversidad de temáticas biomédicas y con la participación de un gran número de oradores convocados por las diversas sociedades científicas. Es de remarcar, que los investigadores jóvenes y becarios tendrán una especial participación lo que denota el compromiso de nuestras sociedades para formar y transmitir el conocimiento científico.

Desde sus comienzos, la existencia y desarrollo de la AAFE se basó en nuclear a los investigadores que trabajasen en temáticas relacionadas con farmacología experimental y clínica tal de impulsar el desarrollo de esta disciplina en el país, estrechar las relaciones y complementar el trabajo de investigación entre farmacólogos y promover el adelanto y divulgación de los conocimientos en farmacología fomentando el intercambio científico entre los miembros de la sociedad y la vinculación entre farmacólogos de la región. La AAFE es una sociedad científica que ha transitado 52 años de actividad ininterrumpida, años durante los que han participado y participan diversos científicos vinculados a la investigación en todas las áreas de la farmacología, generando vínculos con otras sociedades científicas nacionales, así como de la región latinoamericana y a nivel internacional siendo miembro participe de la International Union of Pharmacology (IUPHAR). En este sentido, es nuestro enorme placer contar en esta ocasión con el presidente y secretario de la IUPHAR como disertantes así como también, de miembros de las comisiones científicas de sociedades hermanas del área de farmacología de Brasil y Chile. Es nuestra intención que la AAFE mantenga y potencie su lugar en el campo de la farmacología internacional, manteniendo vínculos ya existentes y generando nuevas colaboraciones para fomentar los aspectos académicos y científicos de la farmacología en un entorno de integración multidisciplinaria.

Actualmente, y siguiendo las tendencias internacionales, los miembros de la asociación trabajamos activamente en farmacología básica y asimismo tenemos una fuerte representación de expertos en farmacología clínica humana y veterinaria. En este sentido, se denota el compromiso de nuestros investigadores con la comunidad, reforzando el rol de los mismos en las decisiones clínicas y farmacológicas, mostrando un compromiso con nuestra sociedad para brindar respuestas en el área de la farmacología que así se requieran. Hemos y continuamos teniendo un rol importante en esta situación tan particular que nos toca vivir, la pandemia de COVID-19, en la que los avances farmacológicos de nuevas moléculas, fármacos de reposicionamiento y la evaluación e identificación de nuevos tratamientos han sido fundamentales para brindar alternativas terapéuticas en un cortísimo plazo de evaluación. Esto denota la necesidad de reforzar y potenciar la participación del farmacólogo clínico en nuestra asociación para satisfacer las necesidades de la sociedad e incluso agencias regulatorias. Es por ello, que nuestra visión es la de ser la asociación científica referente para el asesoramiento técnico-social y regulatorio para la toma de decisiones en aspectos relacio-

nados con la farmacología clínica de medicamentos y productos en desarrollo en poblaciones diversas que así lo requieran en Argentina.

Esperamos que puedan aprovechar los contenidos brindados en esta reunión anual, que puedan participar activamente de las actividades programadas, fomentando el intercambio científico-académico entre miembros de la comunidad científica de las diversas sociedades que hoy nos reunimos con una visión integradora, multi0 y transdisciplinaria.

En nombre de la comisión directiva de AAFE les dejo un saludo afectuoso y los invito una vez más a participar activamente de la reunión anual.

Discurso de la Dra. Hebe Durán, Presidenta Nanomed-ar.

Querid@s compañer@s y amig@s de la comunidad científica,

Nos encontramos este año nuevamente para participar de la Reunión Conjunta de Sociedades de Biociencias. Me dirijo a ustedes como Presidenta de la Asociación Argentina de Nanomedicinas (Nanomed-ar) en representación de nuestra Comisión Directiva. En esta oportunidad compartimos esta reunión con SAIC, SAI y AAFE. Mantenemos nuestro interés en la concreción de estas reuniones conjuntas con otras Sociedades del área Biomédica, con la visión de que la discusión de avances científicos en el área es muy relevante dado el carácter multidisciplinario de la Nanomedicina. La difusión de trabajos en un ámbito donde confluyen científic@s dedicados a temáticas diversas de la Investigación Biomédica resulta sumamente valioso para la generación de nuevas ideas que permitan el desarrollo de proyectos enfocados a la resolución de problemas relacionados con la Salud.

No podemos dejar de mencionar que el contexto de pandemia en que se ha visto inmerso no solo nuestro país, sino el mundo entero durante los últimos dos años, nos ha enfrentado a toda la humanidad a nuevos desafíos para poder salir adelante de una situación global extremadamente dolorosa. La pandemia demostró fuertemente el valor de la Ciencia y la Tecnología y de un fuerte Sistema Público de Salud. Nuestro país, más allá de dificultades, ha estado a la altura de los desafíos tanto en C&T como en Salud, sabiendo lo que esto significó para todo el personal esencial, principalmente en el área de la Salud. Nos queda el sabor amargo de las pérdidas en vidas que esta pandemia nos ha dejado. Desde Nanomed-ar, agradecemos a las y los trabajadores esenciales que han permitido sobrellevar estos tiempos de pandemia y aislamiento de la mejor manera posible. Hoy, en noviembre de 2021, vemos un horizonte de salida, gracias al rápido avance de la implementación de vacunas anti-COVID.

Asimismo, para sobrellevar estos tiempos, debimos reformular las formas de comunicarnos e interactuar para poder seguir trabajando, compartiendo eventos, dando y recibiendo clases en todos los niveles educativos. La virtualidad se nos hizo cotidiana, con todas las dificultades que eso implica, aunque también pudimos obtener algunas ventajas, como el hecho de acercarnos a quienes habitualmente no podemos interactuar por largas distancias y costosos viajes para encontrarnos en un meeting. A modo de ejemplo, en la Reunión Anual 2020 de Nanomed-ar realizada de forma virtual, pudimos contar con la participación de prestigios@s científic@s de diferentes partes del mundo, quienes de forma desinteresada se mostraron abiert@s, no sólo a disertar, sino también a participar en fructíferas discusiones científicas, sin importar en algunos casos las enormes diferencias horarias que llevaron a algunos Investigadores a participar en horarios nocturnos.

Con respecto a nuestra Asociación, realizamos en este evento conjunto, nuestra XI Reunión Anual y estamos cumpliendo 11 años de la creación de Nanomed-ar. Quiero saludar y agradecer a quienes me precedieron en la conducción de Nanomed-ar, científicas y científicos pioneros en el desarrollo de la Nanomedicina en nuestro país.

Quiero recalcar también que nuestra Asociación ha valorizado siempre la posibilidad de dar lugar a jóvenes investigadores en formación y estudiantes de doctorado y de grado, dándoles la oportunidad de presentar sus trabajos en sesiones conjuntas con Investigadores formados de amplia trayectoria, sumado al incentivo de premios a los mejores trabajos presentados en sesiones de posters o mini-orales.

Este año la Reunión Conjunta cuenta con numerosos simposios, conferencias, sesiones de posters que abarcan una enorme variedad de temáticas, sumando además simposios dedicados a la presentación de logros de grupos de investigación del país en la lucha contra la pandemia. El excelente programa que podrán apreciar es fruto de la intensa actividad realizada por los miembros de las Comisiones Directivas de las cuatro sociedades participantes, quienes conjuntamente con la empresa G2 y la Secretaria de SAIC, se cargaron al hombro la laboriosa tarea de ocuparse de todas las actividades requeridas para que esta Reunión sea un éxito en su formato virtual.

Agradezco también a todas y todos los participantes, Investigadores, Becarias y Becarios y Asistentes a la Reunión Anual que se suman a este evento.

En particular, como Nanomed-ar, quiero agradecer a los Coordinadores de Simposios y a l@s miembros del Comité Científico, por su trabajo conjunto en la coordinación y selección de premios y a los subsidios y auspicios que hemos recibido de la ANPCyT, la Fundación Balseiro, la Fundación Argentina de Nanotecnología y la empresa Atom Protect.

Para cerrar y pensando en un contexto más global, como científic@s responsables debemos ser conscientes de la importante necesidad de alertar a los poderes gobernantes de la importancia de la C&T para mejorar las condiciones de vida y las condiciones del planeta que nos alberga. Se requieren políticas de C&T que apunten a adoptar medidas para frenar el cambio climático, mejorar las posibilidades de Salud e implementar políticas que tiendan a lograr condiciones de vida dignas en todas las regiones del planeta. Para esto los que manejan los poderes del mundo necesitan a tod@s los científic@s no solo de nuestras áreas de Salud y Biociencias, sino de todas las áreas del conocimiento. Aportemos nuestros granitos de arena en esta dirección.

Sin más que agregar, les deseamos a tod@s que disfruten de esta Reunión Anual Conjunta y esperamos que en el año 2022, la situación de Salud Pública nos permita la presencialidad y podamos encontrarnos en nuestra Reunión Anual de NANOMED-ar.

CONFERENCIA SAIC I
Chair: Dra. Edith Kordon

REDIRECTING IMMUNE CELLS AGAINST BREAST CANCER

Macarena Román [2], Marta Bort [1], Andrea Miró [1], Alex Martínez-Sabadell [2], Enrique Arenas [2] and Joaquín Arribas[1, 2]
[1] Cancer Research Program, Hospital del Mar Medical Research Institute (IMIM) and [2] Preclinical Research Program, Vall d'Hebron Institute of Oncology (VHIO)

It is currently clear that T lymphocytes can be efficiently redirected against tumor cells through chimeric antigen receptors (CARs) or T-cell bispecific antibodies (TCBs). Despite the potential of this therapeutic strategy, to date only CARs and TCBs targeting certain hematological malignancies have been approved to treat patients; no CAR or TCB has been proven effective against solid tumors yet in the clinic. One of the main hurdles in the development of CARs and TCBs is the scarcity of genuine tumor-specific antigens. Because of this shortage, CARs and TCBs have been directed against tumor-associated antigens, which are also expressed in normal tissues, albeit at lower levels. These CARs and TCBs have caused serious or even fatal toxicities because of their "on-target off-tumor" effects on normal tissues. To avoid these toxicities, current clinical trials using CARs or TCBs targeting tumor-associated antigens, such as HER2, are performed with dosages that prevent intolerable side effects, likely compromising anti-tumor efficacy.
In search for novel tumor-specific antigens, we focused in p95HER2, a truncated isoform of HER2 expressed in a subset of HER2-amplified breast and gastric cancers. We have recently shown that p95HER2 is undetectable in normal tissues, making it a very attractive tumor-specific antigen against which it will be safe to direct T cells through CARs or TCBs. Proving the feasibility of this approach, we have already generated and characterized a p95HER2-TCB. This TCB has a potent anti-tumor effect against p95HER2-expressing tumors, but has no effect on cells expressing normal levels of HER2. Based on this solid foundation, we have recently: i) develop and functionally characterize CARs targeting p95HER2, ii) analyze the efficacy and safety of p95HER2-TCB and p95HER2-CAR on patient-derived models of HER2-positive breast and gastric cancers, iii) arm these CARs to make them effective against p95HER2-positive tumors, and iv) anticipate mechanisms of resistance against these T cell-based therapies.
In summary, we will present novel and safe immune therapies against a subset of HER2-positive cancers.

CONFERENCIA SAI I LEONARDO SATZ
Chairs: Dra. Eloisa Arana y Dra. Romina Gamberale

MECHANISMS OF MALIGNANT TRANSFORMATION OF THE IMMUNE SYSTEM

Ari Melnick[1]
[1]Gebroe Family Professor of Hematology/Oncology, Weill Cornell Medicine.

During the humoral immune response, germinal center (GC) B cells undergo dramatic and rapid-sequence phenotypic changes. Transitioning to the GC phenotype involves deep remodeling of the 3D architecture of the genome and extensive redistribution of epigenetic marks. The GC B-cell epigenome displays loss of chromatin activating marks and gain of repressive marks at promoters and enhancers for genes involved in cellular checkpoints, B cell receptor signaling, interferon response, antigen presentation, and other mature B-cell functions. These genes are not "silenced," but instead are simply held in a poised configuration, where RNA Pol II is present and loaded at promoters but is not actively transcribing nascent mRNAs. This pattern is directed by BCL6 in dark zone GC B cells and lymphoma cells, and is reversible by signals from T follicular helper (TFH) and follicular dendritic cells (FDC) in the light zone. BCL6 mediates this transient repression effect by recruiting (a) SMRT/NCOR-HDAC3 complexes, (b) BCOR-RING1B complexes in cooperation with EZH2, and (c) the LSD1 histone demethylase through the BCL6 repression domain 2 (RD2). MTA3 and CTBP may also be relevant BCL6 corepressors but this has not been validated outside of the cell line context. Activation of BCL6 target genes in the light zone is mediated by the histone acetyltransferases CREBBP and EP300, and the histone methyltrans-

ferase KMT2D. Along these lines, FL and GCB-DLBCL manifest almost universal somatic mutation of chromatin-modifier genes (e.g. KMT2D, CREBBP, EZH2, TET2, and EP300). These data suggest that one of the most critical vulnerable transitional states for pathogenesis of GC-derived lymphomas involves epigenetic remodeling downstream of signals received from TFH and FDCs in the light zone. Since epigenetic marks are inherently reversible, it is likely possible to develop epigenetic therapy regimens to correct the defects induced by these mutations. Drugs such as EZH2 or HDAC3 specific inhibitors represent promising approaches for the treatment of these GC-derived malignancies.

CONFERENCIA AAFE - HOMENAJE AL DR. OTTO ORSINGHER
Chair: Dr. C. Reyes Toso

OTTO ORSINGHER PASSED AWAY ON JUNE 26, 2021.

Francisco Stefano
Universidad de Buenos Aires, Argentina

A scientist and a teacher he has left strong footprints on the Pharmacological Sciences of our country. Born in Santa Fe province, at an early age his family moved to Chaco, Saenz Peña city. He studied at the Faculty of Chemical Sciences, Córdoba and graduated as a biochemist in 1955 and as a pharmacist in 1957. He obtained a Rotary Club scholarship to work at the "Istituto Superiore di Sanità" Rome, under the direction of Amilcare Carpi. The high hierarchy of this Institute was a magnet, attracted eminent scientists giving the possibility to meet and interact with the greats of that time. He developed a close relation with the Nobel Prize Daniel Bovet, Victor Whittaker and James Mc Gaugh. These were the solid basis that constructed the personality of Otto Orsingher. At his return to Argentine was invited to join the group being formed by Ivan Izquierdo at the Pharmacology Department, Faculty of Chemical Sciences of the University of Córdoba. In the optimistic environment prevailing at that time, formed with Izquierdo a duo of notable scientists who will contribute to the growth of Pharmacological Sciences. But things were not as good as thought and Izquierdo left Córdoba in 1973 Orsingher took charge of the Department of Pharmacology and kept alive the working enthusiasm. He studied in rats the effects of perinatal undernutrition on the learning and memory performance of the adult animal and showed the importance of perinatal nutrition in the development of brain functions. His research performed 40 years ago is quoted nowadays by scientist working in this field (Perinatal undernutrition reduces alpha and beta adrenergic receptor binding in adult rat brain- Science, 1982). He also described the antiepileptic effects of cannabidiol, (Effect of cannabidiol and of other cannabis sativa compounds on hippocampal seizure discharges Psychopharmacologia, 1973). Of the one hundred and odd citations that this work has, 20% are in publications after 2017, 44 years later.

Besides his intense teaching activity he participated in the organization the AAFE the Argentine Association for Experimental Pharmacology, Meetings etc.

Appointed as Director of the INAME (The National Institute of Drugs) in1993 he contributed to the transparency and modernization of the institution.

Orsingher was not only an excellent scientist, he was a good man, loyal to colleagues and interested in the advancement of science and did not seek laurels or envy those who seek them.

MELATONIN. A MULTIFACTORIAL THERAPEUTIC RESOURCE WASTE IN THE COVID 19 PANDEMIC.

Daniel P. Cardinali.
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Web site: www.daniel-cardinali.blogspot.com

The current COVID-19 pandemic is the most devastating event in recent history and a great challenge for health policy and management. Considering the public health consequences of COVID-19 pandemic and in the face of essentially no treatment options currently available, the use of melatonin to treat COVID-19 disease has been proposed. Due to its wide-ranging effects as an antioxidant, anti-inflammatory, and immunomodulatory compound, melatonin is uniquely placed to affect the consequences of SARS-CoV-2 infection. Furthermore, indirect evidence points to a possible antiviral action of melatonin by interfering with the SARS-CoV-2 / angiotensin-converting enzyme 2 association. Melatonin is also an effective chronobiotic agent to reverse the circadian alteration of social isolation and to control delirium in severely affected patients. As a cytoprotective, melatonin fights various comorbidities such as diabetes mellitus, metabolic syndrome, and ischemic and non-ischemic cardiovascular diseases that aggravate COVID-19 disease. In view of the evidence on the appearance of neurological sequelae in patients infected with SARS-CoV-2, another possible application of melatonin is based on its neuroprotective properties. Since melatonin is an effective means of controlling cognitive decline in the early stages

of Alzheimer's disease, its therapeutic importance for the neurological sequelae of COVID-19 should be considered. Finally, exogenous melatonin may be an adjuvant capable of increasing the efficacy of anti-SARS-CoV-2 vaccines. In this presentation I will review the experimental evidence indicating that melatonin has a prominent therapeutic role in the COVID 19 pandemic. This multifactorial profile is unique to melatonin and is not shared by any other therapeutic drug candidate in the COVID 19 pandemic. Controlled studies are urgently needed to support this proposal.

CONFERENCIA INAUGURAL
Chair: Dra. Laura Bover

IMMUNE CHECKPOINT BLOCKADE IN CANCER THERAPY: NEW INSIGHTS INTO THERAPEUTIC MECHANISMS

James P. Allison, Ph.D. - Premio Nobel de Fisiología / Medicina 2018
MD Anderson Cancer Center, Houston, USA.

Since the finding that CTLA-4 is an immune checkpoint which inhibits T cell proliferation, the existence of multiple non-redundant pathways that limit T cell responses, including the PD-1/PD-L1 axis, has been shown. Ipilimumab, a checkpoint inhibitor antibody to CTLA-4 that blocks its interaction with B7 molecules on the surface of antigen presenting cells and prohibits T cell co-activation, provides long-term survival benefit in ~20% of late stage melanoma patients. Many patients appear cancer-free after a decade or more. PD-1/PD-L1 antagonist antibodies provide objective responses against several tumor types with response rates of about ~25%. Combination of anti-PD-1 and anti-CTLA-4 increases the response rate to ~50% in late stage melanoma and is now standard of care. The FDA has now approved at least 7 different checkpoint antibodies for a variety of cancers. Still, checkpoint inhibitors have yet to provide benefit to patients with immunologically cold tumors. Additionally, potentially severe immune adverse events limit their use in lower mutational burden cancers that often arise later in life. More recent work in my lab and the Immunotherapy Platform centers on CyTOF and single cell RNAseq profiling of tumor-infiltrating T cell populations that mediate effective responses to current immune monotherapies and combinations. The results of this work provide an avenue to identify rational combination therapies that could prove effective for patients and cancers that currently do not respond to immunotherapy while ameliorating potential side effects of increased immune activity.

CONFERENCIA PLENARIA ALFREDO LANARI
Chair: Dr. Carlos Davio

IRON METABOLISM IN OLIGODENDROCYTES AND ASTROCYTES: FRIEND OR FOE?

JM Pasquini[1,2], MV Rosato Siri[1,2], PV Martino Adami[3], ME Guitart[1,2], MS Marcora[1,2], L Morelli[3] and J Correale[4]
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Recent reports show that astrocytes (AST) are able to create a permissive environment for remyelination through their action on oligodendrocyte (OLG) precursor migration, proliferation, and differentiation. When disrupted, iron homeostasis negatively impacts OLG differentiation and impairs myelination. We demonstrate that iron deficiency (ID) affects not only OLG maturation but also AST. Using gestational iron deprivation, we studied OLG requirements for their progression to a mature myelinating state and energy metabolism in primary cultures of OLG and AST from newly born control and ID pups. In particular, oxygen consumption and extracellular acidification rates were measured using a Seahorse extracellular flux analyzer. Both ID AST and OLG exhibited decreased spare respiratory capacity, which indicates that maternal ID effectively induces mitochondrial dysfunction. Absence of glycogen granules was observed in ID AST and an increase in ROS production was detected in ID OLG. Mitochondrial fission was increased in ID AST, while fusion was prevalent in ID OLG. Electron microscopy also showed abnormal cristae in ID mitochondria in OLG as well as in AST. These findings further prove that the regulation of cell metabolism may impact cell fate decisions and maturation.

An additional model of ID was developed by knocking down the divalent metal transporter 1 (DMT1), a multi-metal transporter with a primary role in iron transport and present in AST and OLG. OLG maturation was compromised in primary OPC cultures treated with conditioned medium from DMT1-silenced AST, which suggests that molecules secreted by AST may be affected.

CONFERENCIA SAICII
Chair: Dra. Ana Maria Eiján

MONOCLONAL ANTIBODIES FOR STUDY OF CANCER: DESIGN, PRODUCTION AND CLINICAL VALORATION

Laura Bover
University of Texas, M.D. Anderson Cancer Center, Houston, Texas, USA

In the early 1970s, the idea of producing identical antibodies specific to a given antigen started to arise among the scientific community. It was successfully accomplished when Köhler, Milstein and Jerne created the process of producing monoclonal antibodies (MAbs) and shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery. MAbs generation for cancer poses a series of major technological challenges: **1)** surface antigens expressed selectively/preferentially on cancer cells need to be identified. **2)** it is necessary to target specific epitopes within these antigens, that result in either tumor cell killing, activation or blocking of checkpoint molecules. **3)** an antibody must be generated with the desired function, or for particular applications (i.e cell internalization). **4)** further characterization and preclinical development of the candidate MAb need to be undertaken. Importantly, the first and most critical step in therapeutic MAbs development is **the identification of antibodies that bind to novel surface antigens expressed preferentially on cancer cells.** Contributing to this achievemet, significant progress in our understanding of the cancer cell surfaceome and how it might be exploited therapeutically began around 40 years ago through a series of studies that identified cell surface differentiation antigens used to identify lineage and functional subsets of mouse leukocytes. In subsequent years, hybridoma technology, fluorescence-activated cell sorting (FACS), and omics tools have greatly expanded our knowledge in that field and helped to identify exploitable targets. Moreover, the advance on immunotherapy research in cancer biology, improved clinical outcomes prompting *Science* magazine to name cancer immunotherapy as its 2013 Breakthrough of the Year and later added another Nobel Prize in 2018 (Allison and Honjo) to the many accomplishments of antibodies. Recognition of the power of immunotherapy has heightened interest and demand improved technologies to provide the cutting-edge, high-quality MAbs required for today's basic, to translational to clinical researchers. Our Monoclonal Antibodies Laboratory at M.D. Anderson Cancer Center has developed significant expertise in generating more than **360 MAbs** for various applications and recently licensed MAbs to Pharmaceutical companies for use in clinical trials**.** We are going to discuss some of our developments during this conference.

CONFERENCIA SAIC III
Chair: Dr. Javier Cotignola

THE CANCER GENOME ATLAS, A USABLE GENOMIC RESOURCE?

Jean C Zenklusen
NIH/NCI/CCG/TCGA

The Cancer Genome Atlas (TCGA) is a joint project of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI). TCGA began in 2006 as a pilot project focused on three cancer types: lung, ovarian and glioblastoma. Due to the success of the initial efforts, TCGA was reauthorized for a full production phase in 2009. In the following decade, TCGA collected more than 11,000 cases across 33 tumor types, and generated a vast, comprehensive data set describing the molecular changes that occur in cancer. Tissue sample collection and data generation were completed in 2013 and 2016, respectively. Network maker papers, which are integrative cross-platform analyses of TCGA data on individual cancer types have been published for 31 of the tumor types to date (https://cancergenome.nih.gov/publications)

The value of the TCGA data set cannot be overstated. Its richness has enabled researchers to catalog specific genomic and molecular changes that occur in cancer, to define a more meaningful taxonomy of cancer types and subtypes, and to even investigate questions that were not imagined at the outset of the project, such as the mining of the data to discover new viruses and other microbial agents. The TCGA marker papers have served as additional resources for understanding the molecular features of these cancers, and a launching pad for individual researchers to deepen the exploration of the data generated.

However, translation to the clinic of the wealth of information gathered during the project has been modest, mainly for the lack of extensive clinical data and outcomes, but also because we have not been able to truly comprehend the complexity of the biological processes that underline cancer, focusing mainly on single gene mutations that are a poor reflection of the biological reality.

CONFERENCIA SAI II
Chairs: Dra. Silvia G Correa y Dra. Marina Palermo

IMMUNE SURVEILLANCE OF THE LIVER BY CD8+ T CELLS

Matteo Iannacone, MD PhD.
San Raffaele Scientific Institute, Milan, ITALY

Kupffer cells (KCs) are highly abundant, intravascular, liver-resident macrophages known for their scavenger and phagocytic functions. KCs can also present antigens to CD8+ T cells and promote either tolerance or effector differentiation, but the mechanisms underlying these discrepant outcomes are poorly understood. Here, we used a mouse model of hepatitis B virus (HBV) infection, in which HBV-specific naive CD8+ T cells recognizing hepatocellular antigens are driven into a state of immune dysfunction, to identify a subset of KCs (referred to as KC2) that cross-presents hepatocellular antigens upon interleukin-2 (IL-2) administration, thus improving the antiviral function of T cells. Removing MHC-I from all KCs, including KC2, or selectively depleting KC2 impaired the capacity of IL-2 to revert the T cell dysfunction induced by intrahepatic priming. In summary, by sensing IL-2 and cross-presenting hepatocellular antigens, KC2 overcome the tolerogenic potential of the hepatic microenvironment, suggesting new strategies for boosting hepatic T cell immunity.

CONFERENCIA SAI III
Chairs: Dr. Fernando Chirdo y Dra. Roxana Schillaci

THE GUT MICROBIOTA IN COLON CANCER AND ANTI-TUMOR IMMUNITY

Wendy Garrett
Irene Heinz Given Professor of Immunology and Infectious Diseases, Departments of Immunology and Infectious Diseases and of Molecular Metabolism at the Harvard Chan School of Public Health.

Cancer has largely been considered a disease of genetic and environmental factors, however, increasing evidence has demonstrated a role for the microbiota (especially its anaerobic bacterial membership) in influencing tumor growth and spread, shaping anti-tumor immunity, and affecting therapeutic response. I will discuss both human data from meta'omic analyses and data from mechanistic studies in pre-clinical models that support that specific anaerobic bacteria act as potentiators or restraints of colonic tumorigenesis.

CONFERENCIA PLENARIA ALBERTO TAQUINI
Chair: Dra. Maria Marta Facchinetti

WHY DOES HEME OXYGENASE-1 (HO-1) MATTER IN THE ONCOGENIC SCENE?

Elba Vazquez
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Currently our view of cancer has evolved to include, in addition to the transformed cells that have deregulated homeostatic mechanisms, a wide spectrum of cells of the tumor microenvironment. The dialogue established between the tumor cells and the microenvironment, is an essential determinant of the characteristics of the tumor progression.

The completion of the human genome sequence has generated great expectations in the development of new cancer therapies. However, considering the heterogeneity of most tumors, a single biomarker does not seem sufficient to predict the disease outcome. Furthermore, the problem widens when analyzing and grading the architectural complexity of the epithelial structures. In this regard, innovative high-throughput "omics" platforms are now identifying and quantifying new specific and sensitive biomarkers for detection, stratification and treatment. Although some progress has been made, there is an urgent need to chart a coherent road map with clearly define milestones to guide therapeutics efforts.

Heme-oxygenase 1 (HO-1), the rate limiting enzyme in heme degradation, has been shown to govern a plethora of biological processes and molecular functions associated with anti-tumoral effects in several cancers, ranging from cell proliferation, invasion and migration impairment, to exerting co-regulatory functions at the transcriptional level and preventing DNA damage. This evidences multiple roles beyond its enzymatic activity. Particularly, for the past decade, we have been evaluating using *in vitro* and *in vivo* models, its antitumoral role in prostate cancer (PCa). Thus, this presentation is intended to describe the most pressing issues of HO-1 in PCa and sub-

sequent progression, from its nuclear presence in cells, to its modulation of cell physiology and metabolism, its impact on cellular architecture and its role in modifying the tumoral microenvironment, favoring a less aggressive phenotype.
Advanced PCa exhibits bone dissemination as the preferable site for progression. Bone metastases are incurable and only palliative treatments are available. Our rigorous approach has defined the participation of HO-1 on bone turn over and remodeling and demonstrated that its modulation on both, prostate tumor cells and bone cells, disrupts their communication altering the tumoral bone niche. A better understanding of how these processes influence the early onset of bone metastasis can shed light into more tailored therapies. Overall, we clear provide sound evidence for HO-1's relevance as a key homeostatic factor against the aggressive and metastatic disease.

CONFERENCIA SAIC IV
Chair: Dra. Georgina Colo

NON-GENETIC MECHANISMS OF MAPK-TARGETED THERAPY IN MELANOMA

Ignacio González López-Cepero[1], Lucía Benito-Jardón[1], Valeria Burdiel-Herencia[1], Yaiza Rodríguez-García[1] and Joaquin Teixidó[1]
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Resistance to MAP kinase-targeted therapy in cancer is a frequent response that causes an important clinical challenge. In melanoma, genetic alterations in *BRAF*, especially the $BRAF^{V600E}$ mutation, are the most frequent activating mutations, leading to Erk1/2 hyperactivation. Inhibitors targeting the $BRAF^{V600E}$ mutation such as vemurafenib (VMF) have improved the survival of melanoma patients, especially when combined with MEK1/2 inhibitors including trametinib (TMT). Yet, melanoma treatment with MAPK inhibitors (MAPKi) leads to an almost universal resistance leading to disease relapse. While genetic alterations such as *NRAS* mutations, changes in *BRAF* splicing and *BRAF* amplification are common mechanisms involved in MAPKi resistance of melanoma, non-genetic mechanisms of therapeutic resistance are now beginning to be unveiled. Linked to their high mutational burden, melanoma cells display one of the strongest plasticity amongst cancers. Thus, it is well accepted that during the development of resistance, melanoma cells display several distinct populations including drug-tolerant persistent cells that can cycle or stay non-cycling, while others show rapid high proliferative behavior representing the initial resistant subset. Using combined *in vitro* and *in vivo* approaches, we are investigating the non-genetic mechanisms involved in resistance of melanoma cells to combined VMF and TMT treatment. To this end we are using $BRAF^{V600E}$ YUMM (Yale University Mouse Melanoma) mouse melanoma cells which can be inoculated into immunocompetent C57/BL6 mice. We have *in vitro* generated several VMF/TMT-resistant YUMM cell lineages, and we have *in vivo* obtained different resistant YUMM cell populations which likely encompass drug-tolerant persistent and fast-appearing resistant cells. We are now biochemically, functionally and genetically comparing these resistant melanoma cell populations, and we will identify the immune cell content within the microenvironment of these different resistant tumors. Our results could provide new insights into therapeutic decisions for melanoma treatment.

CONFERENCIA SAI IV
Chairs: Dr. Gabriel Morón y Dr. Norberto Zwirner

DOMINANT AND REACTIVE ARCHETYPES OF CANCER IMMUNITY

Alexis J. Combes[1,2,3,4], Bushra Samad[1,2,3,4], Jessica Tui[1,2,3,4], Nayvin W. Chew[1,2,3,4], Peter Yan[1,2,3,4], Gabriella C. Reeder[1,2,3,4], Im Kwok[1,2,3], Kevin C. Barry[5], Tristan Courau[1,2,3,4], Rafael J Arguëllo[6], Arjun Arkal Rao[1,2,3,4], Adam B. Olshen[3,7,8], Alexandre Boissonnas[9], Saurabh Asthana[3,8], Vincent Chan[2,3,10,] & Matthew F. Krummel[1,2,3‡]
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abstract not available

CONFERENCIA SAIC V
Chair: Dr. Bruno Buchholz

FIXING A BROKEN HEART: EPIGENETIC REVERSAL OF CARDIAC HYPERTROPHY AND ARRHYTHMIA

Tram A. Tran[1], Qing-Jun Zhang[2], Zhi-Ping Liu[2] and Elisabeth D. Martinez[1]
[1]Department of Pharmacology and [2]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, Texas

Hypertrophic cardiomyopathy affects a vast number of individuals as a result of mechanical stress on the heart, drug toxicities, or underlying genetically-driven heart disease. Increases in the size of myocytes as well as reprogramming of transcriptional toward fetal patterns, are hallmarks of the more lethal, pathological forms of cardiac hypertrophy. Transcriptional remodeling leads to impaired contractility, increased fibrosis and cardiac failure with death. In the USA alone, inherited hypertrophic cardiomyopathy affects one in five hundred individuals. No treatment exists for preventing the onset of the disease in individuals born with these causative mutations. Compared to healthy tissue, tissue from cardiomyopathies has lower histone H3K4me3 and H3K9me3, while H4K20 methylation remains unchanged. We have found that the enzymes responsible for erasing histone tri-methylation, the Jumonji demethylases, are aberrantly high in hypertrophic hearts, and their genetic deletion in mice prevents cardiac hypertrophy in response to mechanical overload blocking fibrotic gene expression. Based on this, we hypothesized that inhibition of cardiac H3K4me3/H3K9me3 Jumonji demethylases would reverse transcriptional maladaptive reprogramming and overcome/prevent hypertrophic cardiomyopathy. We will present genetic and pharmacological data defining the role of Jumonji enzymes in the establishment of hypertrophic cardiomyopathy and therapeutic studies of Jumonji demethylase inhibitors that provide proof of principle for transcriptional reprogramming, increased cardiac function, improvement of arrythmias and prolongation of survival from cardiac sudden death in disease models.

CONFERENCIA SAI V
Chair: Dr. Pablo Baldi y Dr. Martín Rumbo

CONTROL OF IMMUNITY AND INFLAMMATION BY THE MICROBIOTA

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abstract not available

CONFERENCIA SAI DE CLAUSURA
Chairs: Dra. Verónica García y Dr. Emilio Malchiodi

HOMEOSTASIS AND INFLAMMATION

Ruslan Medzhitov
Howard Hughes Medical Institute, Department of Immunobiology
Yale University School of Medicine

abstract not available

CONFERENCIA NANOMED-AR
Chairs: Dra. Hebe Durán y Dra. Mariela Agotegaray

EXTRACELLULAR VESICLES AND EXTRACELLULAR RNA: FROM INTERCELLULAR COMMUNICATION TO BIOMARKER DISCOVERY

Juan Pablo Tosar[1,2]
1. Analytical Biochemistry Unit, Nuclear Research Center, School of Science; Universidad de la República, Uruguay.
2. Functional Genomics Unit, Institut Pasteur de Montevideo.

Extracellular vesicles (EVs) are a heterogeneous group of cell-derived nanoparticles that can transfer proteins, lipids and nucleic acids between cells. In the last decade, they have emerged as key players in intercellular com-

munication and their involvement in several diseases is becoming increasingly clear. They are also been tested as carriers of drugs or therapeutic proteins and nucleic acids and they could soon become one of the pillars of liquid biopsy-based molecular diagnostics. Because they transport RNAs in the bloodstream and other biofluids with high ribonuclease activity, the literature describing EVs and extracellular RNAs (exRNAs) are highly intertwined. However, a significant fraction of exRNAs do not co-isolate with EVs and remain in ultracentrifugation supernatants. Trying to understand the biology and the stability of these nonvesicular exRNAs has led us to design new techniques capable of capturing the whole diversity of exRNAs, including vesicular and nonvesicular RNAs with remarkable differences in stability. These results collectively affect our understanding of exRNA biology, highlight immunological implications of exRNAs and greatly expand the exRNA catalogue beyond those encapsulated in exosomes and other EVs, potentially impacting their use as minimally-invasive disease biomarkers.

CONFERENCIA SAIC VI
Chair: Dr. Juan Miguel Bayo Fina

TRANSCRIPTIONAL REGULATORY MECHANISMS SHAPING HIV-1 PROVIRAL FATE

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HIV-1 integration and transcription are two intimate regulatory steps shaping proviral fate (activation, latency, and reactivation). Upon integration into the genome of CD4+ T cells, the provirus is regulated through a complex transcriptional circuit architecture composed of two successive phases: "host" (driven by cellular activators) and "viral" (driven by the viral activator Tat). While the normal function of the host and viral phases creates a Tat positive feedback loop facilitating viral replication, our studies indicate that dysfunction of the host phase (due to reduced host co-factor availability) blunts the viral phase thereby promoting latency establishment. Given the multiple layers of transcriptional control including complex circuit architecture (host-viral phases), diverse integration landscape, and immune cell state (due to physiologic signals and host co-factor availability) it has become difficult to ascertain their precise contribution to proviral transcription and fate. Thus, a key gap in this emerging field is to elucidate the mechanisms by which different physiologic signals promote the latency-reactivation switch and to define how the integration landscape and immune cell state contribute to the heterogeneity in the transcriptional responses. The long-term goal of our research is to uncouple these multiple layers of control to provide a better understanding of the HIV-1 transcriptional program and to exploit novel host co-factors for therapeutic targeting. I will discuss our recent advancements in this research area with particular emphasis on the roles of host co-factors and integration placement into the human genome.

CONFERENCIA SAIC VII
Chair: Dra. Georgina Coló

IMMUNOTHERAPIES IN TNBC: A PRECLINICAL APPROACH TO IDENTIFY NOVEL APPROACHES

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Immunotherapy has revolutionized the treatment regimens for various cancer types, leading to improved clinical responses in otherwise untreatable advanced cancers. Observations showing accumulation of tumor-infiltrating lymphocytes (TILs) within the tumor microenvironment (TME), as well as work highlighting the efficacy of immune checkpoint inhibitors (CPIs), have sparked interest in the further development of these approaches. Checkpoint inhibitors are currently being investigated in the early-stage setting in a number of phase II/III trials in TNBC with various different anti- PD-1, PD-L1 and CTLA-4 agents. Our efforts have been directed to both the development of novel therapeutic approaches and the understanding of the processes involved in drug-related efficacy. In this presentation, the development of pre-clinical animal models of TNBC will be discussed in the context of their use as key tools to test for novel therapeutic concepts and the mechanisms involved in their response. In addition, an overview of current ongoing projects in our laboratory will be discussed. It is expected, as it's the case in several of our preclinical investigations, that these concepts may translate in the near future into their clinical evaluation, as well as to serve as the bases for supporting their development.

SIMPOSIO AAFE N°1 - IUPHAR
Chairs: Dra. Paula Schaiquevich y Dr. Ventura Simonivich

ROLE OF PHARMACOLOGISTS IN THE TEAM, OVERVIEW OF IUPHAR AND LATIN AMERICA

Ingolf Cascorbi, IUPHAR President
Institute of Experimental and Clinical Pharmacology, University of Kiel, Germany

The International Union of Basic and Clinical Pharmacology (IUPHAR) is the umbrella organization of national and regional societies of pharmacology worldwide. Currently we have 58 members representing about 37,000 pharmacologists. The mission of IUPHAR is, in collaboration with the pharmacology societies from around the world, to support pharmacology research, education and their application to improve global health.

To achieve this goal, IUPHAR strengthens the cooperation of academic organizations related to pharmacology in each country, organizes world congress and supports regional meetings, prepares widely-available drug-related databases and pharmacology education materials, and return profits to each country directly and under the cooperation of ISC, WHO, UNESCO, and other relevant organizations. The new governance structure will guarantee that the various research committees as well as early career scientists will have a voice on the IUPHAR executive committee.

IUPHAR is currently intensifying the collaboration with Latin-American pharmacological societies to strengthen multinational collaborations as well as the visibility of the various activities taking place. The Covid-19 pandemic is an example were international collaboration, exchange of knowledge and launch of mutual research projects requires a common platform facilitating such activities. IUPHAR will act as a mediator between its members and its various scientific committees such as NC-IUPHAR or the Clinical Division as well as education initiatives such as PEP.

NEW DISCOVERIES & IMPACT OF COVID ON DRUG RESEARCH AND PHARMACOLOGY, IMPACT LATIN AMERICA

Michael Spedding
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Therapeutic research and healthcare will be profoundly changed by the SARS-CoV-2 virus. The IUPHAR website (https://iuphar.org/) shows how pharmacological societies have spread information and supplied expert advice to their respective governments. The nomenclature committee of IUPHAR (NC-IUPHAR) listed sites for drug action (https://guidetopharmacology.org). Whereas the rapid production of vaccines has been a major success, drugs have rarely been found to be effective (exception, dexamethasone in RECOVERY trial) despite >91,000 articles on SARS-CoV-2 in Pubmed, almost 2000 agents with in vitro activity against SARS-CoV-2, and 6000 clinical trials listed in ClinicalTrials.gov (cost~6bn$) [1]. Why? The highly lipophilic and basic nature of many these agents can cause phospholipidosis [1], as they accumulate massively in cell membranes, and SARS-CoV-2 enters via ACE2 and lipid rafts, then creates its lipid envelope in endoplasmic reticulum membranes. Thus agents such as chloroquine and hydroxychloroquine may work *in vitro*, but are much less effective *in vivo*. Cell membrane lipids (sphingolipids) and sugars are critical for viral envelopes and for viral recognition and we have worked on specific glycosphingolipids which are critical for age-related diseases (Parkinson's and amyotrophic lateral sclerosis, ALS) as they are neurotrophic, and the enzymes (glucosylceramidases) play major roles in disease progression [2]. Surprisingly they are also critical for envelope viruses entry and exit from cells, so the drugs we are developing for ALS (ambroxol [3]) are also to be tested in COVID-19. The discovery of cheap generic drugs for COVID-19 to accompany vaccination is therefore a major goal, but drug development needs to be carefully prioritized.

1. Tummino, T. A. *et al.* Drug-induced phospholipidosis confounds drug repurposing for SARS-CoV-2. *Science* (2021) doi:10.1126/science.abi4708.
2. Henriques, A. *et al.* Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase. *Hum. Mol. Genet.* **24**, 7390–7405 (2015).
3. Bouscary, A. *et al.* Drug repositioning in neurodegeneration: An overview of the use of ambroxol in neurodegenerative diseases. *Eur. J. Pharmacol.* **884**, 173446 (2020).

PHARMACOGENETICS RESEARCH AND TESTING AT INSTITUTO NACIONAL DE CÁNCER, BRAZIL

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The implementation, current status and perspectives of a pharmacogenomic (PGx) program at the Brazilian National Cancer Institute (INCA), targeting the cancer chemotherapeutic drugs – fluoropyrimidines, irinotecan and thiopurines will be presented. This initiative was designed as a research project, approved by the institutional review board. A dedicated task force developed standard operational procedures, which were successfully applied to test gastrointestinal cancer INCA outpatients and pediatric patients from INCA and seven other hospitals, diagnosed with acute lymphoblastic leukemia. The program has been subsequently expanded to include gastrointestinal cancer patients from two additional cancer treatment centers. We anticipate implementation of routine pre-emptive PGx testing at INCA but acknowledge challenges associated with this transition, such as continuous financing support, availability of trained personnel, adoption of the PGx-informed prescription by the clinical staff and, ultimately, evidence of cost–effectiveness.

SIMPOSIO SAI N°1: COVID-19 IN PREGNANCY, CHILDHOOD AND AGING
Chairs: Dra. Silvia Di Genaro y Dr. Esteban Grasso

MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C): IMMUNE DYSREGULATION AND CORRELATES OF DISEASE SEVERITY

Carrie L. Lucas
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Multisystem inflammatory syndrome in children (MIS-C) is a life-threatening post-infectious complication occurring unpredictably weeks after mild or asymptomatic SARS-CoV-2 infection. We profiled MIS-C, adult COVID-19, and healthy pediatric and adult individuals using single-cell RNA sequencing, flow cytometry, antigen receptor repertoire analysis, and unbiased serum proteomics, which collectively identified a signature in MIS-C patients that correlated with disease severity. Despite having no evidence of active infection, MIS-C patients had elevated S100A-family alarmins and decreased antigen presentation signatures, indicative of myeloid dysfunction. MIS-C patients showed elevated expression of cytotoxicity genes in NK and CD8+ T cells and expansion of specific IgG-expressing plasmablasts. Clinically severe MIS-C patients displayed skewed memory T cell TCR repertoires with expansion of *TRBV11-2*+ T cells and autoimmunity characterized by endothelium-reactive IgG. The alarmin, cytotoxicity, TCR repertoire, and plasmablast signatures we defined have potential for application in the clinic to better diagnose and potentially predict disease severity early in the course of MIS-C.
Ramaswamy et al., 2021, *Immunity* 54, 1–13

TISSUE RESIDENT CD4+T CELLS IN COVID-19 PATIENTS

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abstract not available

COVID-19 IN CHILDREN- AN EVOLVING PICTURE

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Among the most intriguing observations in Coronavirus disease 2019 (COVID-19) are the reduced incidence and mortality rates in children. In fact, and in contrast to other viral respiratory infections such as those caused by influenza and respiratory syncytial viruses, SARS-CoV-2 usually develops as a mild infection in children. The reasons for this remain unclear. Considering that pulmonary infiltration by neutrophils plays a relevant role in the development of severe COVID-19 in adults, we analyzed whether neutrophils in children infected with SARS-CoV-2 expressed a particular phenotypic profile. We found that neutrophils from children with COVID-19 had a distinct signature characterized by a reduced expression of adhesion molecules involved in neutrophil migration together with an increased expression of both, the inflammatory markers CD64, HLA-DR and PECAM-1 and the inhibitory receptors leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1) and programmed

death-ligand 1 (PD-L1). This finding is unusual as neutrophil activation is associated with the up-regulation of adhesion molecules as observed in adults with COVID-19. We hypothesized that this particular signature might prevent neutrophil infiltration in the pulmonary capillaries thus providing protection against tissue injury in children. While the course of SARS-CoV-2 infection in children is usually mild, some children develop severe COVID-19. It has been shown that a severe disease in adults is linked to a delayed kinetic of antibody production. We also studied whether a defective antibody response could be associated with a more severe condition in children. We found that children with severe COVID-19 display a very poor and late antibody response against SARS-CoV-2. This weak antibody response occurs due to a low frequency of circulating Follicular Helper T cells and a systemic inflammatory response revealed by high levels of inflammatory cytokines in plasma.
Vaccines against COVID-19 have shown very high levels of safety and effectiveness. In countries where vaccination was widely performed, such as in Israel and the United Kingdom, the infection rate in children is worsening. In this scenario, it is urgent to characterize the factors determining the predisposition of some children to suffer severe COVID-19.

COVID-19 AND PREGNANCY

Gil Mor
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abstract not available

SIMPOSIO SAIC N°1: GENERATION AND INTEGRATION OF NEURAL STEM CELLS IN SYNAPTIC CIRCUITS: IMPORTANCE IN NEUROLOGICAL DISORDERS
Chair: Dr. Gustavo Paratcha

LRIG1 CONTROLS NEURAL STEM CELL HOMEOSTASIS IN THE DEVELOPING CORTEX

Ana P De Vincenti, Gustavo Paratcha
Instituto de Biología Celular y Neurociencias (IBCN)-CONICET-UBA, Facultad de Medicina, Universidad de Buenos Aires (UBA).

The cell-intrinsic mechanisms underlying the decision of a stem/progenitor cell to either self-renew or differentiate are incompletely understood. Here, we show that Lrig1 is early expressed in the neocortex during the period of cortical neurogenesis. The majority of the progenitor cells expressing high levels of Lrig1 were negative for the proliferative marker Ki67, indicating that Lrig1 expression is inversely correlated with proliferation. In agreement with this, we show that Lrig1 abrogates the self–renewing activity of cortical neural progenitor cells induced by mitogenic signals. Cortical progenitors derived from Lrig1-deficient mice give rise to an increased number and size of mitogen-induced neurospheres. Analysis of Lrig1-deficient mice also shows a significant increase in the number of cycling cells during cortical development in vivo. Notably, Lrig1 restricts proliferation of NPCs by regulating the expression levels of cyclins and cell-cycle inhibitors. Together, our results indicate that Lrig1 deficiency triggers proliferation of cortical precursor cells and suggest that Lrig1 may promote neural stem cell quiescence.

DENDRITIC REMODELING AND SYNAPTIC INTEGRATION OF ADULT-BORN HIPPOCAMPAL NEURONS

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In the mammalian adult hippocampus, new neurons are continuously generated throughout life in the subgranular zone of the dentate gyrus. Increasing evidence point out the contribution of adult-born hippocampal granule cells (GCs) to cognitive processes such as learning and memory, indicating the relevance of understanding the molecular mechanisms that control the development of these new neurons in the preexisting hippocampal circuits. Cell proliferation and functional integration of adult-born GCs is a process highly regulated by different intrinsic and extrinsic factors. In this work, we described a novel role of the Glial Derived Neurotrophic Factor, GDNF, acting through its GFRα1 receptor in the control of dendritic structure and spine density of adult-born granule cells. Our findings reveals that GFRα1 is required for the integration of the new born neurons into preexisting circuits and for spatial memory processing.

NOVEL ROLE OF THE SYNAPTIC SCAFFOLD PROTEIN DLGAP4 IN THE VENTRICULAR SURFACE INTEGRITY AND NEURONAL MIGRATION DURING CORTICAL DEVELOPMENT

Delfina M. Romero [1,2,3,#], Karine Poirier [4], Richard Belvindrah[1,2,3], Imane Moutkine [1,2,3], Anne Houllier[1,2,3], Anne-Gaëlle LeMoing [5], Florence Petit [6], Anne Boland [7], Mariano Soiza-Reilly [8], Binnaz Yalcin [9], Jamel Chelly [10], Jean-François Deleuze [11], Nadia Bahi-Buisson [12,13,14], Fiona Francis [1,2,3]

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Subcortical heterotopias are malformations associated with epilepsy and intellectual disability, characterized by the presence of ectopic neurons in the white matter. Mouse and human heterotopia mutations were identified in the microtubule-binding protein Echinoderm microtubule-associated protein-like 1, EML1. Further exploring pathological mechanisms, we identified a patient with an EML1-like phenotype and a novel genetic variation in *DLGAP4*. The protein belongs to a membrane-associated guanylate kinase family known to function in glutamate synapses. We show that DLGAP4 is strongly expressed in the mouse ventricular zone (VZ) from early corticogenesis, and interacts with key VZ proteins including EML1. *In utero* electroporation of Dlgap4 knockdown (KD) and overexpression constructs revealed a ventricular surface phenotype including changes in progenitor cell dynamics, morphology, proliferation and neuronal migration defects. The Dlgap4 KD phenotype was rescued by wild-type but not mutant DLGAP4. Dlgap4 is required for the organization of radial glial cell adherens junction components and actin cytoskeleton dynamics at the apical domain, as well as playing a critical role during neuronal migration. We hence identify a synapse-related scaffold protein with pleiotropic functions in the telencephalic neuroepithelium, influencing the overall integrity of the developing cerebral cortex.

FROM NEURAL PROGENITORS TO A NOVEL BIOLOGICAL TOOL TO UNDERSTAND SCHIZOPHRENIA PATHOPHYSIOLOGY

Diego Gelman

Instituto de Biología y Medicina Experimental (IBYME–CONICET)

A wide diversity of cortical interneurons is generated from the subpallial region of the developing telencephalon by a combinatorial expression of transcription factors in a precise spatiotemporal program. Most cortical interneurons are derived from the medial ganglionic eminence, that specifies both somatostatin and parvalbumin interneurons. It has been shown that fast spiking parvalbumin interneurons are affected in schizophrenia patients, a fact that supports the GABAergic hypothesis of the disease. An inaccurate developmental program of parvalbumin interneurons may contribute to the emergence of psychiatric diseases, including schizophrenia. Dopamine D2 receptors promotes the inhibitory activity of parvalbumin interneurons, especially during the transition between adolescence to adulthood. The dopaminergic hypothesis of schizophrenia is the most perdurable in the field and postulates an imbalance in the dopaminergic tone between cortical and subcortical regions. Moreover, the main pharmacological treatment of schizophrenia, antipsychotics, targets dopamine D2 receptors. In a converging hypothesis between GABAergic and dopaminergic hypothesis of schizophrenia, we generated a mice line that lacks dopamine D2 receptors exclusively from parvalbumin interneurons. Selective dopamine D2 receptor deletion causes an impaired inhibitory activity in the ventral hippocampus and a dysregulated dopaminergic system. Conditional mutant animals show adult onset of schizophrenia-like behaviors. Prefrontal cortex, striatum and ventral hippocampus recapitulates cellular, molecular and physiological endophenotypes as previously described from post-mortem brain studies. Aripiprazole treatment, a widely used antipsychotic, revealed that a functional dopamine D2 receptor is required on parvalbumin interneurons to regain normal locomotor activity. The absence of a functional dopamine D2 receptor signaling promoting the inhibitory activity of parvalbumin interneurons may drive an excessive dopaminergic neurotransmission and an aberrant adult neuronal circuit, leading to schizophrenia-like phenotypes. A complete understanding of parvalbumin interneuron developmen-

tal program and signaling from dopamine D2 receptor may contribute to identify novel target molecules to finely modulate and promote its inhibitory activity.

SIMPOSIO AAFE N°2 - IUPHAR: NEW DISCOVERIES & IMPACT OF COVID ON DRUG RESEARCH AND PHARMACOLOGY, IMPACT IN LATIN AMERICA
Chair: Dr. Sergio Sanchez Bruni

CHILEAN PHARMACOLOGY DEVELOPMENT: FROM BASIC TO CLINICAL EXPERIENCES

Gonzalo E. Yévenes[1*], Javier A. Bravo[2], Guillermo Díaz-Araya[3], Ramón Sotomayor-Zárate[4], Jenny L. Fiedler[5], Miguel Reyes-Parada[6,7] and Jorge Fuentealba[1*]

The Chilean Society of Pharmacology (SOFARCHI) celebrated their 40th anniversary in 2019, as a non-profit scientific society whose main objective is the promotion of research in pharmacology, from theoretical to experimental, and to clinical points of view.

SOFARCHI encourages the development and dissemination of pharmacology through promoting research among its members, sponsoring undergraduate and graduate courses, and fostering the creation of teaching books on different pharmacological topics. The Society also actively aids the Institute of Public Health, an agency of the Chilean state, in the technical evaluation of medicines that require sanitary registration.

Lastly, the Society has developed a strong outreach program engaging our scientific work to high school students along the country.

Over the last 10 years SOFARCHI has experienced important growth, with special promotion on the young scientists participation, where about 70% involves undergraduate and graduate students that the SOFARCHI has potentiated.

Our members have developed their research from molecular and cellular to pre-clinical and clinical pharmacology topics, like kappa opioid system, dopamine-related compulsive behaviors, chemical properties of amphetamine analogs, the contribution and relevance of P_2X receptors in Alzheimer disease, as well as the clinical experience of Chilean pharmacologists who have worked for more than 10 years with dexmedetomidine, cardiovascular diseases and pain, are successful examples.

In conclusion, we believe that the diversity of research areas reflects the integrative spirit of our scientific society and the translational character of the pharmacological sciences.

THE ARGENTINE SOCIETY OF EXPERIMENTAL PHARMACOLOGY: PAST, PRESENT, AND FUTURE

Paula Schaiquevich

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The Argentine Society of Experimental Pharmacology is one of the oldest scientific societies in Argentina. The society gathers researchers from different areas of knowledge towards a common end: to provide with advanced knowledge in the field of clinical, translational, and basic research in pharmacology.

The lecture will go over the history of the society, examples of discoveries that provided the world with benchmark investigations in the area of experimental pharmacology. We will also discuss the mission, vision and strategic goals of the society that are proposed to be aligned with our changing world to facilitate the education and promotion of new discoveries in pharmacology with national and regional impact.

DEVELOPING ANTI-INFLAMMATORY DRUGS FOR INFECTIOUS DISEASES: COVID-19 AND BEYOND

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In the last few years, we have been trying to understand the mechanisms by which inflammation is shut off and tissue homeostasis is regained. It has become clear that resolution of inflammation is an active process driven by molecules collectively known as mediators of resolution. These molecules include lipid mediadores (eg. lipoxin A4), proteins (eg. annexin-A1, plasmin); peptides (eg. angiotensin 1-7) and other molecules, such as Short Chain fatty acids and Hydrogen peroxide. In the context of infection, we have found that pro-resolving mediators, in general, have a tissue protective effect and do not interfere with the ability of the murine host to deal with infection. For example, treatment with Angio 1-7 or lipoxin A4 analogues decreased pulmonary inflammation and disease severity in mice. In an attempt to study this concept in humans, we evaluated the effects of a pro-resolving molecule, a melanocortin agonist, in COVID-19 patients with moderate disease in a phase 2 trial with 60 patients. We found that the melanocortin agonist decreased time to respiratory recovery and hospitalization. This is the first proof of concept study showing that pro-resolving molecules may be beneficial for humans, a tenet that clearly deserves further investigation.

SIMPOSIO SAIC / SAI: OXIDATIVE STRESS AND INFLAMMATION: FROM PHYSIOLOGY TO THERAPEUTICS
Chairs: Dra. Elba Vázquez y Dra. Geraldine Guerón

IDENTIFICATION OF HEME OXYGENASE-1 AS A DNA-BINDING PROTEIN

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Heme oxygenase-1 (HO-1) is a rate-limiting enzyme in degrading heme into biliverdin and iron. HO-1 can also enter the nucleus and regulate gene transcription independent of its enzymatic activity. Whether HO-1 can alter gene expression through direct binding to target DNA remains unclear. Here, we performed HO-1 CHIP-seq and then employed 3D structural modeling to reveal HO-1 DNA binding domains. We found that HO-1 is a DNA binding protein and identified three probable DNA binding domains. Using the Proteinarium tool, we identified several genes that were the most highly connected nodes in the interactome among the HO-1 gene binding targets. We finally validated that HO-1 modulates the expression of these key genes using *Hmox1* knockout cells. Conclusively, HO-1 protein directly binds to DNA, and regulates targeted gene expression. This provides the foundation for developing specific inhibitors or activators targeting HO-1 DNA binding domains to modulate targeted gene expression and corresponding cellular function.

MODULATION OF AUTOPHAGY BY INFLAMMATORY MEDIATORS AS A POTENTIAL HOST DIRECTED THERAPY IN HUMAN TUBERCULOSIS

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During mycobacterial infection, macroautophagy/autophagy, a process modulated by several immune mediators, is essential for mounting successful host responses. We investigated the role of IL17A and IFNG during the autophagy process in monocytes from tuberculosis (TB) patients infected with *Mycobacterium tuberculosis* (*Mtb*) H37Rv strain. TB patients were classified as high responder (HR) or low responder (LR) according to their T cell responses against *Mtb*. IL17A augmented autophagy in infected monocytes from HR patients through a mechanism that activated MAPK1/ERK2-MAPK3/ERK1 but, during infection of monocytes from LR patients, IL17A had no effect on the autophagic response. In contrast, addition of IFNG to infected monocytes, increased autophagy by activating MAPK14/p38α both in HR and LR patients, promoting mycobacterial killing. Besides the crucial function of cytokines, different lipid mediators are critical in the resolution of mycobacterial infection. However, very limited information regarding the PGE2 pathway in TB patients is available. We detected elevated plasmatic PGE2 levels in patients with decreased T cell responses against *Mtb* (LR) in comparison with individuals who show efficient anti-mycobacteria T cell responses (HR). Furthermore, PGE2 displayed a significant suppressive effect on cell proliferation. Additionally, PGE2 significantly decreased the production of proinflammatory cytokines. Our findings suggested a suppressive/regulatory role for PGE2 on the innate and adaptive immune responses of the human host against *Mtb*. However, different roles for PGE2 during *Mtb* infection has been described. In fact, PGE2 has been proposed as a host-directed treatment to counteract the predominant type I interferon response displayed by severe TB patients. In this context, autophagy arises as a potential mechanism that may explain the positive role of PGE2 during the early stages of *Mtb* infection. Interestingly, we observed that PGE2 treatment augmented the percentage of $CD14^{+}LC3A$, $B\text{-}II^{+}$ monocytes and LC3A, $B\text{-}II^{+}$ neutrophils upon *Mtb*-Ag stimulation. Then, PGE2 reduced the production of proinflammatory cytokines but promoted autophagy in TB patients´ cells cultured with *Mtb*, suggesting that PGE2 might be attenuating the excessive inflammatory immune response caused by *Mtb*. Together, our findings contribute to the knowledge of the role of inflammatory mediators in the human host resistance to *Mtb* and highlight their potential as tools to improve anti-TB treatment.

REGULATION OF INTERLEUKIN-1 BETA (IL-1β) SECRETION IN HUMAN NEUTROPHILS

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Neutrophils are the most numerous leukocytes in human circulation. They are rapid and massively recruited to infection foci to fight against the potentially harmful aggressors. Their mission is fulfilled by employing an array of antimicrobial weapons that includes proteases, microbicidal peptides, and reactive oxygen species (ROS). These granulocytes also contribute to the immune response by releasing pro-inflammatory mediators. However, they are double-edged swords of innate immunity because their inappropriate or dysregulated activation also contributes to the development of inflammatory and autoimmune diseases.

We have demonstrated that human neutrophils produce and release the potent pro-inflammatory mediator Interleukin-1-beta (IL-1β). This cytokine is synthesized as a precursor (pro-IL-1β) that is proteolytically processed to an active isoform and then released. As pro-IL-1β is a leaderless protein, it is synthesized in the cytosol. Our studies showed that an unconventional autophagy-mediated secretory pathway mediates neutrophil IL-1β secretion. We also demonstrated that NADPH oxidase-derived ROS are crucial for IL-1β exportation. Our recent findings indicated that even though caspase-1 is required for neutrophil IL-1β secretion, it does not play a central role in pro-IL-1β processing, being the serine proteases, the enzymes involved in accomplishing this function. Altogether, our studies reveal distinctive features of the regulation of IL-1β secretion in human neutrophils. They also unveil new molecular targets that might be employed for the rational design of new therapies to control inflammation in those pathologies where neutrophil-derived IL-1β plays a key pathogenic role, without compromising broad host defense against pathogens.

ROLE OF EPITHELIAL AND STROMAL CELLS IN PROSTATIC INFLAMMATION

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The prostate gland is the main target of inflammatory diseases in the male urogenital tract. From the classical description of “the prostatic antibacterial factor,” many host defense proteins with potent microbicidal, proinflammatory and anti-tumoral activities have been reported in resident cells, i.e. epithelial and smooth muscle cells, during the prostatic response against injuries. However, these cells also represent the target of the inflammatory damage, leading to the development of a Proliferative Inflammatory Atrophy-like process in the epithelium and a myofibroblastic-like reactive stroma. Consequently, prostatitis has been proposed to play a pivotal role in the pathophysiology of the two more prevalent conditions benign prostatic hyperplasia and prostate cancer.

Available information indicates that both prostate epithelium and stroma express fully CD14, TLRs, inflammasomes and other recognition receptors, being able to mount and coordinate the inflammatory and immune responses afterwards. This is particularly important as the triggers of prostatic inflammation include not only pathogens but also autoimmune, mechanical, hormonal, or neuronal factors. For instance, the uric acid from the reflux and influx of sterile urine induces a NALP3-cas1-dependent inflammatory reaction in experimental models of prostatitis.

As the prostate gland is a strictly androgen-dependent organ whose function is to protect haploid gametes, host defenses and immunity of the gland have unique features in order to maintain a constant balance between response and tolerance to diverse antigens. In this context, epithelial cells secrete, in an androgen-dependent manner, numerous proteins with immunomodulatory activities, which favor the presence of specific regulatory phenotypes of inflammatory cells in the prostate. This anti-inflammatory/immunoregulatory effect of androgens is also observed on prostatic smooth muscle cells, where the inflammatory response to endotoxins is dampened in the presence of physiological doses of testosterone. However, it would be too simplistic to ascribe a specific suppressive role to androgens on prostatic inflammation, with these effects being complex and have just started to be defined.

SIMPOSIO NANOMED-AR-NANOMEDICINE I: NOVEL DIAGNOSIS AND TREATMENT STRATEGIES USING NANOTECHNOLOGY

Chairs: Dra. Romina Glisoni y Dra. María Julia Altube

INHALED NANOMEDICINES FOR THE TREATMENT OF PULMONARY BACTERIAL INFECTIONS

Ana Paula Perez

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The incidence of infectious diseases grows at an exponential rate each year and has a direct correlation with high rates of morbidity and mortality. One of the most frequent infections worldwide are respiratory tract infections, with an increasing number being associated with the ability of bacteria to adapt to the environment and different types of antimicrobial compounds. Improved treatment requires the development of new therapeutic strategies.

The inhalation route maximizes pulmonary concentrations and optimizes therapeutic success while minimizing systemic exposure and adverse events. The use of inhaled antimicrobial therapy has become an important part of the treatment of airway infection with *Pseudomonas aeruginosa* in cystic fibrosis (CF) and similar conditions. However, this therapy frequently fails to eradicate the infection due to the combination of the complex pathophysiology of lung disease, antibiotic-resistant pathogens and biofilm formation.

Inhalable nanomedicines improve drug delivery and therapeutic efficacy by enhancing drug retention at the site of action, mitigating effects of clearance mechanisms and degradation, and reducing necessary dosage and frequency, which leads to greater patient compliance and outcomes. However, to date only one antibiotic-nanomedicine for inhaled administration has entered the market, mainly due to diverse obstacles limiting their implementation.

In this talk the obstacles that inhalable nanomedicines must overcome to be effective in pulmonary bacterial infections will be discussed, as well as examples of inhalable nanomedicines in development. Also, strategies based on the use of lipids and archaeolipids nanomedicines targeting microbial biofilms in respiratory diseases will be commented.

NANO BIO ENGINEERING: ITS ROLE IN THE DEVELOPMENT OF SMARTER THERAPEUTICS

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Background: There is a growing need to enhance our antibacterial arsenal given the rising incidence of antibiotic resistance, the emergence of novel virulent pathogens, and the almost 40-year innovation gap between introductions of new molecular classes of antibiotics. In the face of newly infectious organisms and the global crisis in antibiotic resistance, there is a need to invigorate the basic science and technology of antimicrobial development.

Methods: This work, describes different engineering approaches to resolve some of the challenges in antibiotic development.

Results: A first approach successfully allowed the potentiation of current antibiotics using novel and naturally existing therapeutic adjuvants (silver and supplementary metallic micronutrients) based on a better understanding of the mechanisms of infectious disease, a comprehension of microbe-therapeutic biochemical interactions as well as the microbial genetic responses to therapeutics. A second approach allowed us to design an intelligent and endogenous antimicrobial therapeutic using the interface of Nanotechnology and Synthetic Biology. The final approach involved the design of microbial ecologies that incite a competition environment between different microorganisms. This last approach describes an innovative mechanism to discover antimicrobial molecules through the identification of fruitful competitive biochemical interactions between a set of microorganisms in synthetic and natural ecologies. Through microbial competition we discovered a novel antimicrobial exopolysaccharide with applications not only as a therapeutic agent but also as a delivery system of other therapeutic agents.

Conclusions:

Together, the results here described will play an important role in the future development of antimicrobial agents and treatments against infections. In addition, the therapeutic designs proposed here will prevent and contribute toward the decrease in antimicrobial resistant of existing strains.

MICROFLUIDICS TO INNOVATE IN REPRODUCTIVE MEDICINE

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It is well known in life science that integrating knowledge, methods and techniques from different disciplines is the key to tackle many open problems. Multidiscipline definitely offers a future beyond imagination, with many difficulties and challenges that deserve to deal with to achieve innovations and moving then to people.
In the physics of life, micro-nanofabrication techniques have been extensively used. In particular microfluidics, where *small is better*, an area nowadays more affordable thanks to years of nanotechnology growth. Advantages of its applications are precision, control, small volumes, low cost-energy, fast-processes, green-credentials, portability (POC). In reproductive medicine, microfluidics is just starting to bring new interesting ideas to the field [NatureRev2017], and the road is long to see innovations used in clinics and home.
We work with labs on a chip (LOC) for different microswimmers. Under control in laboratory, microdevices had been essential to characterize bacteria and sperm motility. For first, we propose, model and achieve a human sperm rectifier, sperm-diode/sperm-microratchet [PRE2014] that small that you can handle it at the point of one finger. We were able to generate a spermatic current simply designing an asymmetrical biocompatible device and taking advantage of the preferential cell swimming along surfaces. This was possible thanks to bio-observations and to apply physical concepts. Such physical sperm-transport, free of DNA damage, gives place to cell concentration needed for analysis and selection. It was the first step to recent developments for more efficient in-vitro reproduction methods. We went further in applications, improving counting cell devices, where in opposite, the sperm accumulation close to walls is detrimental. For clinical fertility analysis, we proposed meliorations corrugating borders, to the daily used Makler chamber [Biomicrofluidics2015]. In this talk more details and scope of our multidisciplinary achievements will be presented. Even more recently we have reported an unexpected human sperm velocity recovery under ultraconfined conditions, with a nice agreement between model and experiments [Biomicrofluidics2020][Scilight].
We propose a model of the sperm motility, including cells lateral heading and torque that aligns them close to the walls which we hope be useful to predict and design, even more accurate, miniaturized, portable and low cost microdevices for reproductive medicine.

SIMPOSIO SAI N° 2: TISSUE SPECIFIC IMMUNE RESPONSE I
Chairs: Dra. Pilar Aoki y Dra. Carolina Jancic

T-CELL REGULATORY MOLECULES AND CARDIOVASCULAR DISEASE

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abstract not available

INNATE AND ADAPTIVE GAMMA-DELTA T CELLS

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γδ T cells are highly conserved in jawed vertebrates, suggesting an essential role in the immune system. However, there is no consensus whether γδ T cells are innate or adaptive lymphocytes, or both, or something in between. Several waves of functionally defined subsets of γδ T cells develop very early in the fetal thymus. These show limited clonal diversity and can acquire their effector functions already in the thymus, thereby supporting the idea that some γδ T cells are literally innate T cells. For example, IL-17-producing γδ T cells expressing invariant Vγ6+ TCRs are prominent examples of such innate effector γδ T cells in mice. These Vγ6+ T cells are long-lived and self-renewing in a number of peripheral tissues, where they adopt tissue-specific effector phenotypes and fulfill tissue-specific functions including immune responses to pathogens. Recent evidence suggested that the human fetal thymus also produces long-lived innate effector γδ T cells. In particular, some semi-invariant phosphoantigen-reactive Vγ9Vδ2 T cells may develop as type-1 and type-3 effector T cells already in the first trimester of pregnancy, and then expand after birth and persist in tissues into adulthood.
On the other hand, we found that TCR repertoires of non-Vγ9Vδ2 T cells are extremely diverse, and usually do not overlap between individual donors. Notably, monitoring γδ TCR repertoires in patients undergoing hematopoietic stem cell transplantation provided evidence for adaptive anti-viral immune responses of human Vδ1+ T cells.
Our current projects aim at monitoring the dynamics of γδ TCR repertoires and phenotypes during ontogeny and in

response to microbial exposure. To this end, we recently established a protocol for combined single-cell RNA- and TCR-sequencing. This allows us to track the immune response of individual γδ T cell clones, for example in birth cohorts or when analyzing longitudinal samples of patients showing CMV-reactivation after transplantation.

LAG-3, TIM-3 AND TIGIT: CO-INHIBITORY RECEPTORS IN CHAGAS HEART DISEASE

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In chronic infection with *Trypanosoma cruzi*, i.e. chronic Chagas disease (CCD), evidence of exhausted T cells in circulation has accumulated during the last decades. In fact, the frequency of cells with reduced effector capabilities has been directly correlated to cardiac compromise; while contrariwise, the presence of polyfunctional T cells shows association with therapeutic success.
Although programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) were the pioneer inhibitory receptors to be studied in relation to T cell exhaustion, other molecules were demonstrated to complement their function in a lower hierarchical level, apparently enabling the fine-tuning of T cell inhibition. Among these molecules, T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains T cell immunoglobulin and ITIM domain (TIGIT), lymphocyte activation gene 3 (LAG-3) and T cell immunoglobulin and mucin-domain containing-3 (TIM-3)) have been found to be implicated in chronic infections, and arise as promising targets for immune-enhancing therapies. This talk will focus primarily on the role of these inhibitory receptors in peripheral blood $CD4^+$ T cells from CCD patients in relation to *T. cruzi*-specific and non-antigen-specific activation, and how their blockade impact in the functional capacity of $CD4^+$ T cells in the different stage of the disease.

SIMPOSIO NANOMED-AR-NANOMEDICINE II: ARGENTINE NANOTECHNOLOGY DEVELOPMENTS AGAINST COVID-19

Chairs: Dra. Marisa Taverna Porro y Dra. Leticia Higa

WHEN BIO AND NANO MEET: DEVELOPMENT AND PRODUCTION OF DIAGNOSTIC TESTS IN THE FIGHT AGAINST COVID-19.

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In December 2019, a beta-coronavirus called SARS-*Cov 2* emerged in the Chinese city of Wuhan, causing an outbreak of unusual and severe bilateral pneumonia. The virus managed to spread rapidly, expanding westward with a high contagion rate, unleashing the most important pandemic of the last hundred years. This generated a collapse not only in health systems but also in international trade, cutting the supply chain of medical supplies. The first official case registered in our country occurred at the beginning of March 2020. Faced with this scenario, our laboratory presented a proposal to the National Executive Power for the development and manufacture of molecular diagnostic tests and columns for RNA purification, two critical inputs necessary to meet the growing demand of the national diagnostic network. Thanks to the financing of the Corporación Andina de Fomento (CAF), we established a public-private consortium between IIB-UNSAM, the UNQ molecular biology laboratory, and the companies Productos Bio-Lógicos SA and Chemtest SA who contributed their human and technical resources, and administrative capacities to carry out the task. The consortium with the collaboration of different dependencies of the National State brought from China the critical supplies for the development and production of 700,000 manual and automated RNA purification kits that were distributed throughout the country. Also, an isothermal amplification method of viral genetic material followed by detection of nucleic acid by lateral flow immunochromatographic assay (NALFIA) was developed. The kit, called ELA-CHEMSTRIP, combines bio and nano components developed and manufactured entirely in the country, allowing the detection of the viral genetic material present in a swab sample with a detection limit, sensitivity, and diagnostic specificity equivalent to RT-PCR but without the need for sophisticated thermal cyclers. This technology made it possible to decentralize the COVID 19 diagnosis and implement it even in rural areas where there was no infrastructure for molecular diagnosis. In this way, we took advantage of a unique historical opportunity that allowed us to articulate actions and capacities of both the public and private sectors, converging on a common goal. The challenge for the future is to expand and consolidate these capacities to generate positive feedback that enables the development of a national biotechnology industry facing the challenges of the 21st century.

RESPIRATORY NON-WOVEN CLOTH MASKS WITH ANTIMICROBIAL ACTIVITY

Roberto Candal, Patricio Carnelli, Verónica Cainzos, Lucas Guz, Sofía Fantoni, Elsa López-Loveira, Ana María Llois, Belén Parodi, Griselda Polla, Alicia Vergara-Rubio

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The SARS Cov 2 pandemic produced a worldwide increase in the demand for facemasks. Similar situations occurred in the past, although not as dramatic as in the case of COVID-19 (for example, the outbreak of avian flu H1N1 in 2009). The development of efficient materials for making facemasks accessible to all countries is necessary to provide physicians and paramedics in particular, and the general public, with adequate protection against airborne pathogens.

Non-woven polypropylene fabrics produced by different processes as meltblown, spunbond and others, are considered as appropriate materials for the confection of facemasks. The protection provided by these materials is a consequence of the filtration capability and the presence of electrostatic charges that repel or attract the pathogens, blocking the passage through the mesh. For non-medical uses, the combination of nonwovens with cotton (or other) fabrics provides reasonable protection due to the combination of filtration and electrostatic charges.

The incorporation of additives with antiviral and antibacterial activity at the surface of fabric fibers is a strategy to increase the protection provided by the mask. Throughout use, microbes accumulate on the surface of the mask, increasing the likelihood of self-contamination. This problem is even worse when people without special training use the mask. The presence of antimicrobial agents at the fibers prevents the accumulation of viable pathogens. Silver and copper ions display well-known antibacterial and antiviral properties. Its use dates to ancient civilizations, being robust and affordable. Working at the nanoscale, allows great effects with a very low amount of the active agents.

Cu(II) ions were incorporated at different polypropylene non-woven fabrics, using polyvinyl alcohol as matrix to contain and fix the additives. Different formulations and drying conditions were applied. The effect of the hydrophilicity and density (GSM) of the fabric on the mesh (filtration capabilities was determined). The antibacterial activity of selected samples in terms of copper content was determined. A mask prototype was made with two types of non-woven fabrics additioned with copper ions.

DNA NANOVACCINE AGAINST SARS-CoV-2

Ana Clara Mignaqui[1], Romanela Marcellino[1], Federica Ghersa[1], Joaquín Faraldo[2], María de los Ángeles Carignano[2], Verónica Tatsch[2], Augusto Pich Otero[2], Eduardo De La Puente[2] y Juan Sebastián Pappalardo[1].

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On March 11, 2020, the World Health Organization declared a pandemic caused by the SARS-CoV-2 virus and the world had to adapt to a new reality. In this context, at the Veterinary Nanomedicine Group, we were planning preclinical studies in mice to evaluate a candidate nanovaccine against caseous lymphadenitis, a zoonotic disease that affects sheep, among other species, in the Patagonian region.

A question then arose in the group: would it be possible to use our Nanovaccine Platform to contribute to the development of a vaccine candidate to respond to this pandemic? We contacted Laboratorios Bagó S.A. and after discussing it, we decided to try this together. Then, a Research and Development agreement was signed between both parts, that provided the funds to advance with the experiments. The agreement was structured in stages that would be implemented according to the results. The first stage was a proof of concept of the technology, evaluating the humoral immune response in mice, using our platform to carry plasmid DNA encoding for the Receptor Binding Domain (RBD) region of the Spike protein of SARS-CoV-2. The results were very encouraging and prompted us to move forward. We are currently carrying out the end of Stage 2, which will allow us to evaluate two important aspects of the vaccine: On the one hand, we will study the ability of the vaccine to generate immunological memory, that is, an immune cell mediated memory that allows the vaccinated animal to “remember” the pathogen and trigger a fast immune response that protects it from the disease. On the other hand, we will evaluate the safety of the vaccine candidate, that is, if the vaccine is toxic or generates an adverse effect in laboratory animals.

This vaccine is based on rationally designed liposomes targeted to dendritic cells (DC), carrying plasmid DNA molecules encoding RBD (proprietary technologic platform). Targeting is done via DC-SIGN present on DC, and a targeting molecule decorating the liposomes. This synthetic molecule is novel and is based on pathogen-associated molecular patterns, meaning it can target DC of different species, since, unlike monoclonal antibodies, it is a species-non-specific molecule, which allows its use both in animal health as human health. DC are key cells of the immune system, therefore directing the vaccine load towards them significantly increases the efficacy of the vaccine, since these cells will express the SARS-CoV-2 antigens and trigger the immune response.

SIMPOSIO SAI N° 3 GUILLERMINA FELDMAN: CLINICAL FEATURES, IMMUNOPATHOGENESIS AND THERAPEUTIC STRATEGIES IN AUTOIMMUNE DISEASES
Chairs: Dra. Silvia Danielian y Dra. Virginia Rivero

ADDING NEW CLUES TO THE PATHOGENESIS OF HUMAN SLE

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abstract not available

DYNAMIC OF REGULATORY B CELLS IN RHEUMATOID ARTHRITIS.

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive joint destruction associated with increased pro-inflammatory mediators. B cells play an important role in the development and maintenance of RA. Although IL-10-producing B cells represent a major subset of regulatory B cells (Bregs) able to suppress autoimmune and inflammatory responses, recent reports showed that B cell-mediated immune suppression may also occur independent of IL-10. Interestingly, in inflammatory microenvironments, exogenous ATP (eATP) is hydrolyzed to adenosine, which exerts immunosuppressive effects, by the consecutive action of the ectonucleotidases CD39 and CD73. In this direction, mature B cells constitutively express CD39 and CD73. Additionally, B cells also express the inhibitory molecule PD-L1. All the mentioned molecules convert B cells to potential suppressors.

We evaluated the frequency of PD-L1- or CD39+- expressing B cells in the peripheral blood of RA patients compared to healthy controls (HC) matched for sex and age. Fresh peripheral blood B cells from RA patients and HC were characterized by flow cytometry and their functionality assessed in a co-culture system with autologous T cells. Our results show that PD-L1$^+$ B cells exhibiting T cell suppressive capacity are significantly decreased in untreated RA patients but increase in response to successful treatment. Moreover, we determined that B cells from untreated RA patients conserved CD39-mediated regulatory function. Good responder patients to therapy exhibited an increased CD39 expression on B cells after treatment, while most of the non-responder patients showed a reduction in this ectoenzyme expression. The positive changes of CD39 expression on B cells exhibited a negative correlation with disease activity and rheumatoid factor levels. Our results suggest modulating B regs and PD-L1-PD-1and the ectoenzymes/ADO pathways as a potential therapy target for improving the course of RA.

SIMPOSIO SAIC N°2: TRANSLATIONAL RESEARCH IN ONCOLOGY: PRECLINICAL, CLINICAL AND HEALTHCARE APPROACHES
Chairs: Dra. Zulma Ortiz y Dr. Daniel Alonso

PRECLINICAL ASPECTS IN ONCOLOGY TRANSLATIONAL RESEARCH

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In the field of cancer therapeutics, in spite of the great advances accomplished during the last 25 years, which meant the avoidance of millions of cancer-related deaths, lots of people continue to die from cancer, a fact that indicates the need to search for new therapeutic approaches. Based on that need, we have been working for more than 20 years, aiming to achieve three important goals that are, in our opinion, equally important, namely: therapeutic efficacy, good quality of life and, economic viability, a situation that is not the case at present.

In order to fulfill those objectives we adopted and developed two proposals that posed a shift in paradigms related to cancer therapeutics: i) metronomic chemotherapy that consists in the chronic administration, at regular intervals, of low doses of chemotherapeutic drugs, without extended rest periods. This proposal defies the prevailing paradigm: the higher the dose the better, which tends to be changed for this one: less is more when administered chronically; ii) repurposing drugs in oncology that involves the repositioning of drugs that are already well known and characterized, and were created for other uses, to be utilized in oncology, which also suggests a paradigm shift. From the prevailing: one drug, one target, one illness to the new one: one drug, several targets, several illnesses.

Our work was mainly focused on triple negative mammary tumors, an aggressive subtype of cancer with poor prognosis and less therapeutic options. We investigat-

ed the effect of chemotherapeutic and/or repositioned drugs as cyclophosphamide, doxorubicin, celecoxib, metformin, propranolol, losartan, in several combinations,studying tumor growth, survival, metastasis development, mechanisms of action and toxicity. Moreover, in order to facilitate the future translation of the results into the clinic, we tried to mimic the clinical situation. Thus, we imposed several characteristics to our experimental work such as the use of immune competent animals and syngeneic tumor models, the administration of treatment after tumor development, which is given for the most part per os, and the thorough study of its toxicity. One of the drugs combinations was translated to the clinic in a phase II clinical trial which showed therapeutic efficacy, lack of grade 3 and 4 toxicities, and enabled the identification of early predictors of response. Also, in an ongoing clinical trial for pancreas cancer, we are testing other repositioned drug.

CLINICAL AND PREVENTIVE ASPECTS IN ONCOLOGY TRANSLATIONAL RESEARCH

Marina Antelo, Marcela Carballido, Soledad Iseas, Ana Oviedo, Julián Maqueira, Juan Robbio, Giuliana Testa, Daniel Cisterna, Ana Cabanne, Mirta Kujaruk, Guillermo Mendez, Enrique Roca, Mariano Golubicki

Sección Oncología, Hospital de Gastroenterología "Dr. C. B. Udaondo"

Oncological gastroenterology has grown enormously in these years, with new fields still in full growth such as digestive cancer hereditary syndromes. The scientific community specially dedicated to diagnosis, treatment and prevention of hereditary colorectal cancer (CRC) has gained the greatest strength inside this relatively new subspecialty, being Lynch syndrome (LS) the most frequent disease. Its recognition in every new patient with CRC is essential because annual surveillance colonoscopies and total hysterectomy drastically reduce the mortality related to cancer in these patients, and because identification of the causal mutation in one of the LS genes leads to genetic presymptomatic diagnosis in relatives, focusing screening measures on mutation carriers. And almost equally important, the recognition of CRC with mismatch repair (MMR) deficiency, the hallmark of this disease, renders these patients with advanced disease as candidates for PD-1 blockade-based immunotherapy with significantly better survival than patients with MMR proficient CRC.

Accompanying this subspecialty growth, a Hereditary CRC Registry was created in 2008 in the Oncology Section of the Hospital of Gastroenterology "Dr. C. B. Udaondo", at Buenos Aires, with actually more than 1000 patients registered. During the last 13 years, Oncology, Pathology and Molecular Biology have worked interdisciplinary to set up the various molecular and pathological tests for LS diagnosis: immunohistochemistry for MMR proteins, microsatellite instability, BRAF V600E mutations, MLH1 somatic methylation, point mutations for index patient´s relatives, and next generation sequencing of a cancer multi-gene panel, with a parallel constitution of a Molecular Biology Laboratory. We have obtained several national and international grants that have allowed this and gave us the possibility not only to assist our patients, but also to teach and research, publishing several articles in prestigious international journals.

Despite the variable and inconstant support from our sanitary authorities, we have currently become one of the reference centers of Hereditary Digestive Cancer in Argentina, and we will continue working from our public hospital, and hand in hand with our non-governmental organization called IATTGI, to democratize molecular and genetics studies for patients with digestive cancer, providing the best quality of care to all patients, regardless of their socio-economic status.

CLINICAL ASPECTS IN PEDIATRIC ONCOLOGY TRANSLATIONAL RESEARCH

Guillermo L. Chantada

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Translational research in pediatric oncology is a necessity in middle income countries (MIC) in order to carry out research programs of interest for this subset of countries where many of the world's children live. Since we face particular problems, mostly delayed diagnosis, advanced disease and higher toxicity of treatments used in high income countries, obtaining results adapted to the reality of MIC is essential. However, there are many limitations for translational research in MIC such as limited funding, scant interest from the private sector, insufficient support at hospitals and limited training of health care personnel. Nevertheless, there are many examples of successful MIC-oriented translational research programs in Argentina.

Our experience was based on two programs dealing with two pediatric tumors (neuroblastoma, the most common and fatal pediatric solid tumor) and retinoblastoma (an ocular tumor selected as a priority by the WHO global program). In both cases, there was a strong and fruitful partnership between hospitals and academic centers and in the former a public-private partnership including a local pharma company was also a critical part. In the case of retinoblastoma, international collaboration and funding through non-governmental organizations was essential. In neuroblastoma, our group carried out a repurposing program of an anti-idiotype vaccine (racotumomab) against a ganglioside target. After validating the expression of the target in neuroblastoma, a first-in-

children phase 1 study was completed and published and an intention-to-file phase 2 study has been just completed accrual, recruiting patients with neuroblastoma from 4 countries in Latin America. The drug proved to be non-toxic and effective in generating immune response and may be considered for further pivotal clinical studies. In the case of retinoblastoma, the group identified a molecular biomarker, useful for the determination of tumor dissemination which was helpful to design an ongoing prospective clinical protocol. The group also collaborated in the genomic and transcriptomic characterization of metastatic retinoblastoma and patient-derived cell lines and xenografts were developed. High throughput screening for new agents identified innovative drug combinations. In both cases, the studies were also helpful for the training of researchers and improving local capacity for clinical care and laboratory diagnosis of these tumors.

PREVENTION OF CERVICAL CANCER IN ARGENTINA, CONTRIBUTIONS FROM IMPLEMENTATION SCIENCE

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Cervical cancer is one of the leading causes of cancer death among women in Argentina. Human papillomavirus (HPV) screening allows for self-collection with potential to reduce barriers to screening and increase coverage. Implementation science was used to introduce and evaluate performance and implementation of HPV self-collection in Argentina. Main questions were: Would it be acceptable for women? Would it be effective to increase screening uptake? What were main challenges associated with the strategy when scaled-up?

HPV self-collection was introduced in Argentina through the EMA Project, a mix method study carried out in the province of Jujuy: it included a pragmatic cluster randomized trial and qualitative research to evaluate acceptability and adoption by different stakeholders. The study showed that HPV self-collection offered to women by community health workers (CHWs) during home visits was accepted by women and health providers, and it resulted in a four-fold difference in screening uptake between arms. Between 2015 and 2017 the strategy was introduced in 4 additional provinces.

Main challenges when scaling-up HPV-Self collection were analyzed through a fidelity study carried out in La Matanza (Buenos Aires Metropolitan Area). Results showed that there were adaptations regarding the offer of self-collection, which were the result of the different social context where women live, and their relationship with health promoters. As a result, the contact was shorter, with fewer pieces of information provided to women during the offer. This did not impact the quality of the sample taken by women who did accept self-collection, but it might have an impact on the overall acceptability. We also observed that adherence to follow-up was challenging, with reduced level of triage when compared to levels obtained in the EMA randomized controlled trial.

The ATICA study was designed to evaluate effectiveness of an mHealth-based intervention (text messages used to communicate with positive women and CHWs) to increase adherence to triage by HPV+ women who performed self-collection. The study was an effectiveness-implementation hybrid type I trial and used a mixed-methods approach. Results showed that most women accepted receiving text messages; the intervention was effective at increasing triage adherence among HPV+ women.

Summarizing, the studies showed that when offered by CHWs, HPV-self collection is acceptable to women and health providers, and effective to increase screening uptake. However, when scaled-up further adjustments of the offer will be probably needed. Adherence to Triage by HPV+ can be challenging. mHealth methods are effective to communicate with women with reduced access to the health system, they increase triage, and they are accepted by women.

SIMPOSIO SAIC N°3: MATERNAL-FETAL INTERACTION: FROM FERTILIZATION TO THE NEXT GENERATION

Chairs: Dra. Alicia Damiano y Dra. Alicia Jawerbaum

EARLY RESPONSES OF CHORIONIC VILLI TO ZIKA VIRUS EXPOSURE

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Zika virus (ZIKV) infection has caused severe unexpected clinical outcomes in neonates and adults during the recent outbreak in Latin America, particularly Brazil. Congenital malformations associated with ZIKV have been

frequently reported; nevertheless, the mechanism of vertical transmission and the involvement of placental cells remains unclear. This study applied quantitative proteomics analysis in a floating explant model of chorionic villi of human placental tissues incubated with ZIKV and with ZIKV pre-adsorbed with anti- ZIKV envelope protein. The regulation of specific proteins was measured using immunofluorescence and immunoperoxidase assays. Proteomic data show altered levels of proteins involved in cell proliferation, apoptosis, inflammatory processes, and the integrin-cytoskeleton complex. Antibody-opsonized ZIKV particles differentially modulated the protein expression pattern in placental cells; this phenomenon may play a pivotal role in determining the course of infection and the role of mixed infections. These data fill gaps in our understanding of ZIKV in the placenta and help identify infection control targets.

This work was supported by FAPESP (2018/18257-1, 2018/15549-1, 2020/04923-0) and CNPq (Scientific Productivity Fellowship).

CELLULAR FETAL MICROCHIMERISM: LINKING MATERNAL HEALTH TO OFFSPRING'S CELLS

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The talk will start (Staff AC) with a presentation of how multiple adverse obstetric outcomes linked to placental dysfunction, including preeclampsia and fetal growth restriction, correlate with an increased risk of several non-communicable diseases (NCDs) in women, particularly cardiovascular disease (CVD). The potential mechanisms behind such epidemiological associations will be discussed, as well as the need for improving how we target the women who are at the greatest risk of CVD for intensified follow-up and primary prevention of premature morbidity and mortality. The lack of applicability in traditional clinical scoring systems for assessing young women after pregnancy with respect to prediction of future CVD will be presented, as well as some promising biomarkers for precision follow-up. An improved pathophysiological understanding of the crosstalk between the placenta and the cardiovascular system will likely lead to new opportunities for intervention and prevention of NCDs in women.

The talk will continue (Fjeldstad HES) with the presentation of our hypothesis that placental dysfunction, secondary to poor maternal-fetal tolerance, leads to increased levels of cellular fetal microchimerism (cFMC). cFMC arises during pregnancy when fetal cells with stem cell like properties cross the placenta and are incorporated into the pregnant woman's body longterm. The background for our hypothesis is that circulating cFMC is known to be augmented in preeclampsia, a hypertensive pregnancy complication characterized by placental dysfunction. Furthermore, we will present findings from papers suggesting a link between cFMC and maternal autoimmunity, cancer, wound healing, and potentially cardiovascular disease. As cFMC is known to persist for decades following pregnancy, and is augmented in preeclampsia, which we in turn know is linked to longterm CVD, we hypothesize that there may be a role for these cells in the pathogenesis of cardiovascular and neurovascular disease involving small and larger arteries, possibly by way of inflammatory pathways. Some preliminary cFMC data from our own follow-up studies of women with a history of placental dysfunction will be presented.

FETAL PROGRAMMING OF CARDIOVASCULAR DISEASES IN MATERNAL DIABETES: INSIGHTS IN THE MECHANISMS INVOLVED

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Cardiovascular diseases are increasing at alarming rates in both developed and developing countries. Although lifestyle choices and genetic predisposition are main contributors to cardiometabolic diseases, growing evidence indicates that *in utero* exposure to adverse environmental conditions leads the developing offspring to have numerous risk factors, which may have an impact later in life.

Maternal diabetes is a prevalent pathology that increases the risk of cardiovascular diseases in the offspring. The heart is one of the main target organs affected by this metabolic disease from the embryonic stage and until the adult life. Putative mechanism involved in intrauterine programming of heart damage evidenced in experimental models of maternal diabetes will be presented. The focus will be on altered pathways that regulates cardiac cellular metabolism, the damaging effects of the prooxidant and proinflammatory environment and alterations on the cardiac extracellular matrix remodeling. The role of three main players of these pathways will be discussed: mechanistic target of rapamycin, a cellular sensor for energy metabolism and nutrient availability that controls cellular growth and metabolism, peroxisome proliferator activated receptors, nuclear receptors highly involved in heart metabolic processes and lipid metabolism and the transcription factor forkhead box protein O1 which partic-

ipates in myocardial metabolic stress adaptation, oxidative stress, endothelial dysfunction and other processes related to inflammation and apoptosis.
All these pathways are interconnected evidencing the complexity of this process but also bringing opportunities to facilitate intervention to provide protective effects to prevent the programming of cardiovascular diseases in the offspring of diabetic pregnancies.

SIMPOSIO SAIC N°4: NEW TREATMENTS IN SPINAL MUSCULAR ATROPHY (SMA): FROM BASIC RESEARCH TO PATIENTS TREATMENT
Chairs: Dr. Pablo Gravina y Dra. Verónica Aráoz

CHROMATIN AND SPLICING-CORRECTING ANTISENSE OLIGONUCLEOTIDE THERAPY OF SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is caused by mutations of the SMN1 gene. Humans have a paralog gene, SMN2, that cannot fully compensate for the deficiency in the SMN protein because its exon 7 (E7) is poorly included in the mature mRNA.
A successful approved therapy for SMA restores normal levels of SMN expression using an antisense oligonucleotide (ASO1, nusinersen or Spinraza) designed to increase E7 inclusion in the SMN2 transcript. Our studies aim at understanding the detailed mechanisms by which histone marks that affect chromatin structure, modulate the effects of nusinersen on SMN2 E7 inclusion, mainly through the control of RNA polymerase II transcriptional elongation.
Previously we showed that slow transcriptional elongation promotes *SMN2* E7 skipping. Consistently, intragenic chromatin decondensation elicited by histone deacetylase (HDAC) inhibitors such as valproic acid (VPA) promotes *SMN2* E7 inclusion. VPA cooperates with a nusinersen-like ASO1 to promote E7 inclusion. ASO1 also elicits the deployment of the silencing histone mark H3K9me2 on the *SMN2* gene, creating a roadblock to RNA polymerase II elongation that acts negatively on E7 inclusion. By removing the roadblock, VPA counteracts the undesired chromatin effects of ASO1, resulting in higher E7 inclusion. Combined systemic administration of ASO1 and VPA in neonate SMA mice had strong synergistic effects on SMN expression, growth, survival, and neuromuscular function.
We analyzed the effects of ASO1 and/or VPA on the deployment of the H3K9me2 mark genome-wide by ChIP-seq, obtaining results that support the evidence that the ASO1's chromatin effect on the SMN genes is highly specific.
Based on these observations, we suggest that HDAC inhibitors have the potential to increase the clinical efficacy of nusinersen, and perhaps other splicing-modulatory ASO drugs.

EARLY INTERVENTION AND THERAPEUTIC EXPERIENCE IN SMA: LESSONS LEARNED AND FUTURE DIRECTIONS

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Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disorder characterized by progressive muscle weakness and atrophy as a result of motor neuron degeneration and loss in the anterior horn of the spinal cord. With an incidence of 1 in 10,000 live births and a carrier frequency around 1/50 is one of the commonest severe hereditary disorders of infancy and early childhood. Despite SMA clinically manifests as a continuum, based on age of onset, achieved motor milestones and clinical severity, SMA patients are divided into type 0-IV ranging from very severe congenital forms with short life expectancy due to respiratory failure to adult-onset patients maintaining the ability to walk. SMA is a disease of two genes, a determinant *SMN1* and a modifier *SMN2*. Pathogenic variants in the *SMN1* gene cause SMA disease whereas *SMN2* copy number is variable from 1 to 5 in most SMA patients. Numerous studies show that the higher the number of copies of *SMN2* the greater the amount of complete SMN protein produced, the milder the associated SMA phenotype and vice versa.
Knowledge and advances on SMA genetics allowed the development and approval of tailored therapies that has changed trajectories of the disease with evolving phenotypes. Nusinersen and risdiplam agents are designed to bind specifically to SMN2 pre-mRNA in order to promote exon 7 inclusion increasing the amount of functional SMN protein. Onasemnogene abeparvovec-xioi, a third approved treatment, consists of a gene replacement therapy that restores the expression of normal *SMN1* using a viral vector (AAV9) expressing *SMN1*. While the *SMN2* endogenous target regions comprise splicing regulators and intronic regions, the SMN1 transgene in the

AAV9 is an *SMN1* cDNA lacking intronic or other regulatory elements.
Several clinical investigations demonstrate that early diagnosis and intervention is essential for improved response to these treatments and better prognosis. Therapeutic interventions that are effective at pre-symptomatic or early stages of the disease creates the need for awareness, expedite diagnosis and consideration of newborn screening programs as well as the implementation of an adequate process of communication, genetic counseling and management of expectations for the patient and family.
Supported by Biogen, Roche, Galicia AME and the Spanish Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias and cofunded with ERDF funds (Grant No. FIS PI18/000687) for SMA research grants.

MANAGEMENT OF SPINAL MUSCULAR ATROPHY IN ARGENTINA: CARE EVOLUTION AND CURRENT SITUATION

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, considered one of the leading causes of infant mortality. It is caused by mutations in the *SMN1* gene. Over the past two decades, knowledge on the disease has greatly increased leading to the development of new treatments and the approval of the first drug Spinraza by the F.D.A. (Food and Drug Administration, USA) at the end of 2016. General management and care of SMA patients varies due to heterogeneous health care in Latam as in Argentina. In 1997, the first molecular studies of the *SMN1* gene were performed in our country. In 2006, a program was set up for patient care, teaching, and research in NMD at Hospital Garrahan in order to improve the quality of life of SMA patients. A dedicated and trained interdisciplinary team was set up, consisting of a neurologist, physical therapist, pathologist, clinician, geneticist, palliative care specialist, nutritionist, and endocrinologist, among others. The team covers different aspects of the SMA patient ranging from diagnosis to transition to adult care and end-of-life palliative care for the patients and their families. In 2004, families of patients with SMA have set up a non-profit association in Argentina (FAME; www.fameargentina.com.ar/). This parent association has been a strong advocate for and sponsor of the development of early diagnosis, care, and research, allowing for health professionals in the field to attend international meetings and online courses and participating in the organization of training symposia. Different regional academic-scientific organizations and SMA parent organizations are contributing to the increased quality of the management of SMA patients. In spite of these efforts in Argentina, the treatment gap - the difference between people with SMA and the patients who are actually treated - is still wide. There is a need to create new awareness of the disease so as to get to an early diagnosis and treatment.

SIMPOSIO SAI N° 4: TISSUE SPECIFIC IMMUNE RESPONSE II
Chairs: Dra. Carolina Amezcua y Dra. Maria Silvia Ventimiglia

REGULATION OF ENTERIC IMMUNITY: A CYTOKINE'S CELLULAR STRATIFICATION OF FUNCTION

Ruaidhrí Jackson
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The intestinal mucosal barrier is critical in maintaining a balance of tolerance to the commensal microflora while reserving the capacity to combat invasive microbial infection. Although it is not surprising that immune and intestinal epithelial cells have been discovered to play a major role in orchestrating this equilibrium, they alone do not solely control intestinal homeostasis, and there is an emerging appreciation of the vital interconnectivity of the diverse cell types present in the intestine to synchronize mucosal immunity. Here we have uncovered that the cellular source of IL-18 is essential in dictating divergent functional immune responses in the intestine with major implications for homeostasis and bacterial infection. By combining in situ immuno-fluorescence, smFISH and single cell sequencing, and single cell sequencing analysis, we have identified enteric neurons as a key source of IL-18 expression. Interestingly, by conditional deletion of IL-18 in the enteric nervous system, we have uncovered it's essential and non-redundant role governing landscape of antimicrobial protein expression in the colon. By abrogating neuron-derived IL-18, we observed a breakdown in the sterility of the inner mucus layer and an inappropriate infiltration of the resident microbiota. Strikingly, mice deficient in neuronal-derived IL-18 were highly susceptible to invasive *Salmonella* typhimurium infection. Together, this work identifies enteric neurons as another arm of the innate immune response and highlights a new area of investigation in the mechanism controlling mucosal immunity.

SINGLE CELL RNA-SEQUENCING OF HUMAN AND MOUSE TISSUES UNCOVERS UNIQUE FEATURES OF TH2 CELLS

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Single cell RNA-Sequencing is a powerful technique that has the potential to pinpoint important features of rare cell types at many anatomical locations. In our studies, we have used this tool to probe the heterogeneity of T helper cells in mouse models of allergy and in chronic rhinosinusitis patients with nasal polyps. Here, I discuss the similarities and differences in gene expression profiles in T helper cells between two distinct mouse models of allergy. I outline the heterogeneity of T helper cells in human nasal polyp tissue, a Th2 cell-driven disorder of the nasal cavity that is highly associated with asthma. Lastly, I reflect on the clonal heterogeneity of T helper cells and what this may tell us about T helper cell differentiation from naïve CD4 T cells.

A NOVEL MECHANISM FOR PRETERM LABOR AND BIRTH: CONFLICT BETWEEN REGULATORY AND EFFECTOR T CELLS

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Preterm birth is the leading cause of neonatal morbidity and mortality worldwide, which, in most cases, is preceded by spontaneous preterm labor, a syndrome of multiple etiologies. Intra-amniotic infection is a recognized cause of spontaneous preterm labor; however, the remaining etiologies are poorly understood. We hypothesize that a tight balance between regulatory (Tregs) and effector (Teffs) T cells is required for successful pregnancy, and that a conflict between these adaptive immune cells can lead to preterm labor and birth. First, we report that the targeted depletion of functional Tregs during the first and second pregnancy induces preterm labor/birth and adverse neonatal outcomes. The depletion of Tregs also increases maternal susceptibility to infection leading to preterm birth. Furthermore, the depletion of Tregs causes mild cellular immune responses in the placenta and systemic maternal inflammation, in the absence of a fetal inflammatory response. Next, we show that functional Tregs are reduced at the human maternal-fetal interface in non-inflammatory preterm labor (i.e. idiopathic preterm labor). Such a Treg reduction is accompanied by a concomitant increase in Teffs, which display an activated phenotype and are enriched at the maternal-fetal interface of women with spontaneous preterm labor and birth. Following, we show that the non-specific *in vivo* systemic activation of T cells induces preterm birth by eliciting an inflammatory response notably distinct from that of endotoxin- or RU486-induced preterm birth. Moreover, placental single-cell RNA sequencing-derived signatures of activated T cells can be monitored in the maternal circulation, and such are enhanced in women who underwent spontaneous preterm labor and birth. Collectively, these findings implicate effector and regulatory T cells in the pathological processes underlying preterm labor and birth and accentuate the importance of these adaptive immune cells during pregnancy.

Acknowledgements
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SIMPOSIO AAFE N°3: MICROBIOME: ITS POTENTIAL APPLICATIONS AND CLINICAL EVIDENCE
Chair: Dra. Fatima Nader

MICROBIOTA Y ENFERMEDAD HEPÁTICA: EL EJE INTESTINO-HÍGADO

Adrian Gadano
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Liver cirrhosis is widely prevalent and is associated with high morbidity and mortality. Cirrhosis is a consequence of chronic liver inflammation that is followed by diffuse hepatic fibrosis, which eventually leads to liver failure. The microbiota, including bacteria (bacteriome), but also fungi (fungome) and viruses (virome), are also known to

change during the development and progression of cirrhosis. Several factors appear to affect the microbiota including the aetiology of liver disease, such as alcohol and diet (in the case of metabolic associated fatty liver disease [MAFLD]). This last etiology is particularly interesting since changes in microbiota might be closely related to the patogénesis of MAFLD which currently affects approximately 25 % of adult population in the world.
Chronic liver disease (CLD) may cause cholestasis, which impairs the enterohepatic circulation and affects the microbiota. As CLD progresses, changes in microbiota (dysbiosis) are maintained and further exacerbated, probably by changes in intestinal motility, permeability, barrier function towards the lymphatic and blood compartment, portal hypertension and the immune system. Yet the role of the microbiota seems to be pivotal in patients with decompensated cirrhosis, as many decompensating events are related to microbes or their interaction with the host.
Changes in the microbiota occur early in the development of CLD, even before detectable liver damage, especially in alcohol-related CLD and MAFLD. Different studies have shown shifts in the composition of the gut microbiome in different CLDs. Yet, one common property of these changes, which is easy to assess, is the massive reduction in microbial diversity upon the development of cirrhosis and the even greater reduction upon decompensation. In addition to reduced species diversity, bacterial overgrowth occurs in the small bowel, so-called small intestine bacterial overgrowth (SIBO). The motility of the gut is decreased, which leads to an increase in contact time of bacteria and thereby to fermentation changes in the luminal content. This may lead to changes in the microbial metabolites, which may affect the epithelial cells and the liver itself.

MICROBIOME: GENERALITIES AND CHARACTERIZATION

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The human microbiome can be defined as a microbial community, including bacteria, archaea, fungi and viruses, and its interplay with environmental factors. Over the last decades, the next-generation sequencing and the multi-omics technologies such as metatranscriptomics, metabolomics, and metaproteomics, attempted to analyze and integrate multi-omics datasets to interrogate microbial signatures, combining the environmental and microbial genomics, transcriptomic, metabolic, and proteomic interactions. between microbes and their niche. These omics-based studies, conducted in normal healthy populations and in several diseases, have revealed the genetic and functional traits of the gut microbiota in both eubiotic and dysbiotic conditions. Integration of metagenomic and metataxonomic data with other omics information has become increasingly common, but downstream analysis after data integration and interpretation of complex results remains challenging. Recent analytics programs to analyze and integrate multi-omics datasets and its further utilization in other advanced techniques, such as microbial culturomics and machine learning, have become crucial to provide valuable insights regarding the features of the microbiome. There is growing evidence of an association between a wide range of diseases and disbiotic microbiome, with the potential to develop novel screening, prognostic or therapeutic markers. Furthermore, there appears to be almost limitless potential in using big data for personalized medicine, but the progress in this field is irregular. The emergent analytical methodology needed to integrate microbiome data in other multi-omics investigations, especially with statistical and computational techniques for integrating and representing data through networks. Beyond that, the challenge is to integrate this information in an effective, practical and ethical way to transfer this increasingly technique to the clinic.

MICROBIOME AND COLORECTAL CANCER: WHAT DO WE KNOW AND WHAT DO NOT.

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Colorectal cancer (CRC) is the fourth commonest cause of global cancer-related deaths. Incidence rates have traditionally been highest in developed countries but are increasing in developing countries. CRC cases were related to poor diets with intake of food low in whole grains, low in dairy products and high in red and processed meats. Intestinal microbiota may largely explain the interaction between dietary factors and CRC. It is well known the colorectal carcinogenesis involves genetic and environmental factors. Recent studies suggest that several mechanisms implied in carcinogenesis (i.e., inflammation, genotoxins, oxidative stress, metabolites), are closely linked to the intestinal microbiota.
The human intestinal microbiota is composed of 10^{13} to 10^{14} microbes, which represent 10 times more bacterial cells than human cells containing > 100 times as many

genes as in the human genome. A healthy microbiota plays is crucial for developing the intestinal epithelium and maintaining immunity. Microbiota dysbiosis alters host physiological functions, leading to various diseases. Recent studies analyzed the role of the intestinal microbiota in colorectal carcinogenesis showing differences in the intestinal microbiota compositions between patients with CRC and healthy individuals. Although the causality is yet to be in deep characterized, there are several potential mechanisms implicated. Moreover, microbiome alterations also occur with colorectal adenoma, the precursor of CRC. Additional research suggests that modulating the intestinal microbiome may be a new strategy for CRC prevention and treatment. This presentation will summarize recent advances in understanding the associations between the intestinal microbiota and CRC based on evidence from animal and human studies and it will also describe the clinical value of the intestinal microbiota and novel strategies for preventing and treating CRC.

PROBIOTICS, POSTBIOTICS… CONCEPTS AND MISTAKES

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This year we are celebrating the first 20 years of life of the 2001/2002 of the FAO/WHO Guidelines for probiotics. Since that time the word “probiotics” has been used, in association with “humans” in more than 20000 paper, pointing out the “scientific success” of this concept. It is nowadays recognized by both scientists and regulatory bodies that:

- Probiotics must be viable till the moment of consumption
- Probiotics must be in an amount which is able to provide the beneficial effect which is claimed
- Probiotics must provide a beneficial effect, supported by means of human clinical trials

These qualifications are the basis for the quality control by the regulatory bodies, mainly the first one, as the number of viable cells is a rather critical parameter.

In other words, the definition has a set of “qualifications” enabling us to provide a solid support to research and applications.

Today a plethora of new *somethingbiotics* are also present in the scientific literature dealing with supposedly beneficial bacteria or their metabolites:

Postbiotics, microbiotics, paraprobiotics, skinbiotics, psychobiotics, synbiotics, ect

In my presentation, I will address the relevance to be “prudent” in suggesting new names and also to be scientifically sound in defining the “qualifications” of a definition. We, as scientists, must be aware that our “definitions” can have an impact also on the regulatory bodies, on health professionals not skilled in gut microbiology and finally and probably most importantly on consumers. In several of brand-new definitions these qualifications are unclear or missing. I will use a recent debate on the definition of postbiotics (1,2) to focus on the relevance of “measurable qualifications” to provide a scientifically sound definition.

Just as an example in the case of postbiotics: which is (are) the microbial cell component(s) of unviable that are providing the claimed beneficial effect? How can we measure the presence of this (these) substance(s) during the shelf-life? What about a dose response? etc

I feel this is the moment to have a further advancement of a collaboration among scientists and regulators.

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SIMPOSIO SAIC N°5: NEW PARADIGMS IN ENDOCRINOLOGY
Chair: Dra. Virginia Massheimer

THYROID HORMONES AND IMMUNITY: PATHOPHYSIOLOGICAL EFFECTS

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Growing evidence has revealed a bidirectional crosstalk between thyroid hormones (THs) and the immune system. This interplay has been demonstrated for several pathophysiological conditions of the thyroid functioning, and the innate and adaptive immunity. Many situations primarily affecting the action of THs have an impact on the characteristics and/or functions of immune cells, and are translated to host defense status and related disorders. In turn, immune-related disorders conduct to the most frequent thyroid dysfunctions, which have an autoimmune origin. The connection between these systems is complex and not well-understood (Montesinos & Pellizas, Front Endocrinol 2019, 10:350). Cellular activity of THs is classified as genomic and nongenomic. The former is exerted by the participation of 3,5,3`-triiodothyronine (T3) and its nuclear receptors (TRs). The latter is initiated either by TR isoforms in the cytoplasm and mitochondria or at the plasma membrane, where the integrin $\alpha_V\beta_3$ binds mainly 3, 5, 3` 5` tetraiodothyronine (T4, Bernal et al. Nat Rev Endocrinol 2015, 11 690). It has become clear that immune cells should be considered as TH target cells. Neutrophils, macrophages, B- and T-lymphocytes, and natural killer (NK) cells are affected by thyroid hormone status. Overall, hyperthyroidism tends to activate the immune system while a hypothyroid state results in impaired activation of the immune system. Besides, we provided initial evidence of TR expression in murine dendritic cells (DCs), the main antigen-presenting cells, endowed with a unique capacity to recognize, process and present antigens to naive T cells tailoring adaptive immunity. T3-dependent stimulation of DCs potentiates their ability to develop a Th1 and Th17-type response. Moreover, an increased DC capacity to promote antigen-specific cytotoxic T-cell activity, exploited in a DC-based antitumor vaccination protocol, was revealed (Montesinos & Pellizas, 2019). Given that DCs are main players in the etiopathogenesis of autoimmune disorders, further research would enlighten the involvement of thyroid status at this level. On the other hand, $\alpha_V\beta_3$ integrin is overexpressed and activated in several cancer and blood vessel cells, facilitating the hormone proliferative action and worsening cancer processes (Mousa et al.,Front. Endocrinol 2021, 12:691736). The presentation aims to disclose the current evidence supporting the contribution of THs to the modulation of innate and adaptive immunity.

ADIPOGENESIS AND GLUCOCORTICOID EXCESS

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It is known that Glucocorticoids (GCs) have several effects on the organism, including glucose homeostasis, modulation of the inflammatory response and the regulation of functions of adipose tissue (AT). In chronic state of high endogenous levels of GCs, e.g. Cushing Syndrome (CS), abdominal AT mass and adipocyte size are enhanced and the pattern of adipokine secretion is altered, evidencing that GCs play a pivotal role in the development of metabolic disturbances associated with abdominal AT dysfunction. Adipogenesis can be divided into two sequential steps: commitment of mesenchymal stem cells to adipocyte precursor cells (APCs) and terminal adipocyte differentiation. APCs express the cell surface marker CD34, which characterized adipogenic cells in the stromal vascular fraction (SVF) of the AT. CD34+ cells also express the transcriptional factor Zfp423, that in turn activates expression of PPARγ2 which assures APCs conversion into adipocytes. Adipocyte terminal differentiation depends on APCs adipogenic competency, that is the ability of APCs to differentiate into adipocytes in response to a defined stimulus. Thus, the competency of APCs could be modulated by the expression of Zfp423 and PPARγ2, which could be postulated as adipogenic competency markers. GCs has been widely used as adipogenic inducer of terminal differentiation, mainly by the early induction of two transcription factors, C/EBPδ and C/EBPβ. Afterwards, C/EBPδ and C/EBPβ activate C/EBPα and PPARγ2 expressions, two key pro-adipogenic factors necessary for adipocyte differentiation. Several studies demonstrated that GCs exert their pro-adipogenic effects by binding to mineralocorticoid (MR) and/or glucocorticoid (GR) receptors. Nevertheless, the relative contribution of MR or GR in the biological actions of GCs in AT is still a matter of debate. Nowadays, adipogenesis is considered as a general process that includes the generation of both white and beige adipocytes, each one

from an specific APC residing in AT. Beige adipocytes are characterized by high mitochondrial content and UCP-1 expression, which confers the ability to dissipate energy by heat production (thermogenesis). The appearance of beige adipocytes in White AT is promoted by cold exposure or B3AR stimulation. Here, we will focus on understanding the role of GCs in two processes from AT: the competency of APCs and the involvement of GR and MR in it, and the modulation of White AT browning.

PHYTOESTROGENS: BONE-VASCULAR ACTIONS

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Phytoestrogens (PE) like soy isoflavones, are proposed as a natural therapy to counteract the risk of cardiovascular and bone pathologies. The prevalence of these diseases is higher in postmenopausal women, fact mainly attributed to the decline of ovarian estradiol production. Atherosclerosis is a chronic inflammatory process that, in the later step of plaque generation could conduct to vascular calcification (VCa). On the other hand, osteoporosis represents a skeletal disorder characterized by an imbalance between bone formation and resorption.

In our lab, we investigated the cellular and molecular action of the isoflavone Genistein (Gen) on the events involved in VCa; in bone formation; and on the interactions between bone and vascular cells.

Our data support the hypothesis that Gen prevents *in vitro* VCa. The PE promotes endothelial nitric oxide synthesis, and favors cell growth and survival under oxidative stress. Gen down-regulated the expression of both, endothelial cell adhesion molecules (VCAM-1; ICAM-1) and monocytes integrins involved in leukocyte attachment to vascular endothelium induced by a pro-inflammatory environment. On vascular muscle cells, the PE inhibited cell transdifferentiation into osteoblasts like cells, a key event that triggers VCa. Alkaline phosphatase (ALP) and calcified nodules deposition in the extracellular matrix, markers of transdifferentiation, were significantly reduced after Gen treatment. The reduction of calcified areas evidenced in *ex vivo* assays using aortic tissue, confirmed these results.

In contrast to this anti-osteogenic action observed on vascular tissue, on bone cells Gen promoted osteoblastogenesis by an earlier up-regulation of the ERα and *Runx2* gene expression of calvaria pre-osteoblast. The differentiative effect was accompanied by enhancements in ALP activity, in extracellular collagen deposition, and in matrix mineralization. The molecular mechanisms involved the participation of ER; NOS; ERK and PI3K pathways.

A bidirectional cross link between bone and vascular cells that modulates cells growth and prompts angiogenesis was also evidenced.

Overall, although the isoflavone exerts opposite effects on bone and vascular cells, the action displayed could be favorable for the maintenance each tissue homeostasis. These effects may be relevant in hypoestrogenism stage, where bone loss and vascular diseases could be ameliorated by the administration of estrogens from the plant kingdom.

SIMPOSIO AAFE N°4: IMPACT OF PATENTS ON THE DEVELOPMENT OF NEW PHARMACOLOGICAL STRATEGIES

Chairs: Dra. Myriam Laconi y Dr. Santiago Palma

FREE PATENTS, VACCINES FOR EVERYONE? THEORETICAL FOUNDATIONS FOR AN ETHICAL DILEMMA

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On May 5, 2021, the US administration surprised the world by openly supporting the release of patents in hands of pharmaceutical companies protecting COVID-19 vaccines, an initiative that, if implemented, would allow other companies to replicate the technologies involved anywhere without previous authorization by its developers. The decision, according to the US government, aims to “get as many safe and effective vaccines to as many people as fast as possible”.

Activist and the WHO celebrated the decision, believing that it aims to solve existing limitations in terms of drugs availability in different parts of the world. Nevertheless, pharmaceutical companies pointed out that following this policy will bring them not only economic losses, but also will damage to the global pharmaceutical innovation system.

Although there is a general perception about the benefits generated by the existence of a legal system for the protection of inventions, the reality is that such a system entails social costs that must be taken into account and can only be justified if the general long-term generated benefits exceed the costs. The accepted economic theory stipulates that the patent system comes to solve market failures generated by their nature as “non-rivals” and

"non-exclusive" assets, according to which innovations would not be generated if the economic benefits generated bu them can not be appropriated by their creators. In turn, the economic monopoly granted by patents predicts a cost to society created by the inefficiency of the market, in this case, by the lack of competition for a given product. There are a variety of counterarguments against this theory.

This work aims to introduce the theoretical foundations to address the existing tension between two conflicting positions: on the one hand, the need to have economic incentives that motivate vaccine developers investments in order to have safe, effective, rapidly available vaccines and, on the one hand, the right of people and society to access essential medicines.

PATENTS: PRINCIPLE OF TERRITORIALITY, CLAIMS AS THE SCOPE OF INVENTIONS, FREEDOM TO OPERATE, COMPULSORY LICENSES.

Silvia Eliana Sarris
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Compulsory licenses are a temporary limitation National States can apply to holders' exclusive rights over patented inventions. That implies the possibility of third parties to exploit those patents without holders' consent.
In the pandemic global context caused by Covid-19, several governments have tried to implement compulsory licenses as a tool for national production of vaccines against the virus.
However, it is necessary to analyse whether such attempts constitute an effective solution to achieve the intended purpose, or whether it is required to identify other kind of measures to be carried out.
Intellectual property tends to create incentive for inventors, creators and holders who invest in R&D and/or in acquiring cutting-edge technologies for innovation. In this sense, and specifically regarding patents, national and international regulations grant exclusive rights to patent applicants, requiring in turn a sufficient disclosure of the information related to the invention. Furthermore, exclusive rights are granted for a term of up to 20 years, if granted.
Therefore, after the expiration of this period, or in the event that a patent expires for administrative reasons or whether the owner waives his rights, or if the patent application is never granted, the information becomes part of the domain public and free to operate then within all countries where the patent has not been applied for and granted.
Additionally, not all the information required for production is part of one or more patents (either because it is not novel, or it lacks of inventive step, that is, it is obvious for a person skilled in the matter).
Likewise, there may be information that has not been ever disclosed (confidential information, industrial secrets about production techniques, trade secrets of raw material suppliers, supplies, know-how of highly qualified labour, etc). Consequently, compulsory licenses would not ensure a successful transfer of technology and associated knowledge, necessary for building the required facilities, training human resources for production, and acquisition of necessary materials.
Contrarily, successful technology transfer has been achieved with licensing contracts, such as Sputnik – Richmond, and Oxford - AstraZeneca cases.

PATENT YES OR PATENT NO?: THE SOCIAL CHALLENGE OF SCIENTIFIC PROGRESS TO CONTROL THE PANDEMIC.

Leandro Víctor Sorbello
Universidad Nacional de Cuyo. Mendoza. Argentina

Freedom, equality, fraternity! From that historical premise that prompted the advent of copyright as a legal standard of individuality, to the current covid-19 pandemic. What points in common exist between the promotion of the protectionist regime of intangibles and the social and economic processes of its context.
Beyond a theoretical generality, the conflict of interest assumes specific legal and institutional modes in each case. In particular, the role of patents in economically peripheral countries, although endowed with their own scientific capacities, mainly Cuba and Brazil, is analyzed. On the other hand, it investigates the balance of interest in the case of other countries in the Latin American region, such as Argentina and Chile, with asymmetric strategies in technological negotiation and scientific development.

SIMPOSIO SAI N° 5: UNDERSTANDING AND TRANSLATING HUMAN IMMUNOLOGY: A SYSTEMS BIOLOGY PERSPECTIVE

Chairs: Dr. Pablo Romagnoli y Dra. Mariana Salatino

THE SEXY SIDE OF SYSTEMS VACCINOLOGY FOR INVESTIGATING VACCINE-INDUCED IMMUNITY ACROSS THE LIFESPAN

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School of Health and Biomedical Science, RMIT University, Melbourne, Victoria, Australia

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Systems vaccinology is an emerging field of vaccine research whereby systems biology techniques are used to study vaccine responses, providing novel insights into vaccine mechanisms and effects. It has long been known that males and females respond differently to vaccination but the mechanisms were not known. Systems vaccinology is now being used to elucidate the sex-differential biological pathways induced by vaccination. In addition to inducing a response against the vaccine-targeted disease, vaccines also have non-targeted effects whereby they modulate the immune system and alter susceptibility to non-vaccine targeted infections. For example, measles vaccination and BCG decrease susceptibility to respiratory infections and bacterial sepsis. Systems vaccinology, particularly transcriptomics and epigenomics, has been used to determine the biological mechanisms of vaccine-induced non-targeted effects. The very young and aging individuals have greater susceptibility to vaccine preventable diseases and thus are key target groups for vaccination. This talk will therefore focus on systems vaccinology studies conducted at the extremes of age.

EXPLORING IMMUNE CELL STATES IN CANCER AND INFECTION. NEW METHODS FOR SINGLE-CELL DATA SCIENCE

Santiago J. Carmona

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Different subtypes of lymphocytes and myeloid cells infiltrate tumors and contribute or interfere with cancer progression and cancer therapy. Identification of these cancer-associated immune cell states in individual patients is fundamental to reveal mechanisms of primary and acquired resistance, to identify biomarkers of response and design improved therapies.

Single-cell technologies have recently opened a unique opportunity to explore the immune response at a resolution and scale that seemed inconceivable only ten years ago. Single-cell omics analysis of cancer patients' biopsies is rapidly expanding. However, while the resolution at which we can now profile cellular states has increased dramatically, biological interpretation of these data remains a major challenge in the field. In particular, computational methods to accurately interpret single-cell data in the context of previous studies and prior knowledge are currently lacking.

I will discuss some of the current challenges in single-cell data science, and present novel computational methods we are developing to interpret immunological states across individuals and tissues, in health and disease. I will focus on the construction of reference transcriptional atlases to summarize knowledge from multiple studies, and how they can be used to interpret T cell immune responses.

We envision that meta-analyses of single-cell omics data from patients and mouse models will be fundamental to understand the cellular mechanisms of cancer therapy resistance, to identify biomarkers of response for clinical decision support and to identify novel therapeutic opportunities.

SEXUAL DIMORPHISM OF HUMAN DISEASES: A SYSTEMS IMMUNOLOGY PERSPECTIVE

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Many human diseases are sex dependent. However, the molecular and immunological mechanisms driving this sexual dimorphism are largely unknown. In this seminar, we have systematically assessed the impact of sex on the whole blood transcriptomes of patients with human diseases. Using Systems Immunology, we characterized the sexual differences in leukocyte composition of patients and identified sex-specific gene signatures that helped us unravel the impact of sexual hormones in disease progression and treatment.

SIMPOSIO AAFE N° 5: PREVENTION AND TREATMENT FOR COVID-19: WHAT WE LEARNED AND THE CHALLENGES THAT REMAIN
Chair: Dr. Andrea Errasti

DRUG REPOSITIONING

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Drug repositioning is a strategy that seeks to identify new indications for drugs already approved for another purpose, that is, to treat conditions different from those of their original purpose. The time and high economic investment required for the discovery and development of new molecules have motivated the proposal of strategies to identify “old drugs for new uses” in a variety of therapeutic fields. The surprising outbreak of the SARS-CoV-2 virus pandemic prompted a remarkable effort to find treatment alternatives through the repositioning of existing molecules. Antiparasitic, antiviral, antimicrobial, anti-inflammatory, and antihypertensive drugs have been evaluated as treatments for COVID-19. Ivermectin (IVM), a successful antiparasitic drug widely used in animal and human health (2 billion people treated over 30 years), has been investigated as a model for repositioning towards other therapeutic indications. Its antiviral, anti-inflammatory, immunomodulatory, and antitumor activity has been described. IVM (April 2020) emerged as a promising molecule due to its in vitro antiviral effect on SARS-CoV-2. Since that time, different types of studies have been carried out to evaluate its in vivo activity and measure the clinical benefits of its pre-emptive and / or curative use. The main antiviral mechanism of IVM is based on an inhibition of nuclear transport of viral proteins mediated by importin alpha / beta1. Other targets for a direct antiviral effect and anti-inflammatory / immunomodulatory activity of IVM have recently been described. The direct relationship between systemic exposure and the decrease in viral load in patients treated with high doses at the onset of symptoms (PK / PD ratio) was demonstrated in a multicentre clinical study conducted in Argentina. Today there is an important body of scientific evidence supporting the favourable effect of IVM on SARS-CoV-2. However, divergent paths have emerged between the (sometimes disorderly) attempt of science to provide clarifying evidence on clinical issues, the position of regulatory bodies, and the interest of pharmaceutical corporations. This unresolved dilemma has made it difficult to advance scientifically with the repositioning of a molecule (safe and inexpensive) that undoubtedly deserves to be considered.
Note: For reasons of space, the references that support the technical concepts expressed in this summary are not included, being available at the request of whoever is interested.

VACCINE DEVELOPMENT AND NEW STRATEGIES

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The disease caused by the novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has had an effect of enormous proportions, globally leading to more than 200 million confirmed cases and causing more than 4 million deaths. Most approved vaccines against COVID-19 have to be administered in a prime/boost regimen. Getting a single dose vaccine with suitable stability and storage properties that can reach rapidly the local population, is a major challenge for the scientific community especially in low and middle income countries. We engineered a novel vaccine based on a chimeric human adenovirus 5 (hAdV5) vector. The vaccine (named CoroVaxG.3) is based on three pillars: i) high expression of Spike to enhance its immunodominance by using a potent promoter and a mRNA stabilizer; ii) enhanced infection of muscle and dendritic cells by replacing the fiber knob domain of hAdV5 by hAdV3; iii) use of Spike stabilized in a prefusion conformation. Transduction with CoroVaxG.3 expressing Spike (D614G) dramatically enhanced Spike expression in human muscle cells, monocytes and dendritic cells compared to CoroVaxG.5 that expressed the native fiber knob domain. A single dose of CoroVaxG.3 induced potent humoral immunity with a balanced Th1/Th2 ratio and potent T-cell immunity, both lasting for at least 5 months. Sera from CoroVaxG.3 vaccinated mice was able to neutralize pseudoviruses expressing B.1 (wild type D614G), B.1.117 (alpha), P.1 (gamma) and B.1.617.2 (delta) Spikes, as well as an authentic P.1 SARS-CoV-2 isolate. Neutralizing antibodies did not wane even after 5 months making this kind of vaccine a likely candidate to enter clinical trials

PREVENTION AND TREATMENT FOR COVID 19: WHAT WE LEARNED AND THE CHALLENGES THAT REMAIN:

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Since the beginning of the COVID 19 pandemic, we have faced very different difficulties than those we experienced in previous pandemics. This also brings with it scenarios that in normal moments we would not have been able to imagine.

Evidence-based medicine took a limited place at the beginning, being the experiences of using a drug the usual at the beginning, with no evidence in most cases. The need to do something passed over rationality, thus generating very unfortunate situations for the management of patients. Drug repositioning was many times transferred to the bedside without adequate assessment of the risks and pharmacokinetic profiles, despite many being done outside of properly designed and conducted clinical trials.

The value of interaction and solidarity between different scientific groups allowed in record times develop diagnostic methods, vaccines, treatments. It was also possible to show that a complex clinical trial is not necessary to be able to demonstrate the efficacy or lack of it of a certain treatment, the paradigm of this being the British RECOVERY study, which allowed in a pragmatic environment to define treatments as dexamethasone or tocilizumab that changed the prognosis of the most serious patients infected by COVID 19. Other treatments fell by the wayside, such as hydroxychloroquine, convalescent plasma and ivermectin.

The role of governments in promoting treatments that had no evidence was at least controversial, being something that should definitely be avoided for the future.

The development of vaccines showed the dissemination of two technologies such as mRNA and Adenovirus vectors as the spearhead for the mass immunization of hundreds of millions of people. However, accessibility barriers and anti-vaccine movements reduced the possibility of a faster exit, as well as the appearance of new variants.

The pandemic showed the weaknesses of the health system in all its aspects, as well as the strength of science to be able to overcome the most important medical event of the last 100 years, this being the strongest learning. Science showed us that we can be better prepared for the next pandemic.

SIMPOSIO AAFE N°6: CANNABIS MEDICINAL. SCIENTIFIC EVIDENCE OF PHARMACOLOGICAL ACTIVITY AND NATIONAL REGULATION. CURRENT AFFAIRS AND REGULATORY CHALLENGES OF MEDICINAL CANNABIS IN ARGENTINA

Chairs: Dra. Paula Schaiquevich y Dra. Alicia Consolini

CANNABINOID PHARMACOKINETICS. POTENTIAL INTERACTIONS WITH CONCOMITANT MEDICATION

Marta Vázquez
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Absorption and disposition of cannabinoids vary as a function of the route of administration. The bioavailability of inhaled cannabinoids is 10% to 35%, whereas after oral ingestion the bioavailability is only 4% to 12% due to first-pass metabolism and thus absorption is highly variable. Cannabinoids are highly lipid soluble and are therefore rapidly taken up by fat tissue being this tissue a long-term storage site. They are highly protein-bound in blood and are mainly bound to low-density lipoproteins, with up to 10% present in red blood cells and only 2-3% as free drug. They are eliminated from plasma mainly by metabolism in the liver in a multiphasic manner. The real elimination half-lives of cannabinoids are difficult to calculate from plasma as the equilibrium plasma/fatty tissue is slowly reached. Therefore, variable elimination half-lives are reported in the literature. With increasing cannabis use, the potential for cannabinoids interacting with other therapeutic agents also increases. Therefore, their use in therapy could interfere with the absorption and/or disposition of other drugs that undergo the same metabolic pathways or on the other hand, other medications could interfere with cannabinoids pharmacokinetics. Both cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) or their metabolites are not only substrates but also inhibitors or inducers of CYP450 enzymes and UGTs. Moreover, and according to some *in vitro* studies, these two major cannabinoids can inhibit carboxylesterases involved in the deactivation/detoxification or metabolic activation of some medications. *In vitro* and animal studies have also shown that CBD and THC interact in some way with ATP-binding cassette superfamily (inhibiting or downregulating their expression) and thus, a significant impact on the absorption and disposition of other co-administered drugs that are also substrates of these transporters may be expected. Health care professionals must be aware of drug-drug interactions given that the diseases and conditions most frequently targeted for treatment with medical cannabis are chronic in nature and treated with conventional medications concurrently.

NEWS AND REGULATORY CHALLENGES OF MEDICINAL CANNABIS IN ARGENTINA

Norma Elizabet Belixán
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Since the World Health Organization (WHO) proposal approval to remove cannabis and cannabis resin from Schedule IV of the Single Convention on Narcotic Drugs of 1961, possible therapeutic uses were recognized to these substances.

Consequently, the National Health Authority of Argentina (ANMAT) participates in international meetings promoted by International Narcotics Control Board (INCB) in order to achieve uniformity in the applicable standards of good practices and to guarantee requirements compliance with international control.

At the national range, the great regulatory challenge consists in establishing the applicable regulations and implement effective administrative procedures to promote the development of those industries that involve the cannabis plant and its derivatives in order to guarantee the availability and access to safe and quality products.

SIMPOSIO SAIC N°6: IN HONOR TO PROFESSOR ALBERTO BOVERIS. FROM OXYGEN POISONING TO REDOX SIGNALING CURRENT UNDERSTANDING OF EARLY CONCEPTS

Chairs: Dr. Pablo Evelson y Dr. Cesar Fraga

MITOCHONDRIAL METABOLISM IN BRAIN AGING

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A mitochondrion-centric approach to brain aging requires the coordination of signaling and transcriptional pathways modulated by mitochondrion-released hydrogen peroxide. These interconnected mechanisms are critical in the maintenance of a functional, efficient, and responsive mitochondrial population. The gradual decline in energy metabolism during brain aging results in a hypometabolic state, which was examined in a model that integrates mitochondrial function, insulin signaling, and JNK signaling. Brain aging proceeds with a decrease of glucose uptake (dynamic microPET imaging), which was associated with a decrease in the expression of the insulin-sensitive neuronal GLUT4 and microvascular endothelium GLUT1 (55 kD) but not astrocytic GLUT1 (45 kD). Brain aging was associated with an imbalance between the PI3K/Akt pathway of insulin signaling and JNK signaling and a down-regulation of the PGC1α-mediated transcriptional pathway of mitochondrial biogenesis, thus impairing on several aspects of energy homeostasis. Of note, these effects were observed in cortex- and hippocampal preparations but they are not necessarily cell specific, for astrocytes respond in a different manner: astrocytes showed an age-dependent increase in mitochondrial oxidative metabolism (respiring either on glucose or glucose plus pyruvate) and mitochondrial biogenesis. These metabolic changes were associated with an age-dependent increase in H_2O_2 generation (largely ascribed to NOX2) and NFκB signaling as well as augmented responses with age to inflammatory cytokines. Further, the inflammatory cytokines, IL-1β and TNFα stimulated mitochondrial oxidative metabolism and mitochondrial biogenesis in astrocytes. The significance of this hypometabolic state –associated with mitochondrial dysfunction– is strengthened by impaired of cognitive functions as determined by electrophysiology.

FLAVONOIDS AS REGULATORS OF CELL REDOX HOMEOSTASIS

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The concept that flavonoids could be antioxidants *in vivo* was originated based on their chemical structure which allows the scavenging of free radicals and the chelation of redox active metals. Advancement in the knowledge of flavonoid absorption, distribution, metabolism and excretion (ADME) showed that most flavonoids are minimally absorbed as parent compounds and undergo extensive metabolism by both, the intestinal microbiota and host cells. Thus, only at the gastrointestinal tract flavonoids would reach concentrations compatible with a physiologically relevant antioxidant action. In other organs and tissues, flavonoids are found at very low concentrations (nM) and mostly as metabolites that would not support a direct antioxidant action. On the other hand, a large body of evidence has shown that flavonoid consumption decreases oxidant production and oxidation of cell components in humans and experimental models. As an example, the flavan-3-ol (-)-epicatechin (EC) and its oligomers, the procyanindins (PCA), mitigate oxidative stress and regulate redox homeostasis in conditions such as obesity, inflammation, fatty liver diseases and hypertension. These actions are not mediated by direct anti-

oxidant mechanisms but through the capacity of these compounds to: i) interact with and regulate the activity of proteins involved in oxidant production and antioxidant protection; ii) modulate redox-sensitive transcription factors (TF) at different levels, e.g. modulating kinases and phosphatases, TF-DNA binding; iii) interact with membrane domains, particularly lipid rafts, and iv) regulate oxidant steady state levels. Given the large number of flavonoids existing in the human diet it is essential to relate their ADME with their mechanism of action to finally be able to develop dietary recommendations aimed to the prevention and/or treatment of pathological conditions, especially those characterized by alterations in redox homeostasis.

CHOLESTEROL OXIDATION BETWEEN HEALTH AND DISEASE: FOCUS ON THE ANTIVIRAL PROPERTIES OF ENZYMATIC OXYSTEROLS

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The epidemiologically proved correlation between cholesterol and defined chronic diseases, in particular cardiovascular pathology, took hold of the collective imagination, so that cholesterol has generally been considered as a dangerous molecule. However, this multitasking sterol is not synonim of disease, being actually involved in a number of normal cell and tissue functions. In particular here we focus on two physiological cholesterol oxidation products (oxysterols) of enzymatic origin, namely 25-hydroxycholesterol (25OHC) and 27-hydroxycholesterol (27OHC), that were demonstrated to efficiently counteract not only the replication of enveloped human viruses but also that of non-enveloped ones. In our laboratories, 25OHC and 27OHC blocked in vitro the infectivity of several strains of human rotavirus (HRhV), the agent of severe children's gastroenteritis, as well as the replication of different strains of human rhinovirus (HRV), the main agent of common cold, being active in the low micromolar range and in the absence of cell toxicity. Of note, the two oxysterols showed a strong inhibitory effect also when added to cell cultures prior to viral infection. Very recently, 27OHC, suitably complexed with cyclodextrin (CDX), was proven to strongly inhibit the in vitro replication of two coronaviruses, namely SARS-CoV-2, the agent of COVID-19, and HCoV-OC43, another virus responsible for the common cold, again in the low micromolar range and without exerting cytotoxicity. The 27OHC-CDX complex displayed also preventing effects against the tested viral infections. The present knowledge about the complex mechanism of antiviral action and the likely involvement of the two enzymatic oxysterols 25OHC and 27OHC in both the innate and the adaptive immune response against viruses will be analyzed and discussed.

ENERGY MANAGEMENT AND MITOCHONDRIAL DYNAMICS DURING ENDOTOXEMIA

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Mitochondria are often considered the "powerhouse of the cell" owing to their ability to efficiently generate ATP required to sustain normal cell function. However, the dynamic nature of mitochondria and its involvement in a plethora of cell signaling cascades has renewed the recognition of this organelle in cell metabolism and pathological processes.

Sepsis and endotoxemia are described as a paradigm of acute whole body inflammation, characterized by massive increases of NO and inflammatory cytokines in biological fluids, systemic damage in the vascular endothelium, and impaired tissue and whole body respiration despite adequate O_2 supply. Cardiac mitochondrial dysfunction occurring during these inflammatory syndromes plays a key role in the development of organ damage mainly by decreased ATP availability. Mitochondrial ATP production, O_2 consumption and mitochondrial inner membrane potential are related to blood NO levels and mitochondrial protein nitration, leading to decreased ATP availability and impairment of the contractile state. *In vivo* modulation of NO bioavailability (with c-PTIO or L-NAME) partially restores cardiac and mitochondrial function, leading to propose that NO levels link inflammation with mitochondrial bioenergetics and cardiac contractility state. It is important to mention that mitochondrial dynamics may adjust cellular mitochondrial architecture to cope with energy demands. The occurrence of mitochondrial biogenesis, fusion/fission processes, or mitophagy are thought to be activated in the mentioned conditions. Nevertheless, it has to be taken into account that partial restoration of mitochondrial architecture may not be accompanied by improvement in mitochondrial function. The final scenario in these inflammatory syndromes would derive from the complex relationship between these processes and the final effect in the cardiac functional response.

Unraveling the mechanisms involved in the onset of sepsis and endotoxemia would not only improve the interpretation of the pathology but also would provide new possibilities for the generation of novel therapeutic targets for treatment in the clinic as well.

SUPEROXIDE, PEROXYNITRITE AND MITOCHONDRIA

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Mitochondria are major intracellular sources of superoxide radical ($O_2^{\bullet-}$), the product of the one-electron reduction of molecular oxygen. Different mitochondrial sites of $O_2^{\bullet-}$ formation have been identified, notably components of the mitochondrial electron transport chain. Once formed, mitochondrial $O_2^{\bullet-}$ can either readily dismutate by the action of superoxide dismutases, react with mitochondrial targets such as aconitase or yield peroxynitrite anion ($ONOO^-$) following the diffusion-controlled reaction with nitric oxide ($^{\bullet}NO$). While under most conditions $^{\bullet}NO$ reaches mitochondria secondary its diffusion from extramitocondrial sites, evidence for conditions leading to mitochondrial $^{\bullet}NO$ formation have been also reported. Peroxynitrite (the sum of $ONOO^-$ and ONOOH, p*Ka* = 6.8) is an oxidizing and nitrating species that can cause mitochondrial dysfunction and trigger cell death. Indeed, peroxynitrite is a pathogenic mediator in inflammation and degenerative diseases and it contributes to the aging process. In the presentation I will briefly comment on the following aspects of mitochondrial $O_2^{\bullet-}$ and peroxynitrite: 1) mechanisms of generation, 2) key intramitochondrial targets, 3) catabolic pathways and 4) redox-based therapeutics. The talk will provide evidence at the *in vitro* and *in vivo* levels to underscore the role that $O_2^{\bullet-}$ and peroxynitrite have in mitochondrial dysfunction and the related opportunities for mitochondrial-targeted therapeutics.

SIMPOSIO SAIC N°7: WOMEN IN SCIENCE: ADVANCES IN IMMUNE RESPONSE TO CANCER AND ANTITUMOR IMMUNOTHERAPY

Chair: Dra. Marianela Candolfi

EPIGENETIC REPROGRAMMATION OF THE IMMUNE MICROENVIRONMENT IN BRAIN TUMORS: GRANULARITY AT THE LEVEL OF INDIVIDUAL IMMUNE CELLS IN GENETIC MOUSE MODELS AND PATIENTS

Maria G Castro
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Mutation in isocitrate dehydrogenase (*mIDH*) is a common genetic lesion encountered in glioma patients. Approximately 90% of *IDH1* mutations occur in exon 4 at codon 132, resulting in a change of a single amino acid from arginine to histidine (R132H). Less common *IDH2* mutations occur in an analogous codon at position R172. Although IDH1/2 mutations are always heterozygous, they exert a dominant gain of function enzymatic activity which leads to the production of 2-hydroxyglutarate (2HG). Excessive 2HG production causes DNA hypermethylation via inhibition of methylcytosine dioxygenase *TET2,* and also promotes histone hypermethylation through competitive inhibition of α-ketoglutarate (αKG)-dependent Jumonji-C histone demethylases. This leads to epigenetic reprogramming the transcriptome within the *mIDH1* glioma cells (1).

Several studies suggested that *mIDH1* may play a critical role in shaping the immunological landscape of the tumor immune microenvironment (TME). We demonstrate that mutant isocitrate-dehydrogenase 1 (*mIDH1*) synthetizes the oncometabolite 2-hydroxyglutarate (2HG), which elicits epigenetic reprogramming of the glioma cells' immune transcriptome leading to the reversion of the glioma immunosuppressive microenvironment (2). We show that the efficacy of immune-stimulatory gene therapy (TK/Flt3L) is enhanced in *mIDH1* gliomas, due to the reprogramming of the myeloid cells compartment infiltrating the TME. We uncovered that the immature myeloid cells-infiltrating the *mIIDH1* TME are mainly non-suppressive neutrophils and pre-neutrophils. Myeloid cells' reprogramming was triggered by granulocyte colony-stimulating factor (G-CSF) secreted by *mIDH1* glioma stem-like cells. Blocking G-CSF in *mIDH1* glioma-bearing mice restores the inhibitory potential of myeloid cells, accelerating tumor progression. Also, we demonstrate that G-CSF reprograms bone-marrow granulopoiesis resulting in non-inhibitory myeloid cells within *mIDH1* glioma TME and enhancing the efficacy of immune-stimulatory gene therapy. In conclusion, out results uncover an important role of *mIDH1* on reprogramming the phenotypic and functional diversity of myeloid cells in glioma TME, a feature that can be harnessed to enhance the efficacy of immunotherapies in glioma patients.

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IMPORTANCE OF TYPE I-IFN IN ANTITUMOR-IMMUNE RESPONSE

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An important challenge in cancer immunotherapy is to expand the number of patients that benefit from immune checkpoint inhibitors blockade (CI), a fact that is associated with the pre-existence of an efficient anti-tumor immune infiltration. Different strategies are being proposed to be used in combined therapies with CI that could enhance tumor infiltration and immunity. Competent type I IFN signaling underlies most anti-tumor immune mechanisms and has recently proven critical to the efficacy of several anticancer agents and immunotherapy. In this talk, we will discuss our recent findings regarding the potential of TLR3 agonists as type I-IFN inducers and consequently, as strong modulators of the tumor infiltrate composition, capable of switching the immune suppressive tumor environment to anti-tumor immunity.

DIFFERENTIAL GLYCOSYLATION OF TUMOR-ASSOCIATED IMMUNE CELLS CONTROLS THEIR ACTIVITY AND RESISTANCE TO CANCER THERAPIES

Ada G. Blidner
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During this lecture we will discuss the role Galectin-Glycan interactions in tumor microenvironments, focusing on the modulation of the immune cell compartment as a crucial player in cancer progression and therapy resistance. During more than 20 years, the Laboratory of Glycomedicine and Immunopathology lay the foundations of the role of Galectin-1 as a positive regulator of tumor progression, including immune escape, angiogenesis and metastasis. Notably, we discovered that both pro-tumor and anti-tumor immune responses are sensitive to Gal1 regulation, generating a comprehensive picture of the mechanisms underlying Galectin-Glycan contribution to anti-angiogenic and Immunotherapy resistance.

STRATEGIES TO ENHANCE NATURAL KILLER ACTIVITY IN CANCER THERAPY.

Estrella Mariel Levy
Cancer Research Center-Cancer Foundation, FUCA.

Natural Killer (NK) cells are central components of innate immunity. Because of their potential to induce direct cellular cytotoxicity without prior sensitization and release immunostimulatory cytokines like IFN-γ, NK cells have been shown to suppress both local tumor growth and metastasis in animal models.
Nevertheless, NK cells display impaired functionality and capability to infiltrate tumors in cancer patients. In our group, we observed altered phenotype and functionality in peripheral and tumor-associated NK cells from patients with colorectal (CRC) and breast cancer (BC) (1-4).
NK cells are feasible targets of stimulation to participate in immunotherapeutic approaches like antibody (Ab)-based strategies. In several medical scenarios, NK cells can be activated by tumor-associated antigen-specific therapeutic Abs via CD16a-mediated ADCC effects. Cetuximab is an anti-EGFR chimeric mAb. We determined that altered NK cells in CRC patients can be activated by Cetuximab plus Il-2 or Il-15 (4). Also, we demonstrated that Cetuximab triggers ADCC against Triple-negative BC (TNBC) cells (5). Moreover, we determined that Cetuximab opsonization of TNBC cells increased a cross-talk between NK and dendritic cells (6).
Nowadays, several mAbs are being studied to block the PD-1/PD-L1 axis in different tumors; preliminary data from clinical trials presented promising results for patients with advanced-stage/metastatic TNBC. Unlike other anti-PD-L1 mAbs, Avelumab was designed as IgG1 to trigger ADCC against tumor cells. We demonstrated that Avelumab significantly enhanced NK-cell mediated cytotoxicity against TNBC cells and that tumor cells expressing higher levels of PD-L1 were more sensitive to Avelumab-mediated ADCC. Our study suggests that Avelumab-mediated ADCC, independently of the blockade of the PD-1/PD-L1 pathway, could be a valuable mechanism for tumor cell elimination in TNBC (7).
Emerging evidence suggests that a subset of NK cells have adaptive immune features, including memory-like properties, and enhanced Ab-dependent effector functions. Recently we identified and characterized an adap-

tive (NKa) cell subpopulation in BC patients.
NKa cells from BC patients exhibited increased IFN-γ production compared to conventional NK cells via the anti-HER Ab (Trastuzumab)-mediated ADCC (unpublished data). Our results encourage studying NKa cells as a potential candidate for predicting Ab-based therapy outcomes in HER2+ BC.

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SIMPOSIO SAIC N°8: STATE OF THE ART IN AIDS RELATED MALIGNANCIES
Chairs: Dr. Omar Coso y Dr. Pedro Cahn

KAPOSI SARCOMA HERPESVIRUS: UNDERSTANDING MECHANISMS TO TRANSFORM DIAGNOSIS AND TREATMENT

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It has been 27 years since the discovery of the Kaposi sarcoma herpesvirus (KSHV/HHV-8), in an AIDS related Kaposi sarcoma (KS) lesion. Evidence rapidly accumulated documenting that this virus is the causal agent of KS, being necessary, albeit not sufficient for this disease to develop. Soon after this discovery, viral sequences were also identified in a unique type of lymphoma, called primary effusion lymphoma (PEL). KSHV also causes two diseases that are associated with systemic symptoms due to increased production of cytokines: multicentric Castleman disease (MDC) and KSHV inflammatory cytokine syndrome (KICS). These are distinguished by the presence of systemic lymphadenopathy and characteristic features pathologic only in MCD.
Currently, most cases of KS occur in sub-Saharan Africa, where KSHV infection is prevalent owing to transmission by saliva in childhood, compounded by the ongoing AIDS epidemic. Treatment for early AIDS-related KS in previously untreated patients should start with the control of HIV with antiretrovirals, which frequently but not always results in KS regression. In advanced-stage KS, chemotherapy is the most common treatment, but mortality is high. Diagnosis is challenging in Africa because of limited availability to pathology and immunohistochemistry. To facilitate early diagnosis, we have been working on developing a point-of-care molecular diagnostic assay based on KSHV detection. In addition, in an attempt to improve patient outcomes with KSHV associated diseases, we have been focusing on characterizing viral gene products that are expressed in most tumor cells (i.e. during latency) and that play roles in oncogenesis. One of these is vFLIP, and an extensive effort to indentify inhibitors of this viral protein are underway.

AIDS RELATED MALIGNANCIES IN TRANSGENDER WOMEN AND MEN WHO HAVE SEX WITH MEN IN ARGENTINA

Valeria Fink
Fundación Huésped. Médica especialista en Enfermedades Infecciosas (UBA /Argentina)

HIV and cancer have been intertwined from the very early times of the HIV epidemics. Since the first cases of AIDS associated Kaposi's sarcoma were reported, science has made outstanding progress, in particular with the advent of the combined antiretroviral therapy, switching a deadly disease to a chronic condition. However, cancer remains

one of the main challenges for this population due to its morbidity and mortality.
Men who have sex with men (MSM) and transgender women (TGW) are the populations most affected by HIV in Argentina, with an estimated HIV prevalence of 12-15% and 34% respectively. Despite a national program with access to HIV testing and treatment, the number of annual new HIV cases remains stable. Moreover, 30% of the new HIV diagnosis are late presenters, including people with AIDS defining cancers.
AIDS related malignancies are related to oncogenic viruses, such as HPV, KSHV and EBV, which are more frequent in MSM and TGW. Understanding the biology and epidemiology of these cancers is crucial for developing prevention strategies while ensuring access for HIV prevention, testing and treatment services, in particular for key populations.

MECHANISMS OF ONCOGENESIS BY GAMMAHERPESVIRUS / KSHV-ENCODED ANTIGENS MODULATE HIF1A LEVELS AND THE EPIGENETIC REPROGRAMMING OF INFECTED CELLS TO PROMOTE DNA REPLICATION

Erle Robertson
Harry P. Schenck Professor / Perelman School of Medicine at the University of Pennsylvania (USA)

The cellular adaptive response to hypoxia, mediated by high HIF1α levels includes metabolic reprogramming, restricted DNA replication and cell division. In contrast to healthy cells, the genome of cancer cells, and Kaposi's sarcoma associated herpesvirus (KSHV) infected cells maintains replication in hypoxia. We show that KSHV infection, despite promoting expression of HIF1α in normoxia, can also restrict transcriptional activity, and promoted its degradation in hypoxia. KSHV-encoded vCyclin, expressed in hypoxia, mediated HIF1α cytosolic translocation, and its degradation through a non-canonical lysosomal pathway. Attenuation of HIF1α levels by vCyclin allowed cells to bypass the block to DNA replication and cell proliferation in hypoxia. We demonstrated that KSHV utilizes a unique strategy to balance HIF1α levels to overcome replication arrest and induction of the oncogenic phenotype, which are dependent on the levels of oxygen in the microenvironment. Further studies have also demonstrated that epigenetic reprogramming of KSHV genome in infected cell is essential for establishment of latency by controlling viral gene expression. Unwinding of these epigenetic changes is necessary for reversal of the process of virus latency to induce productive replication. The reversal of these epigenetic changes in physiologically allowed condition such as hypoxia has not been previously studied in KSHV infected cells. Importantly, interdependence of host and viral epigenome remain ambiguous during viral reactivation under this physiologically allowed state, and so we investigated the epigenetic reprogramming of KSHV genome during hypoxic reactivation. We identified dramatic upregulated expression of both transcription activating as well deactivating methylated histone marks especially, H3K4Me3, H3K9Me3 and H3K27Me3. The upregulation of these modified histones are restricted to KSHV positive background suggesting a coordinated regulation by hypoxia and a KSHV-encoded antigen. KSHV-encoded RTA, vCyclin and vGPCR were shown to mediate the up-regulated expression of these modified histones. Further, chromatin sequencing experiment with replication associated proteins such as DNApol1α or DNA modifications such as DNMTs showed qualitative enrichment on KSHV genome rather higher expression or quantitative enrichment. These studies provide new insight and add to our understanding of epigenetic reprogramming during hypoxic reactivation of KSHV.

SIMPOSIO SAI N° 6: NEW INSIGHTS INTO ADAPTIVE IMMUNITY
Chairs: Dr. Rubén Motrich y Dra. Ana Rosa Pérez

TREGS CONVERT ANTIGEN-PRESENTING CELLS TOWARDS A TOLEROGENIC PHENOTYPE VIA CTLA-4-DEPENDENT TROGOCYTOSIS

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Regulatory T cells (Tregs) abundantly express the immune checkpoint receptor CTLA-4, whose Treg-specific deficiency causes severe systemic autoimmunity and inflammation. As a key mechanism of suppression, Treg-expressed CTLA-4 diminishes the expression of CD80/CD86 costimulatory ligands on antigen-presenting cells (APCs). However, the extrinsic function of CTLA-4 has not been fully elucidated yet. Here, we show that Treg-expressed CTLA-4 facilitated Treg-APC conjugation and immune synapse formation. These Treg-APC

conjugates thus provided a stable platform whereby Tregs were able to deplete CD80/CD86 proteins on APCs by extracting them via CTLA-4-dependent trogocytosis. This depletion occurred even with Tregs only expressing a mutant tailless form of CTLA-4, which lacks the cytoplasmic tail portion required for its endocytosis and cycling. The CTLA-4-dependent trogocytosis of CD80/CD86 also accelerated in vitro and in vivo passive transfer of other membrane proteins and lipid molecules from APCs to Tregs without their significant reduction on the APC surface. Furthermore, CD80 downregulation or blockade by Treg-expressed membrane form of CTLA-4 or soluble CTLA-4-immunoglobulin, respectively, disrupted cis-CD80/PD-L1 heterodimers and increased CD80-dissociated free PD-L1 on dendritic cells (DCs), expanding a phenotypically distinct tolerogenic population of $CD80^{lo}$ free $PD\text{-}L1^{hi}$ DCs. Therefore, Treg-expressed CTLA-4 is able to exert dual suppressive effects on T cell immune responses by directly limiting CD80/CD86 costimulatory signal to naïve T cells and by indirectly increasing free PD-L1 available for the inhibition of effector T cells via CTLA-4-dependent trogocytosis. Finally, our results imply that combined blockade of CTLA-4 and PD-1/PD-L1 may synergistically hinder Treg-mediated immune suppression, thereby effectively enhancing immune responses, including tumor immunity.

UNDERSTANDING TYPE 1 DCS ACTIVITY AND SUPPRESSION IN LUNG CANCER

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Abstract not available

BATF REGULATES PROGENITOR TO CYTOLYTIC EFFECTOR CD8 T CELL TRANSITION DURING CHRONIC VIRAL INFECTION

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Abstract not available

SIMPOSIO SAIC N°9: TRANSLATIONAL RESEARCH
Chair: Dra. Laura Bover

NK CELLS: NEXT GENERATION CELL THERAPIES FOR CANCER

Katy Rezvani
The University of Texas MD Anderson Cancer Center, USA

Dr. Rezvani will discuss a new frontier in NK cell therapeutics: engineering NK cells with chimeric antigen receptors. She will discuss the opportunities and challenges of NK cell CAR engineering, and present pre-clinical and early phase clinical data on cord blood-derived NK cells expressing CD19 CAR and IL-15 to enhance their *in vivo* persistence in patients with relapsed or refractory blood cancers. In addition, she will discuss novel strategies for the gene editing of CAR NK cells to enhance their function by targeting immune checkpoints. Finally, she will discuss the approach of precomplexing NK cells with an anti-CD16 bispecific antibody targeting cancer targets to redirect their specificity, thus providing a rapid approach to translate NK cells with CAR-like characteristics to the clinic.

LOW DOSE RADIATION TO OVERCOME RESISTANCE TO CHECKPOINT INHIBITOR THERAPY

James Welsh
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Checkpoint inhibitor (CPI) therapy relies on effector immune cell infiltration and function that often fails due to the physical barrier of the stroma and the immune suppressive tumor microenvironment (TME). High-dose (HD)-XRT is well-known to debulk tumors, killing tumor cells and enhance the efficacy of CPI therapy through disruption of the stroma and systemic priming of T cells. Exposure of tumor cells to HD-XRT releases tumor-associated antigens (TAAs), upregulates MHC class I molecules, and activates the cGAS/STING pathway through DNA damage. However, disruption of the stroma by HD-XRT promotes recruitment of Tregs and myeloid derived suppressor cells (MDSCs) and increased secretion of immunosuppressive cytokines such as TGF-β and IL-10. In contrast, low doce (LD)-XRT, that lacks tumor killing properties, modulates the stroma and TME, boosts the

immune response and synergizes with CPI therapy. Key findings on the effect of LD-XRT include: 1) increase of NK and T cell infiltration, 2) polarization of tumor associated macrophages from pro-tumorigenic M2 phenotype into anti-tumor M1 phenotype, and 3) reduction of TGF-β levels. Consequently, LD-XRT can overcome limitations of CPI therapy. Moreover, in a phase 2 clinical trial in melanoma patients the combination of CPI therapy (ipilimumab) with LD-XRT and stereotactic ablative radiation therapy (SBRT), lesions treated with LD-XRT responded better than un-irradiated lesions (31% vs. 5%, P = 0.0091). In another prospective phase 2 clinical trial in patients with advanced metastatic tumors, where one metastatic mass was treated with HD-XRT and distant metastases were treated with LD-XRT during CPI therapy, we found that LD-XRT (4-7 Gy) delivered in 4 fractions to metastatic lesions increased the infiltration of immune cells and improved responses. The benefit of adding LD-XRT to HD-XRT was evident with (1) a 53% increase of immune-related response [irRC] in irradiated lesions compared with unirradiated lesions (23% HD-XRT+LD-XRT and 11% HD-XRT), (2) extended median progression-free survival (PFS) time (7.0 vs 5.8 months), (3) extended median overall survival (OS) time (15.9 vs 10.5 months) and (4) increased T and NK immune cell infiltration into irradiated lesions. In conclusión, LD-XRT can enhance immunotherapy by increasing the infiltration of T cells into irradiated solid tumors and overcome limitations of CPI therapy.

ACCELERATING CLINICAL PROGRESS THROUGH TRANSLATIONAL CANCER RESEARCH

Bob Bast

Department of Experimental Therapeutics, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Over the last two decades in cancer research, progress in the laboratory has been exponential, whereas progress in the clinic has been more gradual and linear. The challenge for translational cancer research is to accelerate progress in the clinic by facilitating the flow of new ideas and approaches from bench to bedside and of tissue and outcomes from bedside to bench, while moving more effective treatment, detection and prevention into the community. Precision cancer medicine brings together molecular diagnostics with molecular therapeutics to provide the right combinations of traditional, targeted- and/or immuno-therapy for the right patient at the right time to improve outcomes.

To accelerate progress, we must understand the inter-tumor and intra-tumor complexity and heterogeneity of different cancers using “omic” analysis of malignant and benign cells from multiple sites in primary and metastatic cancers, requiring technology and bioinformatics. “Liquid biopsies” of circulating tumor DNA can integrate sampling from multiple sites. More than a thousand new anti-cancer agents are being evaluated in the clinic. As only 1 of 8 anti-cancer drugs that enter clinical trials are ultimately approved for use in the United States, more predictive models are required to estimate efficacy of new agents. Considering that each cell line or xenograft is derived from a single patient, much larger numbers of models must be used. Insights into drug targets and tumor immunology, as well as most of the patients who enter clinical trials, are found in Academe, while the ability to generate new agents and disseminate them worldwide are found in Pharma. Development of new agents might occur more rapidly and at lower cost, if Pharma and Academe collaborated more frequently through strategic alliances or if the capacity for drug development were embedded in more cancer centers such as MD Anderson.

Numerous drugs, antibodies and immune cells are now available to target particular abnormalities in cancers from a single organ or from multiple organ sites. In a fraction of cancer patients, individual drugs can be paired with molecular abnormalities, improving outcomes to some extent. With rare but important exceptions such as chronic myelogenous leukemia, however, effective treatment of most malignancies will require combinations of targeted agents. Relevant combinations have been sought through synthetic lethality, collateral lethality and combinatorial adaptive resistance therapy. Evaluation of combinations requires access to large numbers of patients whose cancers can be genotypically and phenotypically tested to identify subsets who could potentially benefit. Biomarkers have been used for their positive predictive value to identify patients more likely to respond. Emphasis could also be placed on identifying biomarkers with negative predictive value to identify individuals who would not benefit, saving patients toxicity, time and tens of billions of dollars.

Translational cancer research requires steady funding. The majority comes from Pharma, supplemented with governmental and philanthropic sources. Limited funding mandates that we must choose our laboratory experiments and clinical trials with care, developing truly novel agents and choosing clinical trials that will change the standard of care. We must also support career development and retention of physician-scientists and clinician-investigators who are increasingly endangered species, one of the priorities at my own cancer center. Multi-disciplinary teams must be built and maintained. This effort must define the role of all treatment modalities. Academic institutions need to be more deliberate in catalyzing and rewarding translational research through programs such as the Moon Shots. Translational research is key to accelerating development of precision cancer medicine to eliminate the impact of the disease.

SIMPOSIO SAIC N°10: FUNCTIONAL GENOMICS: HOW TO DO SCIENCE WITH A LOT OR LITTLE MONEY
Chairs: Dr. Juan Miguel Bayo Fina y Dr. Carlos Davio

APPLIED BIOINFORMATIC IN FUNCTIONAL ONCOGENOMICS

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Advances in cancer genomics have been propelled by the development of high-throughput sequencing technologies (NGS) for the generation of functional genomics data (e.g., epigenomics, genomics, transcriptomics), providing a deeper understanding of the biological processes of human diseases. In addition, several large-scale cancer-based projects such as The Cancer Genome Atlas (TCGA), the Tumor Alterations Relevant for Genomics-driven Therapy (TARGET), and Genotype-Tissue Expression (GTEx) project have generated genomic data among thousands of samples in almost all human tissue sites. Although a plethora of web applications and software focused on data retrieval, visualization and analyses have been developed, the full integration and interpretation of these multilayer data require specialized knowledge and resources avoiding their massive use. Currently, there exists a diverse number of software platforms, web applications, and cloud-based services that mainly provide general-purpose bioinformatic toolkits and/or workflows for processing and analysis of functional genomics data. On the other hand, several R-based packages were developed to facilitate the integration and analysis of public and preprocessed genomics data. Here, the in-between solutions focused on cancer biomarker discovery are described to facilitate the integration and mining process of public/private oncogenomic data for non-expert users in the field promoting a bioinformatic by biologists.

UBIQUITIN PATHWAY IN TUMOR CELL MIGRATION AND INVASION: A FUNCTIONAL GENOMIC APPROACH

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Change in tumor cell motility is associated to refraction to treatment, high aggressiveness, and increased metastatic potential that is often linked with an increase in malignancy and a significant reduction in patient survival and quality of life and.
Based on these observations we decided to investigate the role of the Ubiquitin-Proteasome System (UPS) in the control of these processes. To this end we performed a genetic screen using an shRNA library against UPS genes and analyzed alterations in the migrating potential of breast cancer (BC) cells.
After the selection process, we characterized the non-migrating population and obtained a list of 30 candidate regulator genes. We focused on two genes, USP19 and HERC1, whose role in tumor cell migration and invasion was not previously described. Our results demonstrated that silencing of these genes reduces the migratory/invasive potential of different BC cell lines. We extended our investigation *in vivo* and confirmed that mice injected with USP19 or HERC1 depleted cells display increased tumor-free survival, as well as a delay in the onset of the tumor formation and a significant reduction in the appearance of metastatic foci. Finally, we performed a retrospective clinical study for USP19 and an *in silico* study for HERC1. Our analysis showed that USP19 protein expression is a prognostic predictor of distant relapse free survival in BC patients and reveal the existence of an inverse correlation between HERC1 expression levels and breast cancer patients' overall survival. Altogether our data provide solid evidence indicating that both USP19 and HERC1 might represent novel prognostic and therapeutic targets in breast cancer.

PUBLIC TRANSCRIPTOMIC DATASETS, GREAT TOOLS TO DO SCIENCE WITHOUT MONEY

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The advent of Next-Generation Sequencing (NGS) has led to a better understanding of the pathophysiological process bridging to a new era of molecular pathology and personalized medicine. Remarkably, the scientific

community now has available genomic, transcriptomic and epigenomic data of thousands of tissues and cell samples derived from both human and animal disease models. This public data was provided in a cooperative effort by multi-institutional consortia such as The Cancer Genome Atlas (TCGA), International Cancer Genome Consortium (ICGC) and the Genotype-Tissue Expression (GTEx) projects together with the research groups that uploaded their data to the public repository Gene Expression Omnibus (GEO). In the last years several web-based platforms and software to download and analyze have been developed. Here, we described several types of analysis that could be performed to open this fascinating new era to biologist without expend a dime.

SIMPOSIO SAIC N°11: CONTRIBUTIONS TO THE DIAGNOSIS, TREATMENT AND PREVENTION OF TYPICAL HEMOLYTIC UREMIC SYNDROME
Chairs: Dr. Ángel Cataldi y Dra. María Marta Amaral

GUT MICROBIOTA-METABOLOME ASSOCIATED TO STEC INFECTIONS

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Background. Intestinal microbiota and metabolites have been proposed to be responsible for the maintenance of intestinal homeostasis and prevention of colonization by enteric pathogens, such as diarrheagenic *Escherichia coli* (DEC). Among these metabolites, short-chain fatty acids (SCFA) are the most abundant products of bacterial fermentation, and their role in the development of diarrheal disease is controversial. Although different investigations have granted a protective factor to SCFA, others have linked them to an increase in the expression of virulence factors of enteric pathogens, and possibly favor the infection process.

Methodology. SCFA levels were determined in stool samples of children under 5 years of age. 30 diarrheal samples positive for a DEC pathotype and 38 stool samples of healthy children were analyzed. The concentration of the SCFA acetate, propionate and butyrate in the stool samples was determined by high performance liquid chromatography (HPLC). Additionally, the V3-V4 region of the 16S rRNA gene was sequenced for taxonomic classification.

Results. From microbiota analysis we obtained that the diarrheal samples presented a distinctive composition with respect to the control samples. Similar results were obtained in STEC positive samples (n=11) compared to other pathotypes and to control samples. A lower bacterial diversity was found in diarrheal samples, (regardless of the pathotype), when compared to the control group. A higher proportion of *Proteobacteria* and a lower proportion of *Firmicutes* and *Bacteroidetes* was found in diarrheal samples, compared to the control group. Compared to healthy children, higher total levels of propionate-producing bacteria were found in diarrheal samples, highlighting the genus *Veillonella* sp. (4.9% vs. 0.2%, p= 0.014) and with respect to acetate production, an increase was observed in the genus *Streptococcus* sp. (3.9% vs 0.6% p< 0.001).

In diarrheal samples, higher levels of acetate, propionate and butyrate were found compared to samples from healthy children. Acetate levels on stool samples were significantly higher in STEC positive samples, compared to the control group. Regardless of the presence of the two SCFA producing genera in higher proportions in diarrheal samples compared to the control group, a positive correlation was found between levels of acetate producers (*Akkermansia*, *Blautia* and *Ruminococcus)*, propionate producers (*Coprococcus*, *Phascolarctobacterium* and *Roseburia)*, and butyrate producer (*Anaerostipes* and *Coprococcus).*

Conclusions: We found a characteristic microbiota associated with diarrheal samples positive for DEC, as well as positive for STEC. We found a significant increase in SCFA levels of diarrheal samples compared to stools of healthy children. SCFA levels could be associated with the presence of SCFA-producing bacteria, regardless of the abundance of the involved taxas. Metabolomic data complement the understanding of gut bacterial composition's relevance.

DIFFERENT FORMATS OF RECOMBINANT ANTIBODIES FOR STEC DIAGNOSIS AND THERAPY

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Antibodies innovative recombinant DNA technologies have enhanced the murine mAb clinical efficacy and, in the preceding decades, have led to regulatory approvals for immunoglobulin and classic monovalent antibody fragment (Fab) molecules, either for therapy or diagnosis. Single chain fragment variable (scFv) is the format where the VH and VL are joined by a flexible peptide linker to prevent their dissociation. Besides the variable chains, Fab fragments have one constant region in each chain. Both fragments retain the parental IgG specific antigen-binding affinity. Recombinant antibodies fragments targeting Stx1 and/or Stx2 were produced in bacteria and have been comprehensively studied. The scFvStx1 and scFvStx2 genes were constructed on the basis of murine hybridoma (mAb 3E2) secreting Stx1 or (mAb 2E11) Stx2 IgG monoclonal antibodies. Both scFv were coupled to latex nanoparticles and provide a toxin assay with a competitive Stx detection limit of 30 ng/mL for Stx1 and 10 ng/mL for Stx2, which has low cost and can be performed in a short-term time. For the therapeutic approach, four Fab fragments were selected against Stx1 and Stx2 from human phage display library, and showed the following dissociation constants analyzed by surface plasmon resonance: B6 $4X10^{-8}$ M and C8 $1X10^{-8}$ M, both specific against Stx1, F8 $1X10^{-8}$ M specific against Stx2 and the C11 which cross-recognizes both toxins with an affinity of $7X10^{-9}$ M to Stx2 toxin and $3X10^{-8}$ M to Stx1. The cross-reaction of FabC11 is due to the binding epitope GKIEFSKYNEDDTF, localized on subunit B of both toxins. The Fabs neutralizing ability was tested either employing purified toxins or bacterial supernatants in renal (Vero and HK-2) and glomerular cells (HGEC) assays. Considering different ranges of neutralization, the FabF8 and FabC11 neutralized the cytotoxicity in 90 and 100% of the Stx2 producing strains, respectively. Also, the FabB6 and FabC8 were able to neutralize the cytotoxicity in 50 and 85% of the Stx1 producing strains, respectively. Moreover, the FabC11 was able to prevent Stx2 toxicity to human kidney cells and in mice. This neutralizing capacity seems to involve receptor binding site blocking, preventing the translocation of effective subunit A into the target cells. Taken together, our results indicate the recombinant fragments are promising molecules to be used therapeutically against Stx1 and Stx2 intoxication, as well as for rapid screening detection of Stx producing strains.

HEMOLYTIC UREMIC SYNDROME: NEW INSIGHTS AND NEW CHALLENGES FOR CLINICAL MANAGEMENT

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Hemolytic uremic syndrome related to Shiga-toxin-producing *Escherichia coli* (STEC-HUS) is a thrombotic microangiopathy characterized by thrombocytopenia, acute kidney injury, and microangiopathic hemolytic anemia. STEC-HUS can be a life-threatening condition requiring prompt diagnosis and treatment: it is one of the leading causes of acute renal injury in the pediatric population, mortality rates can reach up to 5% and long-term renal sequelae remains, on average, around 30%. Remarkably, Argentina is the country with the highest incidence of this disease in the world, where it generates a great impact on public health.

After STEC infection there is no effective intervention capable of decreasing the risk of developing the disease; however, the interval between diarrhea onset and progression to HUS may represent a window for interventions which could optimize the likelihood of good outcomes. Among them, the importance of early identification of STEC infection, the relevance of maintaining a well hydration status during the prodromal phase and analysis of the different accepted case definitions accepted will be discussed.

After the diagnosis of STEC-HUS, rapid identification of high-risk patients can optimize their management and, although treatment of the disease remains supportive, recent evidence provides the rationale for changing the fluid management approach and for the off-label use of eculizumab (a monoclonal antibody against the terminal complement component 5). In this conference, clinical and laboratory markers of poor prognosis and the role of new treatment interventions will be analyzed.

At last, as mentioned above, a high proportion of acute

phase survivors develop chronic kidney disease, being the most important risk factor for such an outcome is the length of the oligoanuric period. However, even patients who had not undergone dialysis might exhibit renal damage during follow-up. Of concern, the progression is variable, with some patients never recovering normal renal function and others experiencing a secondary decline, sometimes after a period of apparent recovery. In this conference, the role of baseline urinary transforming growth factor 1 (TGFβ-1) levels in predicting short-term renal outcomes and the potential role of uric acid as new additional factor that can aggravate the injury caused by the microangiopathic process will be presented based on studies of our own.

SHIGA TOXIN-ENCODING PROPHAGES OF *E. COLI* O157: EVOLUTION, REGULATION AND LINK TO HUMAN DISEASE

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While it is evident that specific sub-types of Shiga toxin can be associated with more serious pathology, there is less known about whether the prophage context of these toxins in specific strain backgrounds can influence the levels of toxin produced. In Scotland we have higher levels of EHEC O157 infections than in the rest of Great Britain (England & Wales) and we now know this is due to higher prevalence of Lineage 1c *E. coli* O157 strains expressing the Stx2a toxin. This toxin is often present in strains that also encode Stx2c, but Stx2a is the predominant toxin produced, with and without Mitomycin C induction, and this is due to differences in the prophages and their kinetics that encode the different toxin genes. Moreover, during non-induced conditions we show that Stx2a toxin is expressed from the prophage while nearby lysin genes are not, so there is a level of RecA-mediated toxin induction with toxin release most likely through outer membrane vesicles (OMVs). This is supported by recent studies and by the fact that both the Shiga toxin A&B subunits have their own predicted Sec signal peptides to move them into the periplasm. In line with some earlier studies, we show that the most of the main *E. coli* O157 lineages and sub-lineages constantly generate large chromosome re-arrangements (LCRs) which are bounded by prophage regions. For our UK strains, the Stx2c-encoding prophage is a main protagonist in such re-arrangements. We propose that this and similar prophages may be 'self-harming', actually producing a DNA cleaving enzyme that leads to a SOS induction in order to drive expression of an activated RecA regulon that includes Shiga toxin. LCRs are then the by-product of such constant RecABCD repair activity. We propose that this 'background' activity is important for the relative toxicity of a strain in the absence of external activators of the SOS response such as particular antibiotics.

SIMPOSIO SAIC N°12: RNAM METABOLISM IN DISEASE: RNA POL II IS NOT ALONE
Chair: Dr. Juan Miguel Bayo Fina

KAP1 A SIGNALING HUB FOR TRANSCRIPTIONAL CONTROL IN CANCER

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Precise control of the RNA polymerase II (RNA Pol II) cycle, including pausing and pause release, maintains transcriptional homeostasis and organismal functions. Despite previous work to understand individual transcription steps, we reveal a mechanism that integrates RNA Pol II cycle transitions. Surprisingly, KAP1/ TRIM28 uses a previously uncharacterized chromatin reader cassette to bind hypo-acetylated histone 4 tails at promoters, guaranteeing continuous progression of RNA Pol II entry to and exit from the pause state. Upon chromatin docking, KAP1 first associates with RNA Pol II and then recruits a pathway-specific transcription factor (SMAD2/3) in response to cognate ligands, enabling gene-selective CDK9-dependent pause release. This coupling mechanism is exploited by tumor cells to aberrantly sustain transcriptional programs commonly dysregulated in cancer patients. The discovery of a factor integrating transcription steps expands the functional repertoire by which chromatin readers operate and provides mechanistic understanding of transcription regulation, offering alternative therapeutic opportunities to target transcriptional dysregulation.

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS ORF57 PROTEIN PROTECTS VIRAL TRANSCRIPTS FROM TWO HOST-MEDIATED RNA DECAY PATHWAYS THAT MODULATE VIRAL GENE EXPRESSION

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In eukaryotes, nuclear RNAs are subject to a number of RNA decay pathways that serve quality control and regulatory functions. Kaposi's sarcoma-associated herpesvirus (KSHV) is a human pathogen that expresses its genes in the nucleus using the host machinery. Consequently, KSHV evolved mechanisms that allow its transcripts to evade host nuclear RNA decay pathways. The multifunctional KSHV ORF57 (Mta) protein increases the nuclear stability of viral transcripts by protecting them from cellular RNA quality control pathways. However, neither the RNA decay pathway(s) inhibited by ORF57 nor the mechanism by which ORF57 protects viral RNAs from degradation is known. In the absence of ORF57, we show that viral transcripts are subject to degradation by two specific nuclear RNA decay pathways, PABPN1 and PAPα/γ-mediated RNA decay (PPD) in which decay factors are recruited through poly(A) tails, and an ARS2-mediated RNA decay pathway dependent on the 5´ RNA cap. In transcription pulse chase assays, ORF57 appears to act primarily by inhibiting the ARS2-mediated RNA decay pathway. In the context of viral infection in cultured cells, inactivation of both decay pathways by RNAi is necessary for the restoration of a subset of specific ORF57-dependent viral genes produced from an ORF57-null bacmid. Mechanistically, our data suggest that the ORF57 interaction with ALYREF protects certain viral transcripts by preventing the recruitment of the exosome co-factor hMTR4. However, other KSHV transcripts are stabilized by ORF57 in an ALYREF-independent fashion. Interestingly, global viral transcriptome analysis revealed a widespread increase in KSHV gene expression when both decay pathways were inactivated simultaneously. The transcripts that accumulated included known ORF57-dependent RNAs as well as many RNAs that are not dramatically changed upon ORF57 depletion. These viral transcriptome analyses suggest that PPD and ARS2-mediated RNA decay pathways appear to play broader and more complex roles in KSHV gene expression.

TRANSCRIPTIONAL REPROGRAMMING OF THE MALARIA PARASITE

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Plasmodium falciparum (Pf) is the most lethal of the five species of malaria affecting 3.4 billion humans annually. Proper transcriptional regulation at each stage of the *Pf* life cycle is essential for the successful completion of the life cycle, for gametocytogenesis and for red blood cell invasion. Epigenetic enzymes are thought to play important and potentially essential roles in this regulation. The methylation state of histones at specific genomic loci, for example, controls the expression of stage-specific transcription factors such as AP2-G, of nutrient uptake channels including the Clag3 gene paralogs, and of pathological multi-gene families such as the highly virulent *var* genes that encode adhesion proteins involved in antigenic variation. These loci are kept transcriptionally repressed by trimethylation of lysine 9 on histone 3 (H3K9me3). Proper temporal activation of these loci or switching to a distinct gene form for antigenic variation *requires* the enzymatic deletion of this H3K9me3 transcriptional silencing mark. Similarly, proper temporal repression of active genes marked by H3K4me3 such as transcriptionally active stage-specific genes or mono-allelic loci for gene switching, requires erasing the activating H3K4me3 mark. Demethylation of trimethylated histones such as H3K9me3 or H3K4me3 can *only* be carried out by Jumonji domain containing histone hydroxylases. No other proteins with this activity are known to date. The *Pf* genome encodes three Jumonji hydroxylases: PfJmjC1, PfJmjC2 and Jmj3. Using a small molecule screening approach, we have identified potential epigenetic vulnerabilities driven by candidate Jumonji histone demethylating enzymes in the malaria parasite *Plasmodium falciparum*. We show that inhibitors of mammalian JmjC histone demethylases kill asexual blood stage parasites and are even more potent at blocking gametocyte development and gamete formation. In late stage parasites, exposure to demethylase inhibitors results in increased levels of tri-methylated lysine residues on histones suggesting inhibition of *Pf* Jumonji demethylase activity. These epigenetic defects coincide with deregulation of invasion, cell motor, and sexual development gene programs, including gene targets coregulated by the PfAP2-I transcription factor and chromatin-binding factor, PfBDP1. Our pharmacological studies of Jumonji activity in the malaria parasite provide evidence that inhibition of these enzymatic activities is detrimental to the parasite.

RNAPII DEGRADATION IN RESPONSE TO UV-INDUCED DNA DAMAGE

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DNA damage caused naturally by UV light exposure in skin cells triggers not only lesion repair mechanisms but also a global gene expression response that includes RNAPII degradation. Recent identification of the lysine residue responsible for RNAPII ubiquitination and degradation uncovered an unanticipated relevance for RNAPII levels in the control of gene expression in UV-treated cells.

Since DNA is the only biopolymer that is neither disposable nor recyclable, it must be repaired when damaged. Among the various repair systems that human cells have, the nucleotide excision repair (NER) system is the one that deals with most of UV light-induced lesions. Damage detection by NER system occurs by two different DNA-sensing mechanisms that then converge on the same machinery that repairs the damage: transcription-coupled repair (TC-NER) and global genome repair (GG-NER). The "last resort" model states that a RNAPII molecule stalled in front of a UV-induced DNA lesion is degraded so as to allow access to the repair machinery and, therefore, RNAPII degradation would be part of the TC-NER system. However, different evidences suggest that the scenario could be different. On the one hand, most of the repair of lesions in template strands in transcriptionally active genes, the only lesions repaired by TC-NER, occurs in the first hours post UV, while degradation of RNAPII is observed hours later. On the other hand, preliminary results from our group show that RNAPII degradation is mainly controlled by the GG-NER system. Using the CRISPR/Cas9 editing system, we generated human keratinocytes unable to recognize lesions through the GG-NER system (GG-NER KO / TC-NER WT cells) and observed a marked inhibition in the degradation of RNAPII in response to UV light. Moreover, impairment of the actual lesion-repair, but its lesion recognition, enhanced RNAPII degradation. While in GG-NER KO cells we observed less RNAPII degradation and enhanced cell viability upon UV, in comparison to wild type keratinocytes, in different lesion-repair mutant cells we observed the opposite: enhanced RNAPII degradation and reduced cell viability upon UV. Consequently, we propose that RNAPII levels determine cell viability and are mainly controlled by an unexplored GG-NER-dependent mechanism.

SIMPOSIO SAIC N°13: TOXICOLOGY AND HEALTH, NEW PERSPECTIVES FOR THE FUTURE
Chairs: Dra. Claudia Cocca y Dra. Andrea Randi

ORGANIC FOOD: IS THERE ANY RISK BEYOND PESTICIDES?

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In recent years, there is a global increasing tendency to consume organic foods instead of the conventional foodstuffs. It is mainly due to the concern raised by the potential adverse health effects derived from the intake of pesticides, fertilizers, hormones and antibiotics, which are widely used in conventional food production. Although organic label forbids the use of these products, environmental contamination can also occur in organic foodstuffs. The main purpose of this literature review was to compare the levels of a number of environmental pollutants such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), mycotoxins, trace elements, or polycyclic aromatic hydrocarbons (PAHs) in organic and conventional food items. In general terms, the presence of nearby anthropogenic sources of pollution might be the key issue influencing the occurrence of environmental pollutants in foodstuffs, regardless their organic or conventional origin. However, few studies concluded that he concentrations of environmental pollutants might vary according to the production system. Specifically, the concentrations of PCBs, HBCD, PBDEs and heavy metals are higher in organic than conventional meat. Likewise, the concentrations of heavy metals are lower in organic fruits and vegetables than those in conventional production. Regarding milk and dairy products, higher concentrations of PCBs and aflatoxin M_1 have been reported in organic than in the conventional ones. In turn, organic milks show lower concentrations of essential elements than conventional ones, while no differences were found in the levels of toxic elements according to the production system. Similarly, the occurrence of mycotoxins in cereals does not vary depending on the production system. With respect to heavy metals, Cd and Pb levels are higher in organic cereals than in conventional ones. In contrast, the concentrations of Hg, Zn and Cu are higher in conventional than in organic cereals. The occurrence of PCDDs and PCBs is lower in organic eggs than those from conventional production. Based on the above, environmental contaminants should be monitored in both conventional and organic foods. Moreover, the safety feature, which has been globally attributed to the consumption of organ-

ic foods, might be questionable depending on the potential environmental contamination of these foodstuffs.
Based on this, we suggest that environmental contaminants should be monitored in both conventional and organic foods. Finally, the safety feature, which has been globally attributed to organic foods, might be questionable depending on the potential environmental contamination of these foods.

CHLORPYRIFOS-INDUCED MOLECULAR MECHANISMS INVOLVED IN BREAST CANCER PROGRESSION.

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Chlorpyrifos (CPF) is one of the most widely used pesticides due to the low cost, high effectiveness, and broad spectrum. CPF is currently considered an Endocrine Disruptor (ED). EDs have been associated with the progression and metastasis of established breast tumors and were pointed out as potential inductors of the epithelial mesenchymal transition (EMT). In this study, we investigate whether CPF promotes molecular mechanisms involved in breast cancer progression. Our experiments were performed using MCF-7 and MDA-MB-231 cell lines, and two CPF concentrations (0.05 and 50 μM). We evaluated the migration and invasion using 2D and 3D models, as well as the expression of EMT biomarkers.
We show that 50 µM CFP induces invasion in MCF-7 and MDA-MB-231 cells (p<0.001) in 2D cultures. In MCF-7-3D culture, we observe that 0.05 μM CPF increases the invasion area after 7-day (p<0.01) and 50 μM CPF induces the invasion area after 5-(p<0.05) and 7-day (p<0.001) when collagen type 1 was used as matrix model. When Matrigel® was used as substrate, we observe that only 0.05 μM CPF increases the invasion area after 2-(p<0.05), 5-(p<0.01) and 7-day (p<0.001). In MCF-7 cells, 0.05 μM CPF induces the migration in 2D models after 48 h (p<0.01) that was reversed adding IC182,780 and PP2 together (p<0.001). 50 μM CPF (p<0.001) promotes the migration in c-SRC-dependent way (p<0.001). In addition, we found that 0.05 and 50 µM CPF increases the migration area in the spheroid in a ERα-dependent manner (p<0.01 and p<0.05, respectively). CPF at 0.05 μM induces an increment of the number and the area of MS1 and MS2 in MCF-7 cells. (p<0.05, p<0.001, respectively). This effect offset by ICI 182,780 (p<0.001) and PP2 (p<0.05). Finally, in MCF-7 cells, 0.05 and 50 µM CPF increase the metalloprotease MMP2 expression (p<0.001) and decrease E-Cadherin and β-Catenin expression (p<0.01) diminishing their membrane location. CPF at 50 µM induces Vimentin expression and Slug nuclear translocation in MCF-7 cells. 0.05 and 50 µM CPF increase MMP2 gelatinolytic activity and expression (p<0.05, p<0.001, respectively), decrease β-Catenin expression (p<0.01) and increase Vimentin expression (p<0.05) in MDA-MB-231 cells. Our results show that CPF modulates EMT-molecular markers, promotes migration and invasion in breast cancer cells in 2D and 3D cultures. Also, CPF at 0.05 μM induces the stemness phenotype. These data support that CPF may represent a risk factor for breast cancer progression.

TOXICODYNAMICS OF FINE PARTICULATE MATTER IN SUSPENSION ($PM_{2.5}$) IN HEALTHY ANIMAL MODELS AND WITH ARTERIAL HYPERTENSION

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According to the WHO, 9 out of 10 people breathe polluted air, and more than 7 million die prematurely because of it. Fine suspended particulate matter ($PM_{2.5}$) is one of the most relevant indicators when estimating this impact since exposure to $PM_{2.5}$ is often chronic, and it induces negative effects even at low concentrations. This is related to its highly random composition, high surface area, persistence in the atmosphere, and high penetration in the respiratory system.
Once in the body, $PM_{2.5}$ causes histological changes, which can be intensified by the activation of response mechanisms. According to the tissue, particles can irritate by mechanical or chemical action, activate the immune system, and promote the release of pro-inflammatory and vasoconstrictor mediators. In the cellular environment, $PM_{2.5}$ constituents trigger the production of free radicals causing oxidative stress, formation of DNA adducts, activation of the endoplasmic reticulum's unfolded proteins response, and apoptosis. These events lead to macroscopic changes that promote the setting or exacerbation of diseases since particles do not restrict to the target organs.
Therefore, many studies still focus on the $PM_{2.5}$ action

mechanisms, although these do not always simulate real exposure conditions, and only a few comprehensively address the pathogenesis. Furthermore, a small number of them studied how $PM_{2.5}$ particles affect organisms with pre-existing pathological conditions, such as hypertension. On the other hand, the $PM_{2.5}$ toxic potential is closely related to its composition, which depends on the emission sources, most of which are specific from each society.
Local studies have demonstrated that long-term exposure to low levels of $PM_{2.5}$ (< 25 μg m^{-3}) induces serum and histological alterations in lungs, liver, and kidneys, and changes in tissues' elemental composition in both animal models. These effects tend to exacerbate when particles levels are higher, mainly in hypertensive animals. Therefore, it can be expected that sustained exposure to $PM_{2.5}$, even at low particles levels, significantly affects the life quality of individuals, especially those who have a hypertensive disease.

GLYPHOSATE HERBICIDE: CARCINOGENIC POTENTIAL AND REPRODUCTIVE TOXICITY

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Glyphosate-based herbicides (GBHs) are the most widely applied pesticides in the world and are mainly used in association with GBH-tolerant crop varieties. Indiscriminate and negligent use of GBHs has promoted the emergence of glyphosate resistant weeds, and consequently the rise in the use of these herbicides. In addition, alarmingly increased levels of glyphosate, the active ingredient of all GBHs, have been detected in environmental matrixes and in foodstuff, becoming a matter of social concern. GBHs are composed of glyphosate and other chemicals known as co-formulants which enhance the herbicide action. Currently, the safety of glyphosate and its formulations remain to be a controversial issue. In 2015, the World Health Organization classified glyphosate as a "carcinogen type 2a" or a probable human carcinogen; however, nowadays its carcinogenic potential is under discussion. Our research work focused on investigating whether glyphosate or GBH could exhibit carcinogenic potential and/or impair female fertility and fetal/placental growth. We performed *in vitro* and *in vivo* studies to evaluate the effects and elucidate the mechanisms of action of the herbicide. For *in vivo* studies, we exposed female rats during high sensitivity periods of life (perinatal and/or early postnatal period), and the effects were tested at adulthood. We analyzed endocrine disrupting endpoints and epigenetic markers using different experimental conditions focusing our attention on the uterus. The results indicate that glyphosate and GBH produce female subfertility by impairing the implantation process, and induce fetal growth retardation in their offspring. Both compounds show endocrine disrupting effects mediated, at least in part, by epigenetic dysregulation of key genes involved in uterine functional differentiation. Regarding carcinogenic potential, we found: i) higher sensitivity to 17β-estradiol in uterine tissue, ii) induction of preneoplastic and neoplastic lesions in mammary gland, uterus and vagina, and iii) stimulation of epithelial mesenchymal transition-related changes via ER-dependent pathway in Ishikawa cells. In conclusion, we provide evidence on adverse reproductive outcomes and carcinogenic properties of the herbicide. As our results indicate similar effects after glyphosate and GBH treatment, the active principle might be responsible for the deleterious effects. Epidemiological studies are a priority to evaluate possible deleterious effects on human health.

ENVIRONMENTAL IMPACT OF CHLORPYRIFOS TO AQUATIC LIFE, WILDLIFE AND HUMAN HEALTH. CONSEQUENCES AND FUTURE PERSPECTIVES.

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Chlorpyrifos (CPY) is one of the insecticides most widely used throughout the world to limit insect and mite damage to a number of important crops, including soybeans, corn, tree, nuts, wheat, alfalfa citrus and vegetables. The fate and movement of CPY and its metabolite chlorpyrifos-oxon (CPYO) determine exposure in terrestrial and aquatic environments. Detectable concentrations of CPY in air, rain, snow and other media have been measured at considerable distances from agricultural sources, which indicates the potential long-range transport in the atmosphere. For that reason, CPY was listed as toxic air contaminant with potential hazard to human health and other living organisms near application sites. In fact, drift of CYP onto water surfaces have been documented in our country, its effects on vulnerable populations including insects, pollinators crustaceans, fish and amphibians depends on frequency, duration and interval between exposures.
The primary target organ for CPY toxicity in humans is the central nervous system, due to the inhibition of acetylcholinesterase, but there are evidences that indicate its toxicological effects in other tissues. CPY or its me-

tabolite TCP has been found in maternal blood, placenta, umbilical cord blood and meconium. The environmental exposure experienced during the very earliest stages of life, at doses that would not inhibit cholinesterase, produced changes in the developing brain architecture and dimension associated with lowered IQ at school age. Studies performed with embryos, fetus and young animals also demonstrate CPY-damage in a wide variety of brain regions, glial cells and neurons. These discoveries demonstrate that not only acute and chronic toxicity studies must be performed when determining the safety of pesticides but a much larger battery of test including functional neurological and behavioral end points have to be included. The risk associated with the intensive use of CPY and other synthetic pesticides require alternative sources of chemicals to be used in safe management of pests. Plants and their secondary metabolites may be exploited as eco-chemical and biorational approach in integrated plant protection programs.

SIMPOSIO SAIC N°14: WOMEN SCIENTISTS IN EMERGING FIELDS
Chairs: Dra. Adriana Casas y Dra. Sandra Ferreira

VACCINE DEVELOPMENT FOR COVID 19, ARVAC CECILIA GRIERSON PROJECT

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At the end of 2019, a novel coronavirus now known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified as the cause of a cluster of pneumonia cases in Wuhan, China. It rapidly spread, resulting in a global pandemic. In February 2020, the World Health Organization named the disease COVID-19.
As the global severity of the pandemic became rapidly apparent, developing effective vaccines for COVID-19 became the top priority of the vaccine research community, many pharmaceutical companies and medical research institutes. Vaccines to prevent SARS-CoV-2 infection are considered the most promising approach for curbing the pandemic and are being vigorously pursued. In our laboratory, we have been working on the development of new adjuvants to improve the immune response of vaccines against several infectious diseases. In May 2020, after the onset of the COVID-19 pandemic, our team focused on developing a vaccine against SARS-CoV-2 that can be produced in Argentina. We have evaluated in vitro and in vivo, different vaccine formulations in preclinical studies and selected those that elicit high neutralizing antibody titers and T cell responses against SARS-CoV-2. Our project aims to develop and produce in our country a recombinant adjuvanted vaccine against COVID-19 that can be stored between 2 and 8 °C. This vaccine might be used as heterologous booster vaccine for current vaccines and/or as a primary immunization.

AIR POLLUTION EXPOSURE EFFECTS ON ACUTE LUNG INJURY DEVELOPMENT

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Air quality is dramatically decreasing worldwide due to faster development of industrialization and increases in fossil fuel burning. Humans are continuously and involuntarily exposed to air pollutants, and considering that there is no safe threshold, adverse health effects occur at exposure levels to which most of the urban population is currently subjected. Therefore, the concern to establish toxicological mechanisms triggered by environmental hazards has increasingly gained attention from the public health perspective. Among the diverse air pollution constituents, particulate matter (PM) has been pointed out as the major component consistently positively correlated with increased morbidity and mortality rates. Since the respiratory system is the entry point for breathable pollutants, not only various chronic respiratory diseases, such as chronic obstructive pulmonary disease or asthma, but also acute illness including pneumonia and allergies are mentioned among the most relevant harmful conditions favored by PM exposure. In that sense, air pollution characterization in terms of composition and physicochemical properties is critical to establish relevant associations with the observed adverse health effects. It is well established that mitochondria represent a key modulator of the redox signaling network, in which an interplay is displayed from the organelle to the rest of the cell. We have focused on the mitochondrial role in altered lung oxidative metabolism associated with an increased reactive oxygen species production, as well as on the inflammatory pathways initiated after PM inhalation. Moreover, in several lung malignancies outcomes both, tissue damage and repair mechanisms, are equally important taking into consideration that the disease extension depends on tissue remodeling. Hence, it seems important to evaluate and emphasize the discussion of the latest insights into the cellular and molecular toxicological mechanisms triggered in the respiratory system by PM exposure, addressing the central role of the alveolar-capillary barrier integrity. Consequently, assessment of PM-initiated oxi-

dative metabolism and mitochondrial function modulation linked to the alveolar epithelial dynamics involved in tissue healing mechanisms, emerge as valuable knowledge in the development of adequate therapeutic approaches aiming to restore the normal alveolar architecture required to ensure proper lung function.

DEVELOPMENT AND IMPLEMENTATION OF A SCALABLE AND VERSATILE TEST FOR COVID-19 DIAGNOSTICS IN RURAL COMMUNITIES

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Rapid and widespread testing of severe acute respiratory coronavirus 2 (SARS-CoV-2) is essential for an effective public health response aimed at containing and mitigating the coronavirus disease 2019 (COVID-19) pandemic. Successful health policy implementation relies on early identification of infected individuals and extensive contact tracing. However, rural communities, where resources for testing are sparse or simply absent, face distinctive challenges to achieving this success. Accordingly, we report the development of an academic, public land grant University laboratory-based detection assay for the identification of SARS-CoV-2 in samples from various clinical specimens that can be readily deployed in areas where access to testing is limited. The test, which is a quantitative reverse transcription polymerase chain reaction (RT-qPCR)-based procedure, was validated on samples provided by the state laboratory and submitted for FDA Emergency Use Authorization. Our test exhibits comparable sensitivity and exceeds specificity and inclusivity values compared to other molecular assays. Additionally, this test can be re-configured to meet supply chain shortages, modified for scale up demands, and is amenable to several clinical specimens. Test development also involved 3D engineering critical supplies and formulating a stable collection media that allowed samples to be transported for hours over a dispersed rural region without the need for a cold-chain. These two elements that were critical when shortages impacted testing and when personnel needed to reach areas that were geographically isolated from the testing center. Overall, using a robust, easy-to-adapt methodology, we show that an academic laboratory can supplement COVID-19 testing needs and help local health departments assess and manage outbreaks. This additional testing capacity is particularly germane for smaller cities and rural regions that would otherwise be unable to meet the testing demand.

HETEROMERIZATION OF G PROTEIN-COUPLED RECEPTORS: HOW MUCH DOES IT INFLUENCE THEIR BIOLOGICAL FUNCTION?

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G protein-coupled receptors (GPCRs) are a remarkably multifaceted family of transmembrane proteins that exert a variety of physiological effects. They are targets for around one third of currently approved and clinical prescribed drugs and represent the largest and most structurally diverse family of transmembrane signaling proteins, with almost 1000 members identified in the human genome. Although GPCRs are able to operate as monomers, there is increasing evidence about the ability of GPCRs to form and function as heterodimers/heteromers that exhibit distinct pharmacological, trafficking and functional properties as compared to their parent monomeric or homodimeric/homomeric GPCRs. Efforts have focused over the past two decades on the identification of GPCR complexes as well as on their signaling properties. More recent findings have provided evidence for the existence of GPCR heteromerization in native tissues and animal models. In our lab we have been investigating how the functional properties of Mas receptor (R), a GPCR with protective actions that belongs to the renin-angiotensin system, are influenced by interaction with others GPCRs like the bradykinin type 2 (B2R) receptor, the dopamine type 2 receptor (D2R) and the MrgDR. MasR-B2R heteromerization influences MasR pharmacology, signaling and trafficking, while MasR-D2R heteromerization is necessary for MasR to display its anti-inflammatory responses. GPCRs heteromerization not only brings forth a plethora of drug target combinations, but also gives an opportunity to carefully tweak the structure and function of one or more GPCRs involved in the complex, with the final goal of improving therapeutic strategies.

SAIC - LEÓN CHERNY AWARD
Juries: Dra. Claudia Perez Leiros, Dra. Graciela Cremaschi, Dra. Cristina Ibarra y Dra. Isabel Luthy

CLASSIFICATION OF THE FUNCTIONAL EFFECT OF GENETIC VARIANTS IN NKX2-5

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NKX2-5 is a homeobox gene of very early expression in humans. It codes for NKX2-5, a transcription factor that regulates the expression of a number of other transcription factors involved in the development and function of the early heart, among other functions. This gene was the first where a single genetic variant (GV) was related to congenital heart disease (CHD).
Our group recently compiled, curated and structured an exhaustive database of all GVs in the gene. In this study, we aimed to expand this information by predicting the effect of some GVs in the functionality of the NKX2-5 protein. In order to do that, we proposed a classification of all possible missense GVs in the structured region of the protein according to the most probable effect they would have on its functionality.
We worked with all the possible amino acid variants caused by a change in a single DNA base pair in the homeodomain (HD) region (n=337). The *in silico* studies included an estimation of protein-DNA interaction, protein stability, prediction of linear motifs that could be affected by the variant and evolutive conservation. Taken together, these results were used to obtain a classification of the possible effect of GVs on the functionality of NKX2-5.
Our analysis determined that 32 GVs may affect the interaction with DNA (9.5%), 10 may have an effect on functionality due to introducing a cysteine (3.0%), 38 may affect a known linear motif (11.3%) and 135 are predicted to affect protein stability and/or a putative linear motif (40.0%). This analysis allowed us to estimate the potential effect of GVs on NKX2-5. In particular, it contributes to a functional prediction of yet unknown GVs, it proposes a mechanism for pathogenesis of known GVs and it helps to better understand the genotype-phenotype relationship in the development of CHD.

THE GALECTIN-1-GLYCAN AXIS PROMOTES DISSEMINATION AND METASTASIS OF BREAST CANCER

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The Galectin-1 (Gal1)/glycan axis controls several hallmarks of cancer. Here we investigated the role of Gal1 in breast cancer metastasis. We found at single cell level (scRNAseq) that Gal1 is synthesized by basal cell lineages and mammary stem cells (SCs) in normal mammary gland, where it promotes epithelial branching (**). Moreover, in the MMTV-NeuHER2/transgenic model, Gal1 was induced in early lesions (EL) compared to primary tumors (PT) (RNAseq). Addition of rGal1 to EL 3D-cultures promoted invasiveness (**) and increased epithelial-to- mesenchymal transition (EMT) markers (RT-PCR). This effect was confirmed in the aggressive Her2+ human cell line JIMT-1, which showed high levels of Gal1 (**, Western) and low levels of α2,6 sialyltransferase-1 (ST6Gal1), an enzyme that incorporates α2,6-linked sialic acid and blocks Gal1 binding (***, RT-PCR), compared with the HER2+ poorly metastatic cell line BT-474. Accordingly, UPLC-HILIC/WAX revealed a Gal1-permissive glycan signature in JIMT1 (***). Treatment of JIMT-1 cells with rGal1, induced a CD44hi/CD-24low cancer stem cell phenotype (***, flow cytometry) and enhanced migration (*), mamosphere formation (**) and EMT markers (RT-PCR). *In vivo*, treatment of HER2+PDX with rGal1 revealed increased lung metastasis (*). Bioinformatics analysis (TCGA) showed that tumors displaying a Gal- 1hi/ST6GAL1low phenotype

had the poorest prognosis. Remarkably, these tumors upregulated transcripts associated with EMT and downregulated those linked to antitumor immunity (GSEA), as validated by the immunosuppressive infiltrate (Mixture). Our findings highlight the relevance of the Gal1/glycan axis in controlling normal mammary gland branching and emphasize its critical role in metastatic spreading of breast cancer. We propose that the Gal1/ST6Gal1 pair might serve as a possible biomarker capable of predicting the outcome of breast cancer patients and as a therapeutic target of novel anti-metastatic therapies ($p<0.05$*;$p<0.01$**;$p<0.001$***).

A CRITICAL PATHWAY IN HUMAN DEVELOPMENT: C19MC MICRORNAS REGULATES FGF2 RESPONSE IN A MODEL OF HUMAN PLURIPOTENT STEM CELLS CARDIAC DIFFERENTIATION

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Human pluripotent stem cells (hPSC) have the capacity to self-renew and differentiate *in vitro* into all cell types of the organism, and it is an established model for early human embryo development. Recently, we found a 56-miRNA-cluster located at human chromosome 19 (C19MC) that downregulates during hPSC cardiac differentiation (CD). To ascertain the role of this primate-specific microRNA cluster, a hPSC-C19MC$^{(-/-)}$ line was generated with CRISPR/Cas9. C19MC$^{(-/-)}$ cells displayed no evident changes in the cell cycle, apoptosis or differentiation markers compared to wild type. Contrarily, C19MC$^{(-/-)}$ cells were significantly impaired to differentiate into cardiomyocytes. Early mesoderm and cardiac RNA markers, like EOMES, TBX6, MESP1, were found altered. In order to further explore the early steps of differentiation, we performed RNA-seq of the cells at the gastrulation stage (0 and 24hs after CHIR99021 incubation). Gene ontology analysis revealed altered signaling pathways, including PI3K-Akt, MAPK and Wnt, and FGF2. As FGF2 is a key pathway in pluripotency, we address its role through two different approaches. First, both wild type and mutant cells were treated with FGF2 for 3 hours before gastrulation. Wild-type phenotype was partly recovered, as evidenced by the presence of contractile cardiomyocytes at day 15. Second, given that FGF2 is an important activator of RAS cascade that phosphorylates ERK1/2 (pERK), we incubated the cells with FGF2 for up to 5 hs in pluripotency media. Mutant cells exhibited an elevated pERK mark in ground conditions, and it was noticeable that the phosphorylation took place faster when they were treated. In summary these findings support a critical role of the C19MC microRNA cluster in early stages of primate differentiation.

CERAMIDE 1-PHOSPHATE SKEWS HUMAN MACROPHAGES´ FATE TOWARDS A M2-LIKE PHENOTYPE BY RESTRAINING THE M1

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Ceramide 1-Phosphate (C1P) is a bioactive sphingolipid released from dying cells after inflammation, increasing locally in the damaged tissue. C1P exerts many biological effects depending on the cell type, including the downregulation of inflammatory mediators and activation markers. Given that macrophages are critical players for both, resolution of inflammation and tissue restoration, here we aimed to decipher the effect of this sphingolipid on human monocytes/macrophages (Mø) under inflamed tissue conditions and predict the Mø fate and behavior. Human CD14+ monocytes (Mo) were isolated from PBMCs of healthy donors, cultured with RPMI+10% FBS + M-CSF (50ng/ml), and stimulated with C1P short chain analog C8-C1P (1 and/or 20μM), together with naturally occurring inflamogens, lipoteichoic acid (LTA) and hyaluronate (HA); the immune-characterization was performed by flow cytometry and qPCR. Principal Component Analysis (PCA) was carried out including all evaluated parameters; for correlation significance, $p \leq 0.05$ was considered. Firstly, C1P-primed Mo (1, 20 or 1+20 μM) gave rise to transcriptionally different Mø compared to the untreated cells. The more explicative dimensions (Dim1= 26.2% & Dim2=20.9%) predict that PDGF, MER, FGF2, PPARG, LXRA, TGFB1, MMP9, VEGFA, and GAS6 are the more contributing variables that segregate C1P-treated from non-treated cells. In addition, when Mo were also challenged with LTA and HA and polarization markers were considered, CD206+CD163+, CD64+CD206/CD163- percentages, CD163, CD206 and CD11b MFI and mRNA levels of MER were referred as the main variables explaining 55.6% of total variation (Dim1= 36.5% and Dim2 = 19.1%). These findings highlight that monocytes primed with a high concentration of C1P, and under inflamed tissue conditions would skew macrophages to a pro-resolving program over the inflammatory license. Integrating, C1P is a key messenger also in macrophages to promote pro-inflammatory deactivation and tissue regeneration.

SAIC – YOUNG INVESTIGATORS BIGAND AWARD
Juries: Dra. Juana Pasquini, Dr. Basilio Kotsias y Dra. Adalí Pecci

EPIGENETIC MECHANISMS UNDERLYING ASTROGLIAL HETEROGENEITY IN REACTIVE ASTROGLIOSIS: TARGETING CHROMATIN REMODELERS AS A POSSIBLE THERAPY TO REDUCE DAMAGE AFTER BRAIN INJURY

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Astrocytes respond to brain injury through a phenomenon called reactive astrogliosis in which a pro-inflammatory and pathological subpopulation of astrocyte has been described, capable of promoting neuroinflammation and neuronal death. Astrocyte pathological conversion with a pro-inflammatory gain of function involves dramatic and stable transcriptomic changes, probably following activation of transcription factor NF-kB. NF-kB interacts with chromatin remodelling enzymes and recruits them to regulatory regions of target genes promoting epigenetic changes in other cell types.
We aim to address the epigenetic mechanisms that are associated with NF-kB activation in reactive astrocytes that might lead to the establishment of an astrocyte pathological identity.
Using immunofluorescence microscopy and PCR analysis in primary cultures of mouse cortical astrocytes with different microglia abundance and exposed to pro-inflammatory stimulus LPS (Lipopolysaccharide), we observed that LPS significantly promoted: 1) Sequential NF-kB activation in microglia>>astrocytes together with morphological and transcriptional changes, 2) A variable intensity of initial NF-kB activation in astrocytes depending on microglial abundance and the release of microglial soluble factors and 3) A microglial-dependent increase in gene activating histone marks H3K9K14ac and H3K27ac and a decrease in the repressive mark H3K9me3. *In vivo* brain ischemia recapitulated the increase in H3K27ac specifically in reactive astrocytes from ischemic penumbra and inhibition of histone deacetylases exacerbated astrogliosis and brain damage.
Our results showing changes in histone mark abundance are highly indicative of chromatin remodeling events in a subpopulation of pro-inflammatory reactive astrocytes. Such epigenetic mechanisms may represent plausible therapeutic targets to reduce astrocyte pro-inflammatory phenotype, neuroinflammation and neuronal loss after brain injury.
Grants: UBACYT, FONCYT, ISN-CAEN, APBIOTECH

CROSSTALK BETWEEN ANGIOGENESIS AND IMMUNE MODULATION: HYPOXIA AS A DRIVER OF THE DIFFERENTIATION OF EXHAUSTED CD8 T CELLS.

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Hypoxia, angiogenesis, and immunosuppression are interrelated events that fuel tumor progression and blunt clinical effectiveness of therapies. During my scientific career, I focused on glycans and glycan-binding proteins as key players in neovascularization and immune responses in cancer, inflammation, and immunity.
Since 2016, I became the head of the "Glycobiology and Vascular Biology" laboratory at the IHEM-CONICET, were we explore several physio-pathological settings converging on the cellular and molecular mechanisms that link hypoxia, neovascularization, and immune responses. In this sense, we are particularly interested in generating relevant systems to study *in vitro* hypoxic-mediated neovascularization and to generate novel molecular diagnostic tools for clinical applications.
Our main areas of research include: 1) The glycome remodeling in virally mediated tumorigenesis in the context of KSHV and HIV co-infection in Kaposi Sarcoma. 2) The effect of hypoxia in inflammation-induced angiogenesis, and the dynamic regulation of intestinal glycome in inflammatory diseases. In this scenario, we also study miRNA expression as mediators of hypoxia-driven epithelial cell glycosylation.
Finally, in an attempt to reconcile seemingly opposite evidence concerning the impact of hypoxia on functional features of exhausted CD8 T cells, we investigate the fine-tuning of CD8 T cell exhaustion by hypoxia and its association with angiogenesis in the tumor microenvironment. In this sense, we found that both hypoxia and VEGF promote the differentiation of PD-1+TIM-3+CXCR5+ terminally exhausted CD8 T cells at the expense of PD-1+TIM-3- progenitor subsets. Moreover, hypoxia accentuated a proangiogenic profile in exhausted CD8 T cells, MDSCs, and hMSCs cells. Altogether, our findings highlight the reciprocal regulation between hypoxia, angiogenesis, and immunosuppression, providing a rational basis to optimize synergistic combinations of antiangiogenic and immunotherapeutic

IMPACT OF PLACENTAL ZIKA VIRUS INFECTION DURING EARLY PREGNANCY: EFFECT ON TROPHOBLAST FUNCTION, METABOLISM AND IMMUNE-TROPHOBLAST INTERACTION. POTENTIAL ANTIVIRAL EFFECT OF VIP

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Objective: Zika virus (ZIKV) infection during pregnancy is associated to fetal growth impairment and altered central nervous system development. Up to date, there is no treatment or vaccines to ameliorate fetal growth defects. We previously demonstrated that the vasoactive intestinal peptide (VIP) modulates trophoblast cell (Tb) function and metabolism at early pregnancy. Our aim is to determine the metabolism and signaling pathways altered by ZIKV in first-trimester human Tb cells, the impact on the Tb-immune cell interaction and the potential antiviral effect of VIP. **Material and Methods:** First-trimester Tb-derived cell line Swan-71 was infected with an isolated local ZIKV strain with/without VIP. Tb migration was assessed in wound healing assays and RNA expression by RT-qPCR. PBMC from healthy volunteers were conditioned with media (CM) from Tb-infected cells to analyze migration and functional profile. **Results:** ZIKV impaired Tb migration and decreased the expression of the neurotrophic factor BDNF. CM of Tb infected cells increased the recruitment of monocytes, CD4+ and NK cells and modified the activation profile of CD14+ cells favoring immune homeostasis maintenance. ZIKV infection increased Tb glucose uptake and modulated the signaling pathway of retinol inducing RIG-1 and RAR-alpha expression while a decrease of RAR-beta was detected. Interestingly, Tb cells infected in the presence of VIP produced lower infectious viral particles ($p<0.01$) along with a decrease of viral RNA in Tb cells. Moreover, VIP ameliorated ZIKV effect on Tb migration ($p<0.05$). **Conclusion:** Zika viral infection might impact early pregnancy by affecting Tb function, altering Tb metabolism and modulating Tb-leukocyte interaction thus sustaining Tb survival and virus persistence in the placenta. VIP emerges as a potential antiviral candidate to reduce the impact of ZIKV placental infection at early pregnancy since it decreases ZIKV propagation and restores Tb cell migration.

OPTIMIZING THE EFFICACY OF CURRENT THERAPIES AND DEVELOPING NEW STRATEGIES FOR ADVANCED LUNG CANCER

Mariana Malvicini

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Tumor microenvironment (TME) is a complex mix of cancer cells, soluble factors, endothelial cells, tumor-associated macrophages, immune cells, cancer-associated fibroblasts, and cancer stem cells (CSCs) surrounded by the extracellular matrix (ECM). Components of the TME interact with tumor cells in order to prolong and maintain cancer progression. Interestingly, a suppressive tumor microenvironment has been identified as one of the main mechanisms of resistance to therapies, including immune and chemotherapy. One of the current clinical challenges is to develop therapeutic approaches that effectively modulate this TME by combining agents to achieve optimal responses to treatments. We have shown that the administration of coumarin 4-methylumbilliferone (4Mu) modulates the phenotype of CSCs, a small subpopulation of cells with the capacity to self-renew and sustain tumor growth. 4Mu is capable of selectively reducing CD47 expression in these cells, eliciting their phagocytosis by antigen-presenting cells, increasing specific antitumor immunity and potentiate the efficacy of immunotherapy against hepatocellular carcinoma. For advanced lung cancer, immunotherapy alone or combined with chemotherapy has changed the course of patients with metastatic disease and its usefulness is being studied in the context of adjuvant and neoadjuvant therapy. However, it has been reported that there is a 60% of primary resistance to treatments. In this context, we study, on the one hand, the mechanisms involved in the efficacy or lack of response to these therapies and, on the other, the possibility of introducing new developments. We propose improve the efficacy of conventional chemotherapy by reversal of the resistance of CSCs involved in recurrence or lack of sensitivity to platinum-taxane therapy using 4Mu and also the use of glucose-functionalized gold nanoparticles (spheres, cylinders and stars) to induce photothermal damage in tumor cells.

HO-1 AS A THERAPEUTIC TARGET IN A RAT MODEL OF MAFLD: KEY ROLE OF KUPFFER CELLS

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Currently, metabolic dysfunction associated with fatty liver affects a quarter of the world population, but no pharmacological treatment has been recommended yet. We have previously shown that depletion of Kupffer cells (KC) in rats fed a sucrose-rich diet (SRD) for 12 weeks attenuates tissue injury and prevents liver inflammation, without changing the degree of steatosis. The aim of this study was to evaluate the effects of hemin treatment (an HO-1 inducer) on liver damage induced by SRD and identify the underlying mechanisms.
SRD-treated rats are presented with IR, hepatic steatosis, and high serum levels of NEFAS, glycemia, and triacylglycerides (TAG). Administration of hemin for the last two weeks of the dietary intervention (15 mg/kg/48h, SRD+H) did not modify these parameters, except for the observed reduction in serum TAG levels. A lower degree of ballooning (histological change compatible with injury) as well as a decrease in oxidative stress parameters (TBARS and 3-nitrotyrosine levels, SOD and catalase activities), UPR (expression of XBP1s, ATF4 and GRP78) and apoptosis (TUNEL and cleaved caspase-3 expression) were also detected in SRD-treated rats. The induction of HO-1 expression in KC by hemin was associated with lower tissue levels of IL1β, TNFα and pP65 compared to the SRD group. Induction of PEPCK as well as the response to pyruvate were blocked by hemin, that also restored the ratio pAkt/Akt altered by SRD. Finally, animals in the SRD+H group showed an increase in the expression of PPARα, CPT1α and ACOX1α (proteins involved in lipid oxidation), and an increase in pAMPK (vs. SRD). In summary, our results lead us to hypothesize that administration of hemin attenuates liver injury induced by sucrose diet by reducing the pro-inflammatory tone of the KC associated with the induction of HO-1. Moreover, hemin treatment is also able to decrease TAG serum levels by increasing lipid oxidation through the stimulation of the AMPK/PPARα pathway in the liver.

SAIC – FARYNA – RAVEGLIA AWARD
Juries: Dra. Ana Maria Eijan, Dra. Edith Kordon y Dra. Claudia Lanari

PIN-POINTING THE KEY PLAYERS IN METABOLIC REWIRING OF PROSTATE TUMOR CELLS TOWARDS PROGRESSION IN THE BONE NICHE

Pablo Sanchis[1,2], Nicolás Anselmino[3], Rosario Lavignolle[1,2], Agustina Sabater[1,2], Estefanía Labanca[3], Juan Bizzotto[1,2], Sofía Lage-Vickers[1,2], Nora Navone[3], Javier Cotignola[1,2], Elba Vazquez[1,2], Geraldine Gueron[1,2].
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Metabolic rewiring is associated with the metastatic cascade, and communication between tumor cells and the bone niche is determinant for tumor progression. Here, we sought to identify key metabolic genes that fuel prostate cancer (PCa) bone metastasis. By an indirect transwell co-culture system of PCa (PC3) and bone progenitor cells (MC3T3 or Raw264.7) we assessed the transcriptomic profile of PC3 cells modulated by soluble factors released from bone precursors. Strong activation of lipid metabolic pathways including PPAR and PI3K-Akt ($P<0.05$) was observed in PC3 cells. Next, we selected the altered metabolic genes for an unsupervised clustering analysis using transcriptomic data from human PCa and bone metastasic samples (GSE74685). Interestingly, those genes could cluster PCa patients in two defined groups: primary PCa and bone metastasis, highlighting that the early transcriptional metabolic alterations triggered in our co-culture model could discriminate primary tumors from bone metastatic samples. Further, the expression levels of four lipid associated genes (*VDR*, *PPARA*, *SLC16A1* and *GPX1*) could be independent risk-predictors of death (HR: 4.96, 2.85, 3.93 and 3.67, respectively; $P<0.05$), and that the combined expression of these four genes correlates with a worst outcome in metastatic patients (HR: 2.65, $P<0.05$) (SU2C-PCF data set). Further, we identified PKA as a master regulator of this lipid-associated signature (Ingenuity Pathway Analysis). Secretome analysis (ESI MS/MS) of conditioned media from these co-cultures revealed critical soluble factors secreted by bone progenitors (Col1a2, Fn1 and Cacna2d1) which could regulate PKA activity to promote the metabolic rewiring of PCa cells. Overall, we identified a novel lipid gene signature triggered during the communication between PCa and bone cells that appears to be critical for survival in PCa patients, pointing out to new attractive druggable targets for the disease.

GLYCOSYLATION-DEPENDENT CIRCUITS SYNCHRONIZE THE PRO-ANGIOGENIC AND IMMUNOREGULATORY FUNCTIONS OF MYELOID-DERIVED SUPPRESSOR CELLS IN CANCER

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Myeloid-derived suppressor cells (MDSCs) favor tumor progression and therapy resistance by reprogramming antitumor immunity and promoting angiogenesis. To elucidate the mechanisms that synchronize these functions, we investigated the role of glycosylation-dependent, galectin-1 (Gal1)-driven circuits in coupling immunoregulatory and pro-angiogenic activities of MDSCs. Flow cytometry and HPLC-HILIC/WAX revealed an activation-dependent glycan profile in monocytic and polymorphonuclear MDSCs (p=0.03) that controlled Gal1 binding and was more prominent in tumor microenvironments. Exposure to Gal1 led to concomitant activation of immunosuppression and angiogenesis programs in bone marrow derived MDSCs. Flow cytometry of Gal1-conditioned MDSCs showed higher expression of immune checkpoint molecules, including programmed death ligand-1 (PD-L1) (p=0.005) and indoleamine 2,3-dioxygenase (IDO) (p=0.037) and greater production of reactive oxygen species (ROS) and nitric oxide (NO) (p=0.02). *In vitro,* Gal1-conditioned MDSCs showed greater T-cell suppressive capacity (p=0.03) and higher IL-10 (p=0.04) and IL-27 (p=0.003) secretion. These effects were accompanied by enhanced endothelial cell migration, tube formation, 3D-sprouting and vascularization (p<0.05). *In vivo*, Gal1-conditioned MDSCs accelerated tumor growth (p=0.001) and fostered immune evasion and vascularization programs in Gal1-deficient colorectal tumors. Mechanistically, mass spectrometry, immunoblot and blocking assays identified the CD18/CD11b/CD177 complex as a bona fide Gal1 receptor and STAT3 as a key signaling pathway coupling these functions. Accordingly, a combined algorithm that integrates Gal1 expression and MDSC phenotype, showed critical prognostic value by delineating the immune landscape and clinical outcome of human cancers. Thus, glycosylation-dependent Gal1-driven circuits favor tumor progression by coupling immunoregulatory and pro-angiogenic programs of MDSCs via CD18- and STAT3-dependent pathways.

TRANSCRIPTOMIC STUDY REVEALS GENES AND BIOCHEMICAL PATHWAYS ASSOCIATED WITH CLINICAL EVOLUTION OF PATIENTS WITH CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is the most incident pediatric cancer. While considerable progress has been made on treatment efficacy and survival rates, about 15-30% of patients relapse and/or die. We aimed to identify gene-expression profiles in childhood ALL that could help better predict disease outcome, response to treatment and therapy-related toxicity. We collected 39 bone marrow samples at time of diagnosis of ALL from 3 hospitals from Argentina. Total RNA was isolated to perform transcriptome analysis (RNAseq). Clinico-pathological characteristics and disease outcome were evaluated and recorded by oncohematologists. We analyzed differential gene expression (DGE) and gene set variation analysis (GSVA) comparing: early response to prednisone, event-free survival, risks group, acute toxicity and minimal residual disease at day 15. We observed that about 30% of dysregulated genes were non-coding RNAs, being long non-coding RNA (lncRNA) the predominant biotype. We identified 6 differentially expressed pathways relevant to ALL biology (p<0.01) and 7 lncRNAs (MIR99AHG, LINC02866, ZNF385D-AS2, LINC02848, MYO18B-AS1, Lnc-PPDPFL-1, Lnc-RIT2-2; padj≤0.05) among ALL risk groups. Because the biological activity of most lncRNAs is still unknown and under the hypothesis that lncRNAs modulate biochemical pathways, we calculated the correlation between significant lncRNA and pathway expressions. We found that MYO18B-AS1 positively correlated with “inactivation of MAPKK activity” (r=0.4;p=0.02) and LINC02866 negatively correlated with “CXCR3 chemokine receptor binding” (r=-0.4;p=0.02) and “transmembrane receptor protein tyrosine phosphatase activity” (r=-0.4;p=0.01). This study identified dysregulated lncRNAs and biochemical pathways that might be relevant in the pathology of childhood ALL. The analysis of these gene-expression profiles at diagnosis might help improving risk stratification, therapy efficacy and reducing the occurrence of relapse and toxicity.

SAIC – MONTUORI-GADOR AWARD
Juries: Dr. Oscar Bottaso, Dra. Daniela Gardiol y Dra. Paola Finochieto

NANOBODIES WITH NEUTRALIZING PROPERTIES AGAINST SARS-COV-2 VIRUS AS PROMISING MOLECULES FOR COVID-19 TREATMENT

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The COVID-19 disease caused by the coronavirus SARS-CoV-2 is the major outbreak in the last decades. Several vaccines have been approved to prevent the disease, however therapeutic and prophylactic molecules than can mitigate its symptoms, especially in cases where vaccines are ineffective or contraindicated, are still a necessity. The virus can infect cells through the interaction of the receptor-binding domain (RBD) of its S protein with the angiotensin converting enzyme 2 (ACE2) receptor. Consequently, the S protein has become the principal target for therapeutic interventions. Llama-derived single domain antibodies or Nanobodies (Nbs) are small molecules with extraordinary affinity for different targets that can be produced at low cost. In this work we present results showing the neutralizing capacity of Nbs directed against the S protein, both *in vitro* and *in vivo*.

A llama was immunized with the pre-fusion and locked S and RBD proteins expressed in HEK-293T cells. Once high antibody titers were obtained, an Nb library was generated. More than 80 Nbs clones against S and RBD proteins were selected by phage display, 52 of them with unique sequences were expressed in *Escherichia coli* WK6 and purified by immobilized metal chelate chromatography, followed by size exclusion chromatography. Ten of those Nbs were able to prevent the transduction of pseudovirus expressing SARS-CoV-2 S protein as well as the infection of Vero cells with the wild-type SARS-CoV-2 virus strains circulating both in Argentina and in the United States of America. Preliminary results have shown that at least 3 of those Nbs are capable of neutralizing the SARS-CoV-2 isolate USAWA1/2020 in a mouse model, with protection ranging from 60 to 80% after a lethal challenge.

In conclusion, we have selected several Nbs capable of neutralizing the SARS-CoV-2 virus. The strong neutralizing activity of some of these molecules makes them potential candidates for intranasal treatment of COVID-19.

ANTIANDROGENS POSE A PROTECTIVE EFFECT AGAINST COVID-19 BY BOOSTING THE HUMAN MYXOVIRUS RESISTANCE GENE 1 (MX1)

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Population-based studies have shown that prostate cancer (PCa) patients undergoing androgen-deprivation therapies (ADT) were partially protected from COVID-19. Men treated with proxalutamide in a recent clinical trial, showed reduced COVID-19 hospitalization rate. In this work we assessed gene expression profiles and androgen regulation of the main host cell receptors described for SARS-CoV-2 and potential antiviral genes involved in response to coronavirus infection.

Multiple bioinformatics analyses were performed to study host cell receptors and antiviral proteins in SARS-CoV-2 infection and the gene expression changes upon ADT was assessed. We used publicly available datasets from: a) SARS-CoV-2 positive and negative patients' nasopharyngeal swabs at time of diagnosis (GSE152075, n=453), b) SARS-CoV-2 infected human cell lines and ferrets (GSE1407507),

c) ChIP-seq experiments evaluating androgen receptor binding (GSE66037, GSE28950, GSE108704).
Results showed that SARS-CoV-2 positive cases had higher *MX1* expression, and multivariable regression showed that *MX1* expression significantly increased with viral load. Also, *MX1* was significantly up-regulated in tracheal samples from ferrets intranasally infected with SARS-CoV-2. Similar results were found in A549 and Calu3 lung cell lines. Since ADT might result in a therapeutic advantage against COVID-19, we next evaluated *MX1* regulation by dihydrotestosterone (DHT). First, comparable *MX1* levels in lung, prostate and salivary gland of healthy humans were observed (GTEx). LNCaP cells treated with DHT showed a decrease ($p<0.05$) in *MX1* mRNA levels. ChIP-seq experiments showcased AR binding sites on the *MX1* sequence upon DHT. Further, comparison of paired PCa patient's samples before and after ADT showed *MX1* upregulation ($p<0.05$) after ADT.
In summary, *MX1* raises as a critical responder in SARS-CoV-2 infection and we demonstrate *MX1* modulation by DHT. We propose *MX1* as a key player in the therapeutic advantage posed by ADT.

THE OTHER SIDE OF COVID-19 PANDEMIC: EFFECTS ON FEMALE FERTILITY

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SARS-CoV-2 invades the target cell by binding to angiotensin converting enzyme 2 (ACE-2). In the human ovary, ACE-2 is expressed in stromal and granulosa cells.
Our objective was to evaluate the effect of SARS-CoV-2 infection on female gonad.
FF (follicular fluid) from patients undergoing ART (n= 80; 21–41 years old; November 2020-April 2021) were divided in two groups: FF from control patients and FF from recovered COVID-19 patients (asymptomatic and with mild symptoms). The levels of IgG antibodies against SARS-CoV-2, IL-1β, IL-10 and VEGF were measured in FF by ELISA.
Using a granulosa cell line (COV434) and an endothelial cell line (EA.hy926), we studied the effect of FF from control and recovered COVID-19 patients. The expression of StAR, ERα and ERβ, 3β-HSD, VEGF, ANGPTs (angiogenesis-related proteins) and γH2AX (DNA damage marker) was evaluated by WB. Proliferation was evaluated by a WST-1 assay. Endothelial cell migration was evaluated by a wound healing assay. We performed Student's t test or one-way ANOVA.
The results showed that 91.3% of post-COVID-19 FF was positive for IgG against SARS-CoV-2. Patients with higher levels of SARS-CoV-2 IgG showed a decrease in the number of retrieved oocytes ($p<0.05$). The levels of VEGF and IL-1β were lower ($p<0.05$) in post-COVID-19 FF, while IL-10 did not differ.
In COV434 cells with post-COVID-19 FF, the expression of StAR, Erβ and VEGF was decreased ($p<0.05$), while ERα and 3β-HSD did not change.
In EA.hy926 cells with post-COVID-19 FF, a decrease in cell migration was observed ($p<0.0001$) without changes in the expression of ANGPTs. Both cell types showed higher expression of γH2AX with post-COVID-19 FF ($p<0.05$). No differences were found in COV434 and EA.hy926 cell proliferation rates between the groups.
In conclusion, these results describe that SARS-CoV-2 infection alters the follicular microenvironment, damaging ovarian function, and affecting reproductive performance in recovered COVID-19 patients.
This project that involves the use of human samples from assisted fertilization techniques has been approved by the IByME Ethics Committee in 2020 (REGISTRATION CODE 2850, October 2020).
This project was carried out between January and June 2021.

SAIC – REPETTO AWARD

Juries: Dr. Eduardo Cuestas, Dra. Paula Dominguez y Dr. Ramón Exeni

EARLY ANTIPARASITIC TREATMENT PREVENTS PROGRESSION OF CHAGAS DISEASE: RESULTS OF A LONG-TERM CARDIOLOGICAL FOLLOW-UP STUDY IN A PEDIATRIC POPULATION

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Objective: To evaluate cardiac involvement in children after pharmacological treatment for Chagas disease (CD).

Methods: A descriptive study of a cohort of pediatric CD patients treated with benznidazole (Bz) or nifurtimox

(Nf) was conducted by convenience sampling, 95 children with at least 6 years post-treatment follow-up and who attended a clinical visit between August 2015 and November 2019 were invited to participate in the study. They were evaluated with 24-hour Holter monitoring and speckle-tracking 2D echocardiogram (STE). As a control of the incidence of ECG non pathological findings a group of non-infected people were included.
Results: In enrolled treated patients: 24-hour Holter showed alterations in 3/95 (3%) patients, but only one was considered probably related to CD involvement. This patient presented a complete right bundle branch block (cRBBB). No contractility damage was found in 79/95 (83%) patients evaluated by STE.
In non-infected cardiological control group: 24-hour Holter showed alterations in 3/28 (10%) patients. No contractility damage was found in 25/28 patients evaluated by STE.
Benznidazole was prescribed in 87 patients and nifurtimox in 8 patients. Baseline parasitemia data was available for 65/95 patients. During follow-up, 59/61 (96%) treated patients achieved constant negative parasitemia evaluated by qPCR. A decrease in T.cruzi antibodies titers was observed and seroconversion occurred in 53/95 (56%) treated patients. These results showed a good efficacy of treatment in parasite clearance.
Conclusions: A good treatment response with a low incidence of cardiological lesions related to CD was observed. This suggests a protective effect of parasiticidal treatment on the development of cardiological lesions and highlights the importance of early treatment of infected children.

AGENTES ANTIMICROBIANOS Y ANTIPARASITARIOS

1. (035) **INHIBITION OF BACTERIAL ADHERENCE TO VASCULAR CATHETER OF THE ANTIMICROBIAL PEPTIDE AP7121**

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Healthcare-associated bloodstream infections are the leading cause of morbidity and mortality in hospitalized patients. Vascular catheter-related infection is its main source. Gram-positive bacteria specially *Staphylococcus* spp., are the most prevalent etiological agents. Bacterial adherence is the starting point of colonization of a surface known as biofilm development. The inhibition of this process is the primary strategy to prevent bacteremia related to medical devices. The aim of this work was to assess the inhibitory activity of the antimicrobial peptide AP7121 on the *Staphylococcus aureus* adherence in vascular catheters. The biofilm-producer strain *Staphylococcus aureus* ATCC 35556 (SA) was used. First, The MIC of AP7121 (MIC_{AP}) for SA was estimated. Upon, 20 mm segments of vascular catheter (n=3) were inoculated with 10^4 CFU/mL of SA. Three different treatment schemes (A: simultaneous, B: previous and C: following bacterial challenge) using 1 x MIC_{AP} were tested. Control groups were included in each scheme. Statistical analysis was made using ANOVA and Kruskal-Wallis test (p <0.05 was considered statistically significant). The MIC_{AP} was 0.48 mg/L. A significant bacterial reduction ($p < 0.001$) of 2 logarithms representing a decrease of 99% of viable SA cells was achieved with schemes A and B. The post-challenge treatment with AP7121 (scheme C) produced a significant reduction ($p < 0.01$) of 1 logarithm representing a decrease of 90% of viable SA cells. The results observed in this work suggest a fast antimicrobial activity of AP7121 that could be beneficial to reduce bacterial adherence on medical devices such as vascular catheter and potential decreasing bloodstream infections -associated. *In vivo* studies are needed to establish the feasibility of this proposal.

2. (043) **METFORMIN INDUCES AUTOPHAGY AND UPR UNDER INTRACYSTIC GLUCOSE DEPRIVATION IN IN VITRO METACESTODES AND IN VIVO EXPERIMENTAL MODELS OF *ECHINOCOCCUS GRANULOSUS***

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Metformin (Met) is an experimental antiechinococcal drug (Loos et al., 2017; 2020), which inhibits the complex I of the respiratory chain in *in vitro Echinococcus* larval stage, resulting in an indirect AMPK activation (by an increased ADP/ATP ratio due to ATP drop) and a subsequent inhibition of TOR signaling with autophagy induction (Loos et al., 2015, 2018). Here, we demonstrated that Met mediated the dephosphorylation of Eg-TOR in Ser^{3122}, which trigger the reduction of its activity in metacestodes, responding also to exogenous insulin and rapamycin. Moreover, Met induced the overexpression of TFEB, a hub transcription factor that regulates lysosome biogenesis, lipid catabolism and autophagy in metazoa. In concordance with the mitochondrial depolarization induced by Met, an increase in total cellular Pi (an activator of PFK1) was detected, with stimulation of glycogen consumption (glycogenolysis) and, increase of glycolysis and the lactate fraction. Cumulatively, this supports the reduction in the glucose intracystic content demonstrated *in vitro*, as well as in two different experiments (50 and 250 mg/kg/day of Met) in the echinococcosis murine model. In this line of evidence, the energy starvation induced by Met causes endoplasmic reticulum stress-mediated unfolded protein response (UPR). In presence of a mannose-specific lectin (Helja-FITC) and using confocal microscopy, we found that parasite cells expanded their ER volume at least 3-fold under treatment with Met. Using thapsigargin, tunicamycin and Met-treated parasites, we validated the IRE activity by XBP1 splicing and demonstrated that Met induced the mRNA of Bip/Grp78d. In this context, we described that UPR induced after Met-treatment can take place upon glucose deprivation in the parasite, suggesting that the anthelminthic effects of Met result from sustained autophagy mediated by activation of the AMPK-TOR-TFEB signaling pathway interdependent with UPR activation, especially through the IRE/XBP arm.

3. (063) **DISPENSE OF ANTIBIOTICS IN ASSOCIATION WITH FIXED DOSES IN A PHARMACY OF AN UNIVERSITY SOCIAL SECURITY INSTITUTE OF CORRIENTES, 2020**

María Teresa Rocha[1], Sergio Daniel Morales[1], Valeria Burgos[1], Mirta Liliana Mierez[1], María Mercedes González[2], Lorena Dos Santos Antola[1].

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Antibiotics (ATB) are essential medicines for human health, but their massive and indiscriminate use increases the development of resistance. At the same time, in the pharmacological market they are offered in associations at fixed doses (AFD) that are not always rational, which exposes the patient to a greater risk of having adverse effects. The objective of this study was to characterize ATB in the form of AFD dispensed on an outpatient basis in an University Social Security Institute, during the year 2020. An observational, descriptive, cross-sectional study of drug use (SDU) was carried out. The dose unit (DU) was used as a quantitative indicator of outpatient dispensing and the Potential Therapeutic Intrinsic Value (PTIV) as a qualitative indicator, methodologies recommended by Laporte and Tognoni. Of a total of 1,364 outpatient dispensations of ATB, 164 (12%) were AFD, 56% for the female sex. Average age: 42 years; range: 2 to 79 years. The 164 AFD contained a total of 2356 DU, corresponding to amoxicillin + ambroxol (516 DU), norfloxacin + phenazopyridine (350 DU), clarithromycin + ambroxol (338 DU), amoxicillin + acetylcysteine (336 DU), amoxicillin + clavulanic acid + ambroxol (336 DU), amoxicillin + diclofenac (280 DU), ampicillin + dipyrone + guaifenesin (200 DU). Qualitatively, 114 (69.51%) had relative PTIV and 50 (30.48%) had unacceptable PTIV. These findings require special attention, because the consumption of this type of AFD is considered irrational according to the Laporte and Tognoni classification; generates potential risk of the appearance of adverse events and pharmacological interactions, increases health costs and their indiscriminate use can become a risk factor for the development of bacterial resistance, a current scourge that gives rise

to numerous global initiatives and campaigns aimed at its solution.

4. (183) ***CANDIDA ALBICANS* CELL WALL PHOSPHOMANNANS PLAY A ROLE IN THE SUSCEPTIBILITY TO AN ANTIFUNGAL MANNOSE-BINDING LECTIN**

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Candida infections constitute a threat for immunocompromised individuals, due to the limited repertoire of drugs for its efficient treatment and the increased emergence of antifungal resistant strains. We have previously isolated a sunflower mannose-binding lectin (Helja) with antifungal activity against *C. albicans,* which is mediated by its interaction with the outer layer of fungal cell wall enriched in mannosylated glycoproteins. This work aimed to investigate the role of the phosphomannans in mediating the fungicidal action of Helja. We have used a *C. albicans* glycosylation defective in cell wall phosphomannosylation (mnn4null), which is correlated with a concomitant reduction in the surface negative charge, to compare the antifungal effect of Helja relative to the wild type strain (wt).
Yeast cells of both strains were incubated in the presence of Helja to follow optical reading at 630 nm and assess fungal growth. Helja inhibited mutant and wt fungal growth in 70% and 25% respectively. Microscopic analysis of the mutant yeast treated with Helja revealed the loss of cell viability and differentials morphological alterations compared to wt, such as loss of the typical oval yeast form, formation of clusters with agglutinated cells and vacuolar collapse. These results suggest that Helja could establish stronger interactions with the cell wall of the mnn4null mutants than with the wt strains, in which the presence of a greater amount of surface negative charge would display a repulsion effect with the anionic lectin (PI 4.65). In conclusion, our results provide new evidence on the nature of the molecular interactions of Helja with the outer mannoproteins and its impact on the antifungal activity, highlighting the relevance to elucidate the mode of action of new natural compounds for effective antifungal therapies design.

5. (218) **LIPOSOMAL FORMULATION FOR TOPICAL APPLICATION IN PHOTODYNAMIC THERAPY AGAINST CUTANEOUS LEISHMANIASIS**

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Cutaneous leishmaniasis (CL) is an endemic parasitic disease in northern Argentina. It does not heal spontaneously, and after prolonged and painful treatments the disease can evolve into a disfiguring and disabling form. The present work aims to obtain a liposomal formulation for topical application for photodynamic therapy (PDT) against CL. The formulation will allow the transport of methylene blue to the viable epidermis where the infected cells are located, to then activate it with light in such a way as to produce site-specific toxicity.
Ultradeformable liposomes, composed of soy phosphatidylcholine and sodium cholate, loaded with methylene blue, were obtained by means of traditional methods. The suspension was characterized physicochemically by dynamic light scattering and zeta potential. Encapsulation efficiency, drug-lipid ratio, and oxygen consumption as an indicator of ROS production after photoactivation were also determined. The cytotoxicity, both intrinsic and after photoactivation, of different concentrations of the formulation was determined by MTT in the cell line HaCaT. The photoactivation was carried out with a red LED light source emitting around $\lambda = 600$ nm.
In the future, the non-cytotoxic concentrations after photoactivation will be employed to assess the antiparasitic activity against promastigotes of *L. braziliensis.*
In conclusion, it is expected to contribute with alternatives therapies against this neglected disease.

6. (250) **THE USE OF LIGHT AS AN APPROACH TO CONTROL THE GROWTH OF *STAPHYLOCOCCUS AUREUS* RESISTANT STRAINS**

Tomás Roberto Sebastián[1], Quiroga Ezequiel[1], Di Venosa Gabriela[1], Buzzola Fernanda[2], Casas Adriana[1], Mamone Leandro[1].
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Photodynamic inactivation (PDI) combines the action of a photosensitizer with visible light, tocause microorganisms inactivation. The main advantage of PDI is its effectiveness in bacterial strains resistant to antibiotics.The 5-aminolevulinic acid (ALA) is a precursor in endogenous biosynthesis of porphyrins, some of which have photosensitizingactivity.
The aim of this work was to employ ALA-based PDI to eliminate planktonic and biofilms cultures of five methicillin-sensitive (MSSA) and six -resistant (MRSA) *S. aureus* strains.
Bacterial viability and porphyrin production were evaluated through colony count and fluorescence spectroscopy, respectively. PDI light source was an array of Tungsten lamps (90J/cm^2).
Viability of *S. aureus* planktonic cultures was reduced around 1-log after ALA-PDI treatment with 1 mM ALA. As a strategy to increase the outcome of PDI, a more lipophilic derivative of ALA, the hexyl ester ALA (H-ALA), was used. H-ALA (0.5 mM) induced bacterial viability reduction up to 4-logs in all strains tested.
The biofilms of RN6390 (MSSA) and ST5-SCCmecI (MRSA, frequently involved in nosocomial infections) were less sensitive to PDI than their planktonic counterparts. In both strains, it was necessary to perform PDI with 2 mM ALA or H-ALA to reduce biofilms viability by 0.5 and 0.75 logs, respectively.
To improve the effects of PDI on biofilms, we proposed a near-infrared laser treatment (NIRT) prior to performing PDI. NIRT thermal effects are capable ofinducing changes on biofilm structure. By this strategy, the viability of RN6390 and ST5-SCCmecI biofilms was reduced by 3 and 4-logs, respectively. NIRT induced increase in porphyrin synthesis from ALA or H-ALA as well as detachment of bacteria.
We conclude that PDI is a promising alternative to eliminate MRSA strains of sanitary relevance. In addition, we verified the synergistic effect of two radiation-based bactericidal strategies.

7. (257) **THERAPEUTIC EFFICACY OF NOVEL MEBENDAZOLE FORMULATIONS ADMINISTERED DURING THE PARENTERAL STAGE OF *TRICHINELLA SPIRALIS* INFECTION TO CBI-IGE MICE SUSCEPTIBLE TO THE PARASITE**

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Treatment of trichinellosis in the chronic phase is critical, as existing treatments may not eliminate the parasite once the larvae establish in muscle cells. Repeated or prolonged treatments are usually required that entail a higher probability of adverse effects. Meben-

dazole (**MBZ**), a low-cost, broad-spectrum benzimidazole widely used against intestinal parasites, is insoluble and poorly absorbed, which could be related to variable clinical results. Two novel MBZ formulations were designed to improve its solubility and absorption rate, a nanoparticulate system (**Np**) and an inclusion complex with β-cyclodextrin citrate (**Comp**). This research aimed to evaluate the *in vivo* anthelmintic activity of the systems compared with pure MBZ, against the encysted parasite, in the parenteral phase of infection. Adult mice of both sexes of the susceptible CBi+ line (CBi-IGE stock) were orally infected with 2 *Ts* L1 infective larvae/g bw (n=6 per treatment, per sex). Animals were non-treated (controls, **C**) or treated with a daily oral dose of **MBZ**, **Np,** or **Comp** (15 mg MBZ/kg bw) on days 27, 28, and 29 post-infection. Mice were euthanized seven days after the last dose to estimate larval muscle load (number of L1 larvae/g fresh tissue, rLL) and larvae reduction rate (LRr, %). No significant differences were observed between males and females in any of the groups. **MBZ** did not show an effective antiparasitic activity since rLL of mice treated with the pure drug did not differ from the controls (mean±SEM, **C**: 891±118.0; **MBZ**: 1107±155.3). The formulations produced a significant decrease in the parasite load (**Np**: 97±25.3; **Comp**: 69**±9.5), compared with that of the controls (P<0.002) or the** MBZ-treated group (P<0.0004). Accordingly, LRr was 0 % for **MBZ**, 82 % for **Np**, and 91 % for **Comp**-treated mice. The therapeutic efficacy achieved by the formulations suggests that both preparations would allow the use of lower doses of the antiparasitic, thus reducing the possible toxic effects of the treatment.

8. (316) **DISPENSE OF ANTIBIOTICS IN ASSOCIATION WITH FIXED DOSES IN A PHARMACY OF AN UNIVERSITY SOCIAL SECURITY INSTITUTE OF CORRIENTES, 2020**

María Teresa Rocha[1], Sergio Daniel Morales[1], Valeria Burgos[1], Mirta Liliana Mierez[1], María Mercedes González[2], Lorena Dos Santos Antola[1].

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Antibiotics (ATB) are essential medicines for human health, but their massive and indiscriminate use increases the development of resistance. At the same time, in the pharmacological market they are offered in associations at fixed doses (AFD) that are not always rational, which exposes the patient to a greater risk of having adverse effects. The objective of this study was to characterize ATB in the form of AFD dispensed on an outpatient basis in an University Social Security Institute, during the year 2020. An observational, descriptive, cross-sectional study of drug use (SDU) was carried out. The dose unit (DU) was used as a quantitative indicator of outpatient dispensing and the Potential Therapeutic Intrinsic Value (PTIV) as a qualitative indicator, methodologies recommended by Laporte and Tognoni. Of a total of 1,364 outpatient dispensations of ATB, 164 (12%) were AFD, 56% for the female sex. Average age: 42 years; range: 2 to 79 years. The 164 AFD contained a total of 2356 DU, corresponding to amoxicillin + ambroxol (516 DU), norfloxacin + phenazopyridine (350 DU), clarithromycin + ambroxol (338 DU), amoxicillin + acetylcysteine (336 DU), amoxicillin + clavulanic acid + ambroxol (336 DU), amoxicillin + diclofenac (280 DU), ampicillin + dipyrone + guaifenesin (200 DU). Qualitatively, 114 (69.51%) had relative PTIV and 50 (30.48%) had unacceptable PTIV. These findings require special attention, because the consumption of this type of AFD is considered irrational according to the Laporte and Tognoni classification; generates potential risk of the appearance of adverse events and pharmacological interactions, increases health costs and their indiscriminate use can become a risk factor for the development of bacterial resistance, a current scourge that gives rise to numerous global initiatives and campaigns aimed at its solution.

9. (319) **ANTHELMINTIC ACTIVITY OF *STEVIA ARISTATA* EXTRACT ON *ECHINOCOCCUS GRANULOSUS*: *IN VITRO* AND *IN VIVO* STUDY**

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Cystic echinococcosis (CE) is a worldwide zoonotic disease caused by *Echinococcus granulosus*, which produces long-term infections in humans and animals. Available anti-parasitic treatment against CE is mostly limited to the use of benzimidazoles, mainly albendazole (ABZ). However, it has undesirable side effects and their efficacy is about 50%. Based on the problematic described, new treatment alternatives are urgently needed. Plants from the *Stevia* genus (Asteraceae) are a potential source of anti-protozoal and anti-microbial compounds. The aim of the present study was to evaluate the *in vitro* and *in vivo* efficacy of the *Stevia aristata* dichloromethane extract against *E. granulosus.* Viable and free protoscoleces or cysts were treated with 100, 50, 10 and 5 µg/ml of the extract. Viability assessment using the methylene blue exclusion test and scanning electron microscopy (SEM) (for protoscoleces) or evaluation of germinal layer collapse (for cysts) was performed. CF-1 mice (n=30) infected with *E. granulosus* were allocated into the following experimental groups (6 months post-infection): (1) Control, (2) ABZ 25 mg/kg, every 24 h for 30 days; (3) *S. aristata* 50 mg/kg, every 24 h for 23 days. At the end of the treatment the weight of the cysts was recorded and samples were analysed by SEM. Protoscoleces viability decreased quickly with 100 *µ*g/ml, reaching 0% after 20 days of treatment. After 4 days of incubation, the collapse of the germinal layer was observed in 60 ± 5.8% and 83.3 ± 12.0% of cysts treated with 50 and 100 *µ*g/ml, respectively. Whilst ultrastructural damage was observed in the cysts obtained from *S. aristata* or ABZ treated mice, no significant differences in the weight of the cysts were obtained (P > 0.05). In conclusion, *S. aristata* treatment caused high protoscolicidal and cysticidal effects, but not significant reduction in the weight of the cysts in experimentally infected mice.

10. (366) **NANOBODIES WITH NEUTRALIZING PROPERTIES AGAINST SARS-COV-2 VIRUS AS PROMISING MOLECULES FOR COVID-19 TREATMENT**

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The COVID-19 disease caused by the coronavirus SARS-CoV-2 is the major outbreak in the last decades. Several vaccines have been approved to prevent the disease, however therapeutic and prophylactic molecules than can mitigate its symptoms, especially in cases where vaccines are ineffective or contraindicated, are still a necessity. The virus can infect cells through the interaction of the

receptor-binding domain (RBD) of its S protein with the angiotensin converting enzyme 2 (ACE2) receptor. Consequently, the S protein has become the principal target for therapeutic interventions. Llama-derived single domain antibodies or Nanobodies (Nbs) are small molecules with extraordinary affinity for different targets that can be produced at low cost. In this work we present results showing the neutralizing capacity of Nbs directed against the S protein, both *in vitro* and *in vivo*.
A llama was immunized with the pre-fusion and locked S and RBD proteins expressed in HEK-293T cells. Once high antibody titers were obtained, an Nb library was generated. More than 80 Nbs clones against S and RBD proteins were selected by phage display, 52 of them with unique sequences were expressed in *Escherichia coli* WK6 and purified by immobilized metal chelate chromatography, followed by size exclusion chromatography. Ten of those Nbs were able to prevent the transduction of pseudovirus expressing SARS-CoV-2 S protein as well as the infection of Vero cells with the wild-type SARS-CoV-2 virus strains circulating both in Argentina and in the United States of America. Preliminary results have shown that at least 3 of those Nbs are capable of neutralizing the SARS-CoV-2 isolate USAWA1/2020 in a mouse model, with protection ranging from 60 to 80% after a lethal challenge.
In conclusion, we have selected several Nbs capable of neutralizing the SARS-CoV-2 virus. The strong neutralizing activity of some of these molecules makes them potential candidates for intranasal treatment of COVID-19.

11. (441) **ROL OF LACTOBACILLUS PLANTARUM IN THE TREATMENT OF METICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS INFECTION IN DIABETIC FOOT ULCERS**

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Introduction: Diabetic foot infections and delayed wound healing are suggested as the major therapeutic difficulties, due to the increasing global antimicrobial grugs resistance issues. In previous studies we have shown that topical application of *Lactobacillus plantarum* (Lp) cultures favored the healing process . In this work we investigated the effect of Lp on the microbicidal activity of polymorphonuclear cells (PMNs) infected with Staphylococcus aureus (*S. aureus*).
Material and methods: Diabetic patients with ulcers were treated with a topical application of Lp. Biopsies were taken at day 0, 10 and 30 post- treatments. Histopathological and bacteriological studies were performed. At the same time, circulating blood polymorphonuclear cells (PMNs) were isolated from patients. PMNs were stimulated with Lp and culture supernatant to perform the following tests: phagocytosis by flow cytometry, NETosis by fluorometry and microbicial activity. Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) isolated from the ulcer was used as infectious strain in all tests. The effect of Lp on the biofilm and cell viability of *S. aureus* was measure.
Results: PMNs from healthy patients was used as control group. Increased phagocytic activity of PMNs was observed in healthy patients only stimulated with LPS (230 ifm vs 402 ifm p <0.001). The same was also observed for diabetic PMNs (210 ifm vs 460 p <0.001). The PMNs of healthy patients showd greater induction of NETosiss (4000 RFU) vs PMN of diabetics (2000 RFU). A significant increase in NETosis (6000 RFU) and microbicial activity of PMNs of diabetics stimulated with Lp or with supernatant was observed. *S. aureus* were able to produce biofilm. The effect of Lp on mature biofilm, showed significant differences in terms of biomasa reduction when compared to the controls.
Conclusions: The treatment with Lp improve wound healing, suppressing of *S. aureus* infection at wound sites and promoting host innate immunity.

12. (487) **ALOE VERA, A NATURAL GROWTH INHIBITOR OF *C. DIFFICILE***

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Aloe Vera (AV) gel exhibits antimicrobial and anti-inflammatory properties, making it an alternative therapy for intestinal disbiosis. The inner leaf gel contain active compounds with prebiotic activity on beneficial microbiota, while others compounds inhibit the growth of pathogenic bacteria.
Clostridioides difficile (*C. difficile*), is a Gram-positive bacillus, anaerobic, spore-forming, that constitutes the major cause of hospital-acquired diarrhea, often in association with previous antibiotic use. *C. difficile* infection (CDI) treatment is based on oral administration of metronidazole and vancomycin. The emergence and spread of *C. difficile* isolates resistant to multiple antibiotics, especially the hypervirulent ribotype 027 strains, are becoming an increasing problem for CDI treatment.
We evaluate the AV effect on *C. difficile* growth, and its impact in combination with metronidazole and vancomycin. The antibacterial activity of AV was determined by broth microdilution assays using the hypervirulent *C. difficile* (027/BI/NAP1) strain. The bacteria were cultured in anaerobiosis for 48h, seeded in triplicates in 96 microwell plates in the presence of AV ± antibiotics. After 48h, *C. difficile* growth was determined in a microplate reader at 600nm. Two varieties of AV were used, *Aloe barbadensis Miller* and *Aloe saporina*, in final concentrations of 1, 5, 10, 20, 25 and 30%. The minimal inhibitory concentration (MIC) for metronidazole and vancomycin was calculated and the AV gel was used in combination with 0.25, 0.5, 1 and 2μg/ml of each antibiotic. The results indicated that AV gel inhibits *C. difficile* growth ($p<0.01$) and significantly reduces the MIC of metronidazole and vancomycin ($p<0.05$). This first evidence positions AV as a potential promising combination therapy against *C. difficile,* reducing the concentration of antibiotics treatment and the detrimental consequences on the beneficial microbiota. More studies are underway to strengthen this hypothesis.

BIOINFORMÁTICA, GENOMA, PROTEOMA Y BLANCOS TERAPÉUTICOS

13. (065) **CHARACTERIZATION OF THE ORAL AND ANAL MICROBIOME OF MEN WHO HAVE SEX WITH MEN (MSM) AND TRANSGENDER WOMEN (TGW) IN ASSOCIATION WITH HIV INFECTION.**

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MSM and TGW are highly affected by HIV in Argentina. **Aim** to provide the first microbiome portrait of oral and anal mucosa from a cohort of HIV- and HIV+ MSM and TGW from Argentina. **Methods** Eighty samples of DNA derived from 40 oral swabs (OS) & 40 anal

swabs (AS) were provided by 45 participants. Shotgun metagenomics sequencing was conducted with the Illumina NovaSeq 6000 System. NGS data analysis was performed using CosmosID, Human3, and R/Bioconductor. **Results** In AS *HPV types* were among the most frequent virus, while *Prevotella copri* and *Prevotella corporis* were among the most abundant bacteria. OS were characterized by the prevalence of *KSHV*, *EBV*, *HSV-2* and *HHV-7*, among others; while species of *Haemophilus*, *Rothia*, and *Neisseria* were the most frequent bacteria. Comparisons between HIV+ and HIV-, considering MSM and TGW, together or separately, indicated a distinctive set of differentially abundant taxa for each comparison (LogFC>1.5, p<0.001). In OS *KSHV* was predominantly abundant in HIV+ patients independently of sexual orientation. AS of HIV+ patients showed enrichment mainly in *HPV* types. For bacteria, species of *Prevotella*, *Leptotrichia*, *Veillonella*, *Fusobacterium, Dialister* were significantly abundant in HIV+ patients independently of the condition MSM or TGW. Next, we analyzed the functional profiling to describe the metabolic potential of the microbial community in a multivariable association between phenotypes and microbial features. Distinctive pathways (n~200) defined OS and AS (q < 0.05). Moreover, we identified differential pathways (q<0.05) associated with HIV condition and anal intraepithelial lesions, such as *ADP-L-glycero-beta-D-mannoheptose* and *preQ0 biosynthesis,* respectively. **Conclusion** Our results reinforce the occurrence of oncogenic viromes in this high HIV-risk population and show that metabolic pathways generated by bacteria associated with HIV-infection could modulate environmental conditions related to inflammation and carcinogenesis.

14. (066) **IDENTIFICATION OF LONG NON-CODING RNAS ASSOCIATED TO THE CMS MOLECULAR SUBTYPES AS PREDICTIVE BIOMARKERS OF RESPONSE TO THERAPY IN COLORECTAL CANCER.**

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The consensus molecular subtypes (CMSs) of colorectal cancer (CRC) define tumor heterogeneity at the gene-expression level. The clinical utility of the CMS classification resides in the possibility to estimate survival (prognostic value) and select patients for both chemotherapy and currently used targeted agents (predictive value). Long Non-coding RNAs (lncRNA) have been largely associated with cancer constituting an essential approach for the search and identification of these biomarkers. **Aim**: To identify lncRNA signatures with prognostic and predictive value associated with each CMS subtype. **Methods**: We performed an integrative bioinformatics analysis on GDC-TCGA-CRC dataset (n=674), considering CMS and SFM based-classification that discriminate tumor microenvironment and drug sensitivity. We first classify tumors in CMSs. We annotated lncRNAs (n=14084) and applied DESeq2 for the comparison of each CMS (CMSk) versus the rest (CMSK-k; pvalue <0.01, LogFC> 1). The obtained lists of the up-modulated lncRNAs exclusive of each CMS were evaluated according to CRC molecular features; the immune, stromal or epithelial tumor component; and the prognostic and predictive value (p<0.05). Furthermore, we evaluated their association to systemic and targeted therapies (SFM) and the potential to be detected in peripheral blood of CRC patients. **Results**: we identified lncRNAs that recapitulate the intrinsic features of the CMS: lncRNA-CMS1 associated with poor prognosis, immune component, resistance to chemotherapy and response to anti-EGFR therapies. LncRNA-CMS2/CMS3 associated with good prognosis, epithelial component and response to anti-EGFR/VEGF. LncRNA-CMS4 showed high expression in mesenchymal-like tumors with poor prognosis but responsive to traditional chemotherapies. Many of these lncRNAs are detected in peripheral blood of CRC patients. **Conclusion**: CMS-lncRNA signatures predictive of therapy response constitute valuable biomarkers to be assessed in preclinical models.

15. (078) **BIOINFORMATIC CHARACTERIZATION OF IMMUNE CELL TYPES IN THE BONE MARROW OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS FROM ARGENTINA THROUGH ANALYSIS OF TRANSCRIPTOME DATA**

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Acute lymphoblastic leukemia (ALL), the most incident pediatric cancer, is characterized by the overproduction of immature lymphoid blasts in the bone marrow (BM). While considerable progress has been made on treatment efficacy and survival rates, about 15-30% of patients relapse and/or die. Immunotherapies are promising as complementary treatments to chemotherapy, yet the most relevant therapy targets and patient subsets remain unclear. *Aims*: To characterize immune cell types in the BM microenvironment of ALL samples using predictive bioinformatic tools on transcriptome data. *Methods*: we performed RNA-seq on BM samples collected at ALL diagnosis (N=32). The proportion of immune cells was estimated using MIXTURE through two gene expression signatures (LM22, TIL10). A “cytolytic score” reflecting $CD8^+$ cytotoxic T lymphocytes (CTLs) and Natural Killer cells (NK) abundance was calculated as the geometric mean of 5 genes specifically expressed in CTLs/NK. Gene set enrichment analysis using ImmuneSigDB and Reactome was performed with GSVA package in R. *Results*: Relative proportions of B and T cells were concordant with B- and T-cell ALL subtype, respectively. Cytolytic score was positively correlated with $CD8^+$ T cells and NK proportions (Spearman Rho>0.38, p-val<0.05). Higher $CD8^+$ T cell and NK could be associated with worse event-free survival (hazard ratio=5.39, 95%CI: 0.64-44.98, CoxP p-val=0.08). 12.5% (4/32) of samples showed a cytolytic Z-score > 1, and half of them relapsed or died. Gene set enrichment analysis for >1 vs. <1 cytolytic Z-score resulted in statistically significant enrichment in genesets related to activation of $CD8^+$ T cells, immune cell trafficking, and BM niche signaling. *Conclusions*: we identified a subset of B-ALL patients with increased abundance of $CD8^+$ T cells/NK, suggesting potential candidates for immunotherapies. Given the small sample size, these observations should be confirmed in additional patients.

16. (135) **GENOMIC ANALYSIS OF CLUSTER *FX-MIR*: POSSIBLE IMPLICATIONS IN FRAGILE X-ASSOCIATED DISORDERS**

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The Fragile X Mental Retardation-1 (*FMR1*) gene consists of 17 exons spanning 38 kb of the Xq27.3 chromosome and codes for the protein fragile X mental retardation protein (FMRP).
FMR1 is involved, by different molecular mechanisms, in 3 genetic disorders. The absence of FMRP due to an expansion of > 200 CGG repeats in the 5′UTR of the gene (full mutation), is responsible for the Fragile X syndrome (FXS) while the premutation state is associated with the Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) and Fragile X-associated Primary Ovarian Insufficiency (FXPOI).
Recently, a microRNA (miRNA) cluster adjacent to *FMR1* (*Fx-mir*) has been described in placental mammals and it has been shown that a number of miRNAs in the cluster target *FMR1* in human and mouse, regulating its expression. In this work we described the *Fx-mir* cluster and the putative targets of its miRNAs in *Rattus norvegicus* (rat). In particular, we were interested in studying whether some of the *Fx- mir* miRNAs target *Fmr1* in the rat as well.
We used public access databases and performed a reciprocal best

hit sequence identity analysis using the human *Fx-mir* miRNAs as bait in order to find orthologous miRNAs in the rat. We found a total of 18 miRNAs located in the *Fx-mir* cluster in rat and among them, 8 with a conserved seed region and chromosome location between both species.
Next, to study putative targets of *Fx-mir* miRNAs, we searched for miRNAs predicted to target *Fmr1* using the gene of interest as input in specialized target finding softwares. We also made the reverse search, finding every possible target for each miRNA of *Fx-mir*. We found that miR-880 is a possible regulator of *Fmr1*.
Finally, we extended the search to all of the X chromosome genome sequence, finding 8 more candidates predicted to target *Fmr1* that might be of interest.

17. (155) **ALTERATIONS IN THE INTERFERON PATHWAY WITHIN COVID-19 INFECTION**

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus that emerged in late 2019 in Wuhan, China. Although much attention has been placed in virus host cell receptors, little has been described about the anti-viral proteins. It is well accepted that type I interferon (IFN) is essential in fighting viral infection by induction of IFN-stimulated genes (ISGs), which work in synergy to inhibit viral replication via multiple mechanisms. In this work, we aimed at evaluating the expression profiles of several genes associated with the IFN pathway in response to the infection with SARS-CoV-2 in COVID-19 positive patients vs. COVID-19 negative patients. We performed bioinformatics analyses in a case-control study from SARS-CoV-2 positive (n=403) and negative (n=54) patients. Samples were obtained from nasopharyngeal swabs. We analyzed the differential expression of the IFN-associated genes alongside with their correlation with other clinical parameters such as age, sex and viral load. Results show a significant downregulation of IFGNR1, STAT6, JUN, MAP3K1, CEBPB, and RAPGEF1 in COVID-19 positive patients. We also found a significant correlation between most of the genes and the viral load. No significant differences were observed between gene expression and age or sex. In summary, our study findings support the role of IFN and IFN-associated genes in SARS-CoV-2 infection, pointing out to new potential drugable targets in order to achieve a better anti-viral response.

18. (177) **COMBINED ACTIVITY OF IVERMECTIN PLUS ATORVASTATIN ON NUCLEAR LOCALIZATION OF IMPORTIN ALPHA AND THERAPEUTIC TARGET EXPRESSION PROFILING IN HOST CELLS FROM NASOPHARYNGEAL SWABS OF SARS-COV-2-POSITVE PATIENTS**

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Nuclear transport and vesicle trafficking are key cellular functions involved in the pathogenesis of RNA viruses. Among other pleiotropic effects on virus-infected host cells, ivermectin (IVM) inhibits nuclear transport mechanisms mediated by importins and atorvastatin (ATV) affects actin cytoskeleton-dependent trafficking controlled by Rho GTPases signaling. In this work we first analyzed the response to infection in nasopharyngeal swabs from SARS-CoV-2-positive and -negative patients by assessing gene expression of the respective host cell drug targets importins and Rho GTPases. COVID-19 patients showed alterations in KPNA3, KPNA5, KPNA7, KPNB1, RHOA and CDC42 expression compared with non-COVID-19 patients. An *in vitro* model of infection with Poly(I:C), a synthetic analog of viral double-stranded RNA, triggered NF-κB activation, an effect that was halted by IVM and ATV treatment. Importin and Rho GTPases gene expression was also impaired by these drugs. Further, by confocal microscopy we analyzed the effects of IVM and ATV on nuclear to cytoplasmic importin α distribution, alone or in combination. Results showed a significant inhibition of importin α nuclear accumulation under IVM and ATV treatments. For gene expression analysis we performed two-tailed Welch's t tests or Wilcoxon rank sum test. For correlations Spearman's rank coefficient was calculated. In cellular studies, Mann-Whitney or t-tests were used. In case of more than 2 experimental groups, ANOVA or Kruskal-Wallis analysis were used. Differences were considered statistically significant at a level of $p<0.05$. Data processing and statistical analysis was performed using Prism 6.1 Software and R. These findings confirm transcriptional alterations in importins and Rho GTPases upon SARS-CoV-2 infection and point out to IVM and ATV as valid drugs to impair nuclear localization of importin α when used at clinically-relevant concentrations.

19. (186) **HUMAN GUT MICROBIOTA ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS: A PILOT STUDY IN AN ARGENTINE POPULATION**

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BACKGROUND: The ability of a microorganism, including commensals, to trigger disease is highly dependent on the host's activation state, the location of the particular microorganism, as well as the genetic predisposition of the individual. In this sense, studying possible changes in the intestinal microbiota related to the initiation, progression and response to treatment of patients with autoimmune diseases, such as Systemic Lupus Erythematosus (SLE), is an interesting field which promotes a comprehensive view of chronic inflammatory processes of increasing incidence worldwide. In the present study we aim to describe the unknown gut microbiota of SLE-patients of the Argentine population in comparison with healthy individuals in order to find novel SLE-biomarkers in our region.
METHODS: We evaluated 24 non-SLE-controls and 13 SLE-patients, from the metropolitan area of
Buenos Aires, Argentina. Fecal DNA was extracted and hypervariable regions V3-V4 of the bacterial 16SR-gene were sequenced using a MiSeq-Platform. Sequences were analyzed with the QIIME2 environment and differential functional pathways were evaluated using PICRUSt. RESULTS: In SLE-patients we found no significant differences in alpha diversity compared to non-SLE-control. However, Beta diversity was significantly different between groups (UniFrac distances, PERMANOVA, p-value <0.05). Additionally, functional metabolic pathways were analyzed and it was found that SLE patients have different metabolic capabilities compared to the control group. Six metabolic pathways were found from the Metacyc database mainly associated with the degradation of aromatic compounds and fatty acid biosynthesis. CONCLUSIONS: Overall, our study provides new knowledge on the gut microbiota composition of our population, allowing the association of local changes in gut

microbial diversity in SLE.

20. (195) **TRANSCRIPTOMIC STUDY REVEALS GENES AND BIOCHEMICAL PATHWAYS ASSOCIATED WITH CLINICAL EVOLUTION OF PATIENTS WITH CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

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Acute lymphoblastic leukemia (ALL) is the most incident pediatric cancer. While considerable progress has been made on treatment efficacy and survival rates, about 15-30% of patients relapse and/or die. We aimed to identify gene-expression profiles in childhood ALL that could help better predict disease outcome, response to treatment and therapy-related toxicity. We collected 39 bone marrow samples at time of diagnosis of ALL from 3 hospitals from Argentina. Total RNA was isolated to perform transcriptome analysis (RNAseq). Clinico-pathological characteristics and disease outcome were evaluated and recorded by oncohematologists. We analyzed differential gene expression (DGE) and gene set variation analysis (GSVA) comparing: early response to prednisone, event-free survival, risks group, acute toxicity and minimal residual disease at day 15. We observed that about 30% of dysregulated genes were non-coding RNAs, being long non-coding RNA (lncRNA) the predominant biotype. We identified 6 differentially expressed pathways relevant to ALL biology ($p<0.01$) and 7 lncRNAs (MIR99AHG, LINC02866, ZNF385D-AS2, LINC02848, MYO18B-AS1, Lnc-PPDPFL-1, Lnc-RIT2-2; $padj \leq 0.05$) among ALL risk groups. Because the biological activity of most lncRNAs is still unknown and under the hypothesis that lncRNAs modulate biochemical pathways, we calculated the correlation between significant lncRNA and pathway expressions. We found that MYO18B-AS1 positively correlated with "inactivation of MAPKK activity" ($r=0.4$;$p=0.02$) and LINC02866 negatively correlated with "CXCR3 chemokine receptor binding" ($r=-0.4$;$p=0.02$) and "transmembrane receptor protein tyrosine phosphatase activity" ($r=-0.4$;$p=0.01$). This study identified dysregulated lncRNAs and biochemical pathways that might be relevant in the pathology of childhood ALL. The analysis of these gene-expression profiles at diagnosis might help improving risk stratification, therapy efficacy and reducing the occurrence of relapse and toxicity.

21. (216) ***GENE HUNTER*: A NOVEL WEB-TOOL TO VISUALIZE SIMULTANEOUSLY DIFFERENTIAL GENES EXPRESSION ACROSS MULTIPLE DATASETS**

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Bioinformatics are becoming a prominent component of cancer research, resulting in an increased number of publicly available datasets to study and making it possible to evaluate gene expression across a variety of populations or disease stages. However, having to search for a specific gene in multiple datasets can often be time consuming and unfriendly to novel bioinformaticians. Our aim was to develop a user-friendly search tool to access simultaneously data of differential expression analyses for a particular gene or even gene signatures across multiple datasets.
For this purpose, 7 publicly available transcriptomic prostate cancer (PCa) datasets ($n_{total}= 875$) were selected to either be used by researchers interested in PCa or as an example to understand the tool before adapting it to their specific need, and differential expression analyses were performed in R, using the limma package. A *Shiny*-based tool, that can be accessed through a user interface, was then built to execute the search.
Our *Shiny* app includes a search bar that allows researchers to look for either a specific gene or a family of genes within all the datasets. Search results are presented in tables containing information on the comparison made for the analysed dataset, the gene ID and symbol, t and B statistical parameters, the log Fold Change, the p-value, adjusted p-value and the dataset's GSE identifier. Additionally, users can also plot different variables to visualize gene expression in all selected datasets more easily. Among the options, clinical significance of a gene can be assessed by overall survival and Kaplan-Meier plots.
In summary, *Gene Hunter* is a novel *Shiny*-based tool with the potential to ease high-throughput analyses in basic cancer research. It does so by providing the opportunity to explore differential expression between tumoral conditions, while straddling the limits of individual studies. This platform has the potential to extend to dataset comprehending other pathologies.

22. (234) **META-ANALYSIS OF HVEM EXPRESSION IN BREAST AND BRAIN CANCER**

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HVEM is an immunological checkpoint with dual immunomodulatory function; while its binding to LTα and LIGHT favors T cell activation, its binding to CD160 and BTLA suppresses their function. Thus, HVEM has emerged as an interesting therapeutic target for enhancing antitumor immune responses. Since HVEM expression has been detected in breast cancer (BRCA) and glioma biopsies, we performed a meta-analysis of transcriptomic data from The Cancer Genome Atlas to assess the expression of HVEM in these tumors. In BRCA biopsies, we found that HVEM expression is higher in normal vs. tumoral tissue, being lower in triple negative BRCA (TNBC) biopsies than in other BRCA subtypes. In TNBC, the expression of HVEM correlated with the expression of lymphocytic activation markers such as HLA-DR (r: 0,7109) and CD69 (r: 0,6013), but also with exhaustion markers as CTLA4 (r: 0,6349), PDL1 (r: 0,5331), LAG3 (r: 0,6547) and TIM3 (r: 0,6663). Even though HVEM expression did not show association with TNBC patient survival, its expression was positively correlated with the expression of gene signatures corresponding to helper and cytotoxic T cells, Tregs, macrophages and dendritic cells (DC). As for glioma biopsies, HVEM expression was higher in gliomas carrying wild-type IDH, an enzyme whose mutation has been recently associated with better prognosis. In addition, HVEM expression correlated with the aggressiveness of glioma subtypes, being higher in glioblastoma (GBM). In GBM, HVEM positively correlated with HLA-DR (r: 0,5021), CD69 (r: 0,4460), CTLA4 (r: 0,3721), PDL1 (r: 0,3725) and TIM3 (r: 0,5136). Although HVEM expression was not associated with patient survival, it correlated with the expression of helper and cytotoxic T cells, DC and macrophages. These results suggest that the pathways triggered by HVEM may have different outcomes depending on the tissue and tumor subtype, and that this checkpoint should be studied in depth as a target for cancer treatment.

23. (235) **AUTOMATED INMUNOHISTOCHEMICAL STAINING QUANTIFICATION IN HUMAN BIOPSIES: PRELIMINARY RESULTS USING DEEP LEARNING**

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Among the current challenges in histopathological assessment for diagnosis in clinical contexts is an accurate determination of the actual tissue malignancy. This task is often performed using microscopy over immunohistochemical (IHQ) staining applied on tissue samples, on which several specialists judge the tissue condition following specific criteria. However, this task is proven to be prone to high inter- and intra-subject variance, which raises the need to elaborate more robust tools and frameworks to assist on this task. The recent influx of deep learning technologies, which are proven to be successful in a variety of contexts, appears to be an adequate alternative in this context. In this aim, we present a joint effort between research groups from Cancer Biology Laboratory (INIBIBB-CONICET) and the Imaging Sciences Laboratory (LCI-UNS-CONICET). Starting with IHQ stained images taken with Olympus CX31 microscope from thyroid and breast cancer biopsies, we applied a Mask C-RNN network for cell nuclei detection. For this purpose, we retrained the net with a series of labeled examples provided by the biochemical specialists. After this initial detection, a ROI was determined surrounding the nuclei, within which the proportion of diaminobenzidine stain (brown-colored precipitation) is computed as a proxy indicator of the Immunoreactive Score (IRS). For this, a Random Forest classifier was trained using stain/no stain labeled pixels also provided by the experts. The results appear promising in the sense that the resulting system is able to consistently provide malignancy assessment even in difficult cases or when the quality of the microscopy acquisition is below standard.

24. (237) **ANTIANDROGENS POSE A PROTECTIVE EFFECT AGAINST COVID-19 BY BOOSTING THE HUMAN MYXOVIRUS RESISTANCE GENE 1 (*MX1*)**

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Population-based studies have shown that prostate cancer (PCa) patients undergoing androgen-deprivation therapies (ADT) were partially protected from COVID-19. Men treated with proxalutamide in a recent clinical trial, showed reduced COVID-19 hospitalization rate. In this work we assessed gene expression profiles and androgen regulation of the main host cell receptors described for SARS-CoV-2 and potential antiviral genes involved in response to coronavirus infection.
Multiple bioinformatics analyses were performed to study host cell receptors and antiviral proteins in SARS-CoV-2 infection and the gene expression changes upon ADT was assessed. We used publicly available datasets from: a) SARS-CoV-2 positive and negative patients' nasopharyngeal swabs at time of diagnosis (GSE152075, n=453), b) SARS-CoV-2 infected human cell lines and ferrets (GSE1407507), c) ChIP-seq experiments evaluating androgen receptor binding (GSE66037, GSE28950, GSE108704).
Results showed that SARS-CoV-2 positive cases had higher *MX1* expression, and multivariable regression showed that *MX1* expression significantly increased with viral load. Also, *MX1* was significantly up-regulated in tracheal samples from ferrets intranasally infected with SARS-CoV-2. Similar results were found in A549 and Calu3 lung cell lines. Since ADT might result in a therapeutic advantage against COVID-19, we next evaluated *MX1* regulation by dihydrotestosterone (DHT). First, comparable *MX1* levels in lung, prostate and salivary gland of healthy humans were observed (GTEx). LNCaP cells treated with DHT showed a decrease ($p<0.05$) in *MX1* mRNA levels. ChIP-seq experiments showcased AR binding sites on the *MX1* sequence upon DHT. Further, comparison of paired PCa patient's samples before and after ADT showed *MX1* upregulation ($p<0.05$) after ADT.
In summary, *MX1* raises as a critical responder in SARS-CoV-2 infection and we demonstrate *MX1* modulation by DHT. We propose *MX1* as a key player in the therapeutic advantage posed by ADT.

25. (242) **ELIGLUSTAT INHIBITS GLUCOSYLCERAMIDE SYNTHASE AND GLOBOTRIAOSYLCERAMIDE EXPRESSION WITHOUT INTERACTING WITH SHIGA TOXIN 2**

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Shiga toxin-producing *Escherichia coli* is responsible for Hemolytic Uremic Syndrome (HUS), a cause of renal failure in children. Renal damage has been associated with Shiga toxin (Stx), which binds to the globotriaosylceramide (Gb3) receptor on the plasma membrane of target cells. We have previously shown that Eliglustat (EG), an inhibitor of glucosylceramide synthase (GLS), the first step of glycosphingolipids pathway, also inhibits Gb3 expression and prevents the cytotoxic effects of Shiga toxin type 2 (Stx2) in human cortical renal tubular epithelial cells (HRTEC) primary cultures. The aim of this work was to clarify the mode of interaction of EG in the active site of GLS and its possible interaction with Stx2 and Gb3, and compare to Gb3 expression in HRTEC treated with EG and Stx2. For this, a computational procedure called molecular docking was carried out with Smina software and Gibbs free energy was calculated for determining the stability of the conformation formed between the ligand and the receptor. On the other hand, the expression of Gb3 receptor was analyzed by TLC in samples of HRTEC treated with EG (50 nM, 24 h) in the presence and absence of Stx2 (1 ng/ml). Docking analysis showed that EG presents an *in silico* 9-fold selectivity over GLS in comparison with Stx2, suggesting a stronger affinity between EG and GLS. These results were according to TLC assay, which showed that EG significantly inhibits Gb3 expression at 24 h. Besides, HRTEC cultures co-treated with EG+Stx2, showed a similar significant decrease in Gb3 expression. The incubation of HRTEC with Stx2 alone maintained the same Gb3 expression level as control non-treated cells. These results demonstrate that Stx2 does not interfere with Eliglustat effect on Gb3 inhibition. Study supported by PUE0041, CONICET.

26. (248) **INTERACTION EVALUATION OF A SUNFLOWER MANNOSE-BINDING LECTIN WITH VIRAL SURFACE GLYCOPROTEINS OF INFLUENZA AND SARS-COV-2**

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Each year, influenza virus infections cause more than half a million deaths worldwide. The novel coronavirus (SARS-CoV-2) has caused over 4.6 million deaths as of September 2021. The influenza virus hemagglutinin (HA) and coronavirus spike (S) glycoproteins mediate virus entry. HA and S are heavily glycosylated, making them potential targets for carbohydrate binding agents such as lectins. We have previously isolated a mannose-binding sunflower lectin (Helja) that showed the ability to inhibit hemagglutination mediated

by influenza, suggesting the binding of Helja to the HA glycoprotein. Here we evaluated the interaction of Helja both to influenza virus antigens and to the receptor binding domain of S (RBD). Using a ligand blot assay, the interaction of Helja with antigens of the H1N1 and H3N2 variants of the influenza A was demonstrated. Also, using an experimental strategy based on the interaction of Helja with mannose-agarose matrices, the ability of the Victoria variant antigens of influenza B to detach the lectin bound to the affinity matrix was observed. We used a similar experimental approach to detect the presence of a protein recognized by anti-Helja antibodies in the fractions eluted from affinity matrix by competence with RBD. These findings suggested the interaction of Helja with viral surface glycoproteins of influenza and SARS-CoV-2 that play a key role in the entry of both viruses into the host cells. Future research could contribute to designing of Helja-based new antiviral agents.

We aknowledge Auge G, Blaustein M, Corapi E, Cossio L, Craig P, D'Alessio C, Fernández N, Ferreiro D, Gándola Y, Gasulla J, Gorojovsky N, Gudesblat G, Herrera M, Idrovo T, Iglesias M, Kamenetzky L, Nadra A, Pignataro F, Roman E, Rubinstein N, Santos J, Velazquez Duarte F, Zelada A from FCEN-UBA-CONICET; Elias F, Pavan F, Ibanez I from Inst. Milstein; Pavan C, Bredeston L, Ruberto L from FFyB-UBA-CONICET; Noseda D. from UNSAM-CONICET for providing proteins and antibodies.

27. (270) **CLASSIFICATION OF THE FUNCTIONAL EFFECT OF GENETIC VARIANTS IN NKX2-5**

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NKX2-5 is a homeobox gene of very early expression in humans. It codes for NKX2-5, a transcription factor that regulates the expression of a number of other transcription factors involved in the development and function of the early heart, among other functions. This gene was the first where a single genetic variant (GV) was related to congenital heart disease (CHD).

Our group recently compiled, curated and structured an exhaustive database of all GVs in the gene. In this study, we aimed to expand this information by predicting the effect of some GVs in the functionality of the NKX2-5 protein. In order to do that, we proposed a classification of all possible missense GVs in the structured region of the protein according to the most probable effect they would have on its functionality.

We worked with all the possible amino acid variants caused by a change in a single DNA base pair in the homeodomain (HD) region (n=337). The *in silico* studies included an estimation of protein-DNA interaction, protein stability, prediction of linear motifs that could be affected by the variant and evolutive conservation. Taken together, these results were used to obtain a classification of the possible effect of GVs on the functionality of NKX2-5.

Our analysis determined that 32 GVs may affect the interaction with DNA (9.5%), 10 may have an effect on functionality due to introducing a cysteine (3.0%), 38 may affect a known linear motif (11.3%) and 135 are predicted to affect protein stability and/or a putative linear motif (40.0%).

This analysis allowed us to estimate the potential effect of GVs on NKX2-5. In particular, it contributes to a functional prediction of yet unknown GVs, it proposes a mechanism for pathogenesis of known GVs and it helps to better understand the genotype-phenotype relationship in the development of CHD.

28. (333) **LARGE SCALE BIOINFORMATIC ANALYSIS OF CRISPR-CAS13 AS NOVEL SARS-COV-2 ANTIVIRAL**

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Objectives: The aim of this study was to assess the alignment rate of two distinct CRISPR-Cas13 crRNAs collections in one million SARS-CoV-2 genomic sequences.

Materials and Methods: The crRNAs collections were designed using the SARS-CoV-2 reference genome and lacked alignment to the human transcriptome. The first collection had crRNAs targeting the NSP13 (Helicase), while the second collection had crRNAs targeting the overlapping regions NSP1-NSP2, NSP2-NSP3, NSP3-NSP4, NSP4-NSP5 and NSP5-NSP6 that code for the cleavage sites of the viral proteases PLpro and Mpro. Each collection had five crRNA 23-nucleotides long. The SARS-CoV-2 database consisted of one million sequences retrieved from GISAID belonging to the period December 2019 - March 2021. The crRNA alignment to the database was made by command line BLAST (v2.11) and only alignments with 100% complementary rate were consider in the t-test analysis.

Results: The first collection had an alignment rate of 99.44% (SD±0.22%), while the second one had an alignment rate of 99.46% (SD±0.28). There was no significant difference between collections (p-value: 0.8). Four crRNAs of either collection were needed to obtain a 100% coverage on the million sequences.

Conclusion: Bioinformatic research revealed a high alignment rate of crRNAs to a million SARS-CoV-2 sequences, thus validating CRISPR-Cas13 as a reliable antiviral. There was no difference in the alignment rate of crRNA collections designed to target either a single NSP or overlapping regions of many NSP, so viral regions with known high conservation rate should suffice for an optimal design of crRNAs. In this research we found that 4 crRNAs were enough to target all studied viral sequences, which is in accordance to previous studies showing that 5 crRNAs would be necessary to target individual species of coronavirus. Here we provide the first large scale analysis regarding the alignment success of crRNAs in one million viral sequences.

29. (356) **EXPLORING THE ROLE OF THE GALECTIN-1-GLYCAN AXIS IN AN EXPERIMENTAL MODEL OF COLITIS-ASSOCIATED COLORECTAL CANCER**

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Objective. To understand the mechanisms underlying the protumorigenic role of Galectin-1 (Gal1) and associated glycans in colitis-associated colorectal cancer (CACRC).

Materials & Methods. We performed transcriptomic analysis of tumor samples from WT and Gal1 KO mice in an experimental model of CACRC (azoxymethane/dextran sulfate sodium, AOM-DSS). Total RNA was extracted, purified, and sequenced. Sequencing data was pre-processed with FastQC and Trimmomatic, and pseudo-counts were estimated using Kallisto. Downstream analyses were run using R software v3.6. Immune infiltrate deconvolution was performed using MIXTURE with ImmuCC-seq signature. Enrichment analyses were performed using GSEAbase package. KO vs. WT comparisons were performed using Wilcoxon test.

Results. We recently reported the immunosuppressive role of Gal1 in AOM-DSS through modulation of the $CD8^+$ regulatory T cell compartment, with significantly decreased tumor number and volume in Gal1 KO mice. Here, we performed transcriptomics analysis in WT and Gal1 KO mice treated with AOM-DSS. Differential expression analysis and functional enrichment showed downregulation of angiogenesis-associated pathways ($p<0.05$). Moreover, perturbation-response analysis of 14 key cancer-associated processes showed that Gal1 KO mice presented lower scores for VEGF, Hipoxia, TNFa and NFkB, and higher score of Trail pathways ($p<0.01$). Immune infiltrate composition in KO mice showed a higher absolute score together with higher $CD8^+$ T cell, B cell and monocyte pro-

portions, and lower macrophage proportion (p<0.05). Lastly, *Lgals1* expression was highly correlated with a set of 148 genes which included *Il1b*, *Tmem176b* and *Nfkbia*.
Conclusion. Our results shed light into the mechanisms associated with the protumorigenic role of Gal1 in CACRC. We propose that Gal-1 can promote an immunosuppressive TME while promoting angiogenesis and immune-evasion related pathways that contributes to tumor growth and disease progression.

30. (378) **IDENTIFICATION OF 5-FLUOROURACIL RESISTANCE REVERSING COMPOUNDS BY COMPUTATIONAL DRUG REPOSITIONING FOR COLORECTAL CANCER TREATMENT**

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Computational drug repositioning consists of the use of different bioinformatics algorithms to identify known compounds that may be candidates for treating a new pathology. In this work we used the Recursive Feature Elimination algorithm (RFE) to select a set of genes associated with tumour recurrence condition after 5-fluorouracil (5-FU) based chemotherapy in a group of colorectal cancer (CRC) patients. In combination, we used the RankProd algorithm, to filter the selected genes that were also differentially expressed (FDR>0,05). The list of genes was entered in the Library of Integrated Network-Based Cellular Signatures (LINCS) database to identify those compounds with the greatest probability to reverse the resistance-associated gene expression profile obtained.
Among the proposed candidate drugs, we selected irinotecan (antineoplastic), ivermectin (antiparasitic) and amitriptyline (antidepressant). By MTT-based proliferation assays evaluated their effect on the proliferation capacity on 5-FU sensitive and resistant CRC cell lines.
We found that both drugs further reduced the proliferation of 5-FU resistant CRC cells (P<0,05). Our preliminary results suggest that ivermectin and amitriptyline have a potential 5-FU resistance reversal effect which have yet to be studied.

31. (382) **IDENTIFICATION OF LONG NON-CODING RNAs (lncRNAs) DYSREGULATED DURING PROSTATE CANCER PROGRESSION**

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Most of the human genome is transcribed into non-coding RNAs which perform a myriad of tasks in cells. Their dysregulation is responsible of the development and progression of different pathological processes, including cancer. Prostate cancer is known for having a wide spectrum of clinical outcomes, ranging from an indolent tumor to a lethal metastatic castration resistant cancer (CRPC). But the biology of the processes leading to these phenotypes is still mostly unknown. The aim of this study was to identify lncRNAs involved in the progression of prostate tumors to CRPC. We browed public repositories and downloaded raw RNAseq data from primary prostate adenocarcinomas (pre- and post-androgen deprivation therapy (ADT); n=40 and n=11, respectively) and CRPC (n=8). We performed differential expression of 17,009 lncRNAs using R/Bioconductor. We identified 12 lncRNAs that responded to ADT and were further dysregulated in CRPC: *PCA3, PCAT18, PCGEM1, LINC01095, GABRG3-AS1, HECW2-AS1, NKILA*, *LOC100506474, SLCA4-AS1, LOC101927870, LOC101929532,* and *LOC105377503*. Interestingly, three of them were widely reported as players in prostate cancer development and progression, validating the pipeline used in this study. Unsupervised clustering analysis revealed that the 12-lncRNA-expression pattern could cluster post-ADT and CRPC samples apart. Primary tumors pre-ADT were more heterogeneous and clustered together with post-ADT or CRPC tumors. This suggests that primary prostate tumors that might potentially progress to CRPC could be detected at the time of diagnosis according to their lncRNA expression profile. In addition, we looked into promoter methylation of these lncRNAs in different types of tumors. Overall, we observed promoter hypomethylation in the tumors compared with normal counterpart. These results warrant further analysis in more samples and in-vitro experiments to validate the findings and determine the role of these lncRNAs in the pathogenesis of prostate cancer.

32. (394) **ENRICHMENT ANALYSIS ASSOCIATED TO VAV3 EXPRESSION IN MELANOMA**

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Skin cutaneous melanoma (SKCM) is the most severe form of skin cancer, originated from the malignant transformation and proliferation of melanocytes.
According to the existing paradigm for the Rho GEFs (guanine nucleotide exchange factors), the Vav subfamily is commonly assumed to be involved in protumorigenic pathways in tumor cells. Interestingly, we have recently proposed an unexpected tumor suppressor role for Vav3 in melanoma. In this tumor type, Vav proteins play important roles during development, tumor growth and metastasis.
In this work, we explore molecular functions associated to Vav3 in melanoma by bioinformatics. First, by survival analysis tools and using human patient databases, we found that Vav3 expression correlated with patient survival (p<0.001). To explore pathways associated to Vav3 expression levels we created two groups of patients with low and high levels of Vav3. The clinical and molecular information was obtained from the dataset Skin Cancer Melanoma from The Cancer Genome Atlas (SKCM-TCGA). We used TCGABiolinks and edgeR packages in R, to identify differentially expressed genes (DEGs; |logFCl>1 and FDR<0.01).
The molecular function of this group of DEGs was obtained using the Panther Classification System of the Gene Ontology Consortium. Of the 579 genes categorized by molecular function, 29% of them were associated to “binding function”, especially “protein binding” (51.2%), and 18.5% to “catalytic function”, especially “hydrolase activity” (57.9%). With this set of DEGs we performed a Gene Set Functional Enrichment Analysis (GSEA). As expected, we observed an enrichment for the Rho GTPase pathway in patients with high Vav3 expression, and pathways related to both innate and adaptive immune system (FDR<0.25), such as the interleukin-2 and gamma interferon pathways.
Our preliminary data suggest that Vav3 could be associated to a transcriptional program that controls immune responses in melanoma.

33. (423) **ALTERATION OF THE PRENYLATION PATHWAY IN CANCER CELLS: ROLE OF ICMT ON THE TUMOR MICROENVIRONMENT**

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Posttranslational modification by the prenylation pathway is a regulatory mechanism that affects the C-terminus of key proteins in cell behavior. Mounting evidence points at a critical role for ICMT in cancer. Our previous work showed that ICMT overexpression enhanced tumorigenesis *in vivo* using xenografts in immunocompromised mice. In this work, we wondered if ICMT may enhance tumorigen-

esis in inmmunocompetent mice. To answer this question, we used an orthotopic model of breast cancer in BALB/c mice. Our results showed that ICMT enhanced tumor aggressiveness. We took advantage of bioinformatic tools to analyze the interplay between cancer cells and the tumor microenvironment (TME). We first analyzed the expression of ICMT in normal and tumor tissues in public databases using GEPIA 2. We found that *ICMT* expression is increased in tumors. Interestingly, the analysis of GEO datasets showed that *ICMT* mRNA levels were significatively increased in tumor cell lines co-cultured with fibroblasts, suggesting that the TME contributes to ICMT overexpression in cancer cells. By using algorithms available in TIMER 2.0, that allow to identify different cell types in tumor samples through deconvolution of cell-specific transcriptional programs from microarray databases, we found that *ICMT* overexpression in tumors enhanced infiltration of cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs). We also studied if the combination of *ICMT* overexpression with CAFs or TAMs infiltration affects clinical outcome in breast cancer by correlation analysis. We found that cases with low ICMT expression and low infiltration of these cell types displayed a significant increase in survival. Moreover, we found that cases with high ICMT expression and augmented presence of M2-like macrophages showed reduced survival. Collectively, our results showed that ICMT overexpression enhances tumor progression and suggest that it contributes to reshape the TME.

34. (460) **MOLECULAR CHARACTERIZATION OF A PATHOGENIC STRAIN OF JUNÍN VIRUS**

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The Junín virus (JUNV) is the etiological agent of Argentine hemorrhagic fever. The genomic sequence of JUNV pathogenic variant P3441 (P) is unknown.
With the objective to characterize the P variant at a molecular level, BHK cells were infected with the P at MOI 1. At 3 days post-infection, cells were harvested and TransZol reagent (TransGen Biotech) was used to extract total RNA. Subsequently, 2 μg of viral RNA was incubated with 1 μl Reverse Transcriptase (Superscript IV, Invitrogen) and random primers (50 μM, Invitrogen) for 10 min at 25ºC followed by incubation at 50ºC for 50 min. Then, 1 μl of cDNA was used as a template in each subsequent PCR using 17 pair of primers to amplify the full genome. The amplified fragments were submitted to Macrogen (Korea) for capillary electrophoresis sequencing followed by analysis using the Ugene software.
Results showed 59 genome differences between P and the vaccine attenuated Candid 1 (C#1) strain including some with potential biological functions such as the N protein R476, within a Z binding domain; D511 and L546, conserved in several pathogenic strains and found in the Z-interacting domain; and the Z protein V64 within Z RING domain. L protein showed more than 40 differences with C#1 but their relevance is uncertain since none is within functional domains. GPC proved to possess all the point mutations that have been studied by other groups. Then, specific primers were designed to amplify the 4 JUNV ORFs. Fragments of the expected size were obtained for ORFs codifying Z, N and GPC. In the case of L, four fragments were obtained. The fragments were subsequently cloned in the pGEM-T vector (Promega) following the manufacturer's instructions. Up to the present time, all ORFs have been cloned except for one L fragment, which is still in progress.
We conclude that our approach was useful to know the genome sequence of this viral variant as well as to make molecular tools for further studies.

35. (463) **TITLE: CHROMATIN SPATIAL ORGANIZATION DURING DECIDUALIZATION**

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Decidualization is a process of endometrial trans-differentiation crucial to sustain pregnancy. The events involved are regulated by steroid hormones progesterone and estradiol (E2) via the ligand activated transcription factors progesterone receptor (PR) and estrogen receptor (ER). Endometrial specific transcription is also regulated by HOXs and FOXO transcription factors. In this work we used High-throughput Chromosome Conformation Capture (Hi-C) to analyze the changes in chromosome conformation organization and RNA-seq to explore changes in gene expression signatures at different time points of *in vitro* decidualization of human t-HESC cell line. Decidualization was induced by exposure to cAMP, E2 and either progesterone or the synthetic progestin R5020. PGR and FOXO1, both crucial transcription factors in decidualization, showed >4 fold change increase after 3 days of treatment. On the other hand the decidualization markers, PRL and IGFBP1, both displayed >20 fold change increase after 3 days. At day 1 the inflammatory signature was differentially upregulated (FC>2, FDR<0.01) in hormone-dependent decidualization. This signature was changed at 3 and 6 days, indicating distinct differential states. Global chromosome conformation compartments analysis through PCA of the Pearson correlation contact matrices showed extensive remodelling of A and B compartments and positive correlation (p-value<0.01) between B to A compartment changes and differential gene expression during human decidualization. In particular, the Hox genes cluster showed dramatic changes in looping conformation in this region and a correlation with changes in decidual specific genes expression after 6 days of treatment, indicating the increased interaction frequency between this cluster and the neighboring gene promoters. These results explore novel and global human endometrial enhancer-promoter relationships involve in human decidualization.

36. (470) **COMPARATIVE GENOMICS TO EXPLORE ZP EVOLUTION**

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The mammalian fertilization process has been extensively described by both physiology and molecular biology. Zona Pellucida (ZP) and IZUMO1R oocyte proteins play a key role in this process, facilitating the interaction and fusion between the oocyte and the sperm, respectively. Their interactions with sperm counterparts may constitute a prezygotic reproductive isolation mechanism. The story of protein evolution has greatly changed with the large number of genomes reported as their sequencing advanced. We have improved the phylogeny of gamete interaction proteins using chromosome-resolved genome assembled by Hi-C long-range sequencing. ZP isoforms involved in sperm-recognition, ZP3 and Zp2, structural cross-linker ZP proteins, ZP1 and Zp4, and IZUMO1R evolution analysis were studied among mammals, carnivora, caniformia and feliformia groups. In order to detect adaptive changes in these proteins pairwise sequence identity and similarity analysis were performed using multiple sequence alignments. Positive selection signals were find using codeml software from PAML package. The conservation of all carnivora gamete interaction proteins was significantly higher (p-value < 0.05) in felids compared to canids except for the case of ZP4. ZP3 and ZP2, display a similar pattern of evolution along their phylogenies showing adaptive changes in the canids subtree but not among felid species. On the other hand, both ZP1 and ZP4 display a different evolutionary history, showing signatures of positive selection inside felids subtree. The fusion protein IZUMO1R did not show positive selection among all studied phylogenetic groups. These results strengthen the idea that ZPs are proteins that provide

a more specific-specificity isolation than IZUMO1R, which is specialized in fusion events.
Altogether, our findings indicate that sperm-oocyte interaction and fusion proteins lack the degree of diversification necessary to fix a prezygotic reproductive isolation barrier in felidae.

37. (498) **PROTEOMIC STUDY OF BREAST CANCER CELL LINE AFTER HEMIN TREATMENT**

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Hemin is a ferriporphyrin (C34H32ClFeN4O4) with antitumoral effects on prostate, breast and colon cancer. It is widely used to increase the expression and activity of hemoxigenase-1 (HO-1). HO-1 is a microsomal enzyme that catalyses the degradation of heme group and can be translocated to subcellular compartments. Our laboratory, among others, has demonstrated that HO-1 regulates several processes related to cancer progression. The aim of this work was to study the protein expression modification due to hemin treatment in a murine breast cancer cell line (LM3). After hemin or vehicle (control) cell treatment, we performed Mass Spectrometry (MS) and Perseus proteome analyses. The proteome analysis showed that 1033 from 7292 proteins detected were modulated after hemin treatment. We observed that 595 proteins were significantly increased, including HO-1, and 353 proteins were significantly decreased in the group treated with hemin respect to their controls ($p<0.05$). ANOVA significant proteins reveal an upregulated group of proteins related with lipid and iron metabolism. In the group of proteins whose expression decreased after hemin treatment, we found cytoskeleton-related proteins. The Perseus-MS-data analysis revealed that hemin treatment regulates adhesion proteins like vimentin and talin, actin and tubulin cytoskeletal proteins and their stabilizing proteins. In addition, from MS data after hemin treatment, we found an increase in some cancer suppressor proteins such as PTEN and SMAD2/3. Finally, we found that proteases involved in HO-1 nuclear translocation were upregulated after hemin treatment. We further corroborated some of the *in-silico* analysis in LM3 cell line by immunofluorescence and Western blot techniques. In addition, we used a syngeneic LM3 mice model to detect by immunohistochemistry some of the regulated protein. These results show the multiple physiological effects that pharmacological modulation with hemin has in a breast cancer cell line.

38. (522) **A PROTEOMIC STUDY REVEALS NEW MARKERS OF PROLACTIN-MODULATED OVARIAN FUNCTION IN PLAINS VIZCACHAS**

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Prolactin (PRL) modulates the expression of the LH receptor in the ovary and, thus, the cascades of steroidogenic enzymes that synthesize and produce ovarian steroids. When pathological hyperprolactinemia occurs, the pulsatile release of GnRH decreases and alters the pituitary production of FSH and LH. Furthermore, it directly impairs the endocrine activity of ovarian follicles. To analyze which ovarian factors, aside to the aforementioned enzymes, respond to a high PRL environment, an ovarian proteomic study of vizcachas with sulpiride-induced hyperprolactinemia was performed. For this, ovarian protein extracts from hyperprolactinemic (HPRL) and control (CTL) females (n=5 per group) were used. Briefly, equal amounts of protein were analyzed using MALDI-TOF/MS and then, LC-ESI/MS (Orbitrap). The resulting peptides were identified with Proteome Discoverer Software using the Rodentia UniProt Database, and functional enrichment analysis was performed using DAVID, STRING and FunRich softwares. Proteins differentially expressed in each treatment were depicted in a volcano plot (t-test, $p<0.05$). Functional enrichment analysis showed that 24 proteins were differentially expressed in HPRL ovarian tissue compared to that of CTLs. Among those, some cytoskeleton regulation markers such as annexin 2 (ANXA2), Actin related protein 2/3 complex subunit 5 like (Arpc5l), and Myosin regulatory light chain 12A (MYLC12A) prevailed in HPRL-ovaries, while other markers related to mitochondrial function as Dynamin-1-like protein (DNM1L), Succinate-CoA ligase (SUCLG2), and Mitochondrial fission 1 protein (FIS1) were down-regulated. In addition, the interactomes showed different network topology with different nodal peptides in HPRL vs CTL treatments. The present work showed an ovarian expression profile that significantly varies under a hyperprolactinemic environment. Finally, this report provides new markers for future investigations on PRL-dependent modulation of ovarian function.

39. (565) **ANALYSIS OF REGULATORY CIS-ELEMENTS AND FOXA1/GATA2 TRANSCRIPTION FACTORS BINDING BEHIND MRP4/ABCC4 LEVELS IN PANCREATIC CANCER**

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The multidrug resistance-associated protein 4 MRP4/ABCC4 is highly expressed in pancreatic ductal adenocarcinoma (PDAC), and linked to increased proliferation, a mesenchymal phenotype and poor prognosis. This study aimed to determine epigenetic and molecular mechanisms that control ABCC4 expression levels in PDAC. Supported by bibliographic data, we selected pioneer transcription factors (TFs) FOXA1 and GATA2 as candidates to regulate low vs high ABCC4 expression. We queried ChIP-seq and RNA-seq data available from PDAC cell lines in GEO, and from patients tumors at TCGA (PAAD database). ABCC4 showed negative correlation with FOXA1 and positive correlation with GATA2 in PDAC cell lines and patients tumors. Next, we searched for regulatory cis-elements in ABCC4 gene by looking for enhancer marks H3K27ac/H3K4me enrichment, indicative of active clusters of TF binding sites, and also analyzed FOXA1 and GATA2 enrichment available in PDAC and prostate cell lines. We detected two candidate clusters, one in the distal promoter (-13.5 kb) and one in the intron1 (29.9 kb), that showed positive enrichment for both FOXA1 and GATA2, and different epigenetic landscape in low vs high ABCC4-expressing PDAC cell lines. To test the in silico results, we generated low-grade HPAF2 and high-grade PANC1 xenografts in NGS mice, and evaluated FOXA1 and GATA2 immunostaining and enrichment at the selected clusters in ABCC4 gene (ChIP-qPCR). HPAF2 tumors showed glandular differentiation and high FOXA1 staining, whereas PANC1 tumors showed compact indifferentiated cells and high GATA2 staining. Consistently, we found specific FOXA1 enrichment in HPAF2, and specific GATA2 binding in PANC1, at the selected clusters. These findings characterize the role of pro-epithelial pioneer TF FOXA1 in maintaining low levels of ABCC4 expression in low-grade PDAC, and a novel role of GATA2, which could mediate the increased ABCC4 expression in high-grade PDAC and contribute to the mesenchymal phenotype.

40. (566) **COOPERATION BETWEEN GLUCOCORTICOID AND RETINOIC ACID RECEPTORS ON TRANSCRIPTIONAL REGULATION ENHANCES DIFFERENTIATION IN ACUTE MYELOID LEUKEMIA**

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In Acute Promyelocytic Leukemia (APL) cell differentiation is arrested and retinoic acid (RA) therapy alone ends in relapse. Our previous results showed that the glucocorticoid dexamethasone (DEX) significantly enhances RA-induced myeloid differentiation. APL-NB4 cells were treated with RA 0.1 µM in the presence or absence of DEX 0.5 µM and RNAseq analysis was performed. Differential gene expression (FC>1.5, p-adj<0.05, LR>10) between hormone-treated and control (CTRL) samples from three biological replicates was calculated with DESeq2. From a total of 6983 regulated genes, 6244 were regulated by RA+DEX, 5610 by RA and 204 by DEX. Most up (2479) and down-regulated genes (2322) between RA and RA+-DEX samples are shared. Gene Set Enrichment Analysis (GSEA) revealed that expression of genes associated with myeloid development (FDR q-val<1E-6) and hematopoietic stemness inhibition (FDR q-val<1E-6) signatures was markedly enhanced upon addition of DEX. Some of the 565 RA+DEX-potentiated and induced genes (RA+DEXup) are AIM2, FOS and TLR6. Concomitantly, peaks obtained from Transposase-Accessible Chromatin (ATAC)-seq analysis were associated to genes using ChIPpeakAnno, within 1mb from TSS. RA/RA+DEX induced differential peaks, compared to CTRL, in 491 RA+DEXup genes, while only 53 of them were associated with regulated peaks due to addition of DEX. Moreover, 40.7% (200/491) of these genes have a peak in the promoter, whether differential or not, and at least one differential peak in the distal region. These distal regions are enriched for glucocorticoid receptor response elements. Furthermore, this 200 gene subset is responsible for the enrichment of GSEA signatures myeloid development (p-val<6E-15) and hematopoietic stemness inhibition (p-val<2E-16). Taken together, these results suggest that DEX has little effect in chromatin accessibility and may regulate transcription on RA-mediated accessible regions during NB4 cell differentiation.

41. (587) **COVID-19 PATIENT STRATIFICATION: AN APPROACH THROUGH THE PLASMA PEPTIDOME USING THE MALDI-TOF-MS TECHNOLOGY**

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The COVID-19 presents a dynamic nature with very differentiated pathophysiological phases could culminate in sepsis. However, clinically, it's difficult to define what stage the patient, or detect those patients with an advanced risk of progressing to severe sepsis. Our aim was to investigate the usefulness of plasma peptidome fingerprints as instrument for stratification of patients with COVID-19 using MALDI-TOF technology. In this cut of the project, as a result of the monitoring of 7 patients admitted to the Hospital de Cuenca Alta Nestor Kirchner -July and September 2021- 46 plasma samples were included. These were divided into hospitalization (H) (n = 27) and UCI (n = 19), classification used as severity proxy. Supervised analyzes and machine learning algorithms were performed to discriminate between the classification stages in patients. Also, clinical and biochemical parameters were also evaluated. Peripheral blood leukocytes count was increased in patients during stay UCI associated to neutrophilia on the 5 day (Neutrophils/ml (median (IQR)): UCI=14027 (13892-18090) *; H=8954 (3998-10250); *p<0.05). Inflammation markers such as acute phase reactants showed an elevated ferritin (F) levels in UCI patients (F (ng/ml) (mean±SD): UCI=2300±173*; H=1216±556; *p<0.05). The 413 spectra dataset was randomly partitioned into a training set (60%) and a test set (40%). Two ML classification methods were applied, Binary Discriminant Analysis (BDA) and Random Forest (RF), using to 5, 10, 15 and 20 peaks selected based on the ranking of the best predictors. Accuracy (A), sensitivity (SE), and specificity (SP) were used to evaluate the performances. The predictive value of models was for BDA (top 10 peaks) A:78,5%, SE:81,8%, SP:75,0% and RF top (15 peaks) A: 89.5% SE: 100.0% SP:85.7%. These results show the potential of peptidome fingerprints obtained by MALDI-TOF as a tool to develop predictive models that allow stratification patients according to the disease severity..

BIOTECNOLOGÍA FARMACÉUTICA

42. (068) **IMPROVED ANTIVIRAL THERAPY: DEVELOPMENT OF FUNCIONAL DE-IMMUNIZED VERSIONS OF INTERFERON ALPHA FOR EMERGIN VIRAL INFECTIONS**

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Human interferon α (hIFN-α) are a multigene family of proteins that constitutes the current FDA approved therapy for chronic Hepatitis B and C virus infections. Additionally, numerous studies have highlighted these cytokines as candidates for the treatment of emerging viral diseases such as Zika, Chikungunya or Dengue virus infections. Moreover, the pegylated forms of hIFNα2a and hIFNα2b have been proposed as therapeutic alternatives to treat infections caused by the acute respiratory syndrome coronavirus2 (SARS-CoV-2). However, a major disadvantage of hIFN-α2b therapy is given by its short plasma half-life resulting in the occurrence of severe side effects. To optimize the cytokine's pharmacokinetic profile, our group has developed a highly O-glycosylated IFN, GMOP-IFN, by fusing the N-terminal end of the cytokine to a peptide containing four potential *O*-glycosilation sites.

Considering the significant number of reports existent about neutralizing antibodies (NAb) induction after repeated administrations of hIFN-α, and in order to develop a safer and more efficient IFN therapy, in this study we applied the DeFT (De-immunization of Functional Therapeutics) approach to generate functional, de-immunized versions of GMOP-IFN.

Two variants out of four displayed reduced *ex vivo* immunogenicity, while preserving antiviral function. Moreover, both IFN analogs exhibited null specific antiproliferative activity, which constitutes an additional highly favorable characteristic when considering the dramatic consequences associated to hematologic disorders commonly produced by hIFN-α therapy.

Altogether the results obtained in this work indicate that the new de-immunized GMOP- IFN variants are promising candidates for antiviral therapy, exhibiting reduced immunogenicity, proven *in vitro* antiviral activity while lacking antiproliferative activity.

43. (222) **INCORPORATION OF *L. rhamnosus* CRL1332 TO FEMALE HYGIENE PRODUCTS AND THEIR CHARACTERIZATION**

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Lactobacilli play a basic role in the physiology of the urogenital tract of healthy women maintaining the vaginal pH below 4.5. This acidic environment in combination with other antimicrobial substances

secreted by lactobacilli create a barrier against the income of pathogens. However, vaginal microbiota can be unbalanced by different exogenous and endogenous factors. An excellent alternative to reconstitute the vaginal ecosystem and prevent/treat urogenital infections is the application of products containing probiotic lactobacilli. In previous works, the immobilization of *L. rhamnosus* CRL1332 in polymeric nanofibers by electrospinning was optimized. The aim of this work was to evaluate the incorporation of *L. rh.* CRL1332-nanofibers with/without bioprotectors in female hygiene products. Acidification capability of *L. rh.* CRL1332-nanofiber was evaluated. Tampons, panty liners and textile substrates were covered with BVLAB-nanofibers by adapting the electrospinning set up. *L. rh.* CRL1332-nanofibers covering the different substrates were quantified during storage. Morphological and physical-chemical characterization of coated products were carried out. The vibratory system was suitable for tampons, while a translational movement device was optimal for panty liners/textile substrates. *L. rh.* CRL1332-nanofibers vacuum packed under refrigerated conditions remained viable (> 10^7 CFU/g) for up to 360 days and retained the acidifying capability. The bacteria immobilized in nanofibers on the different substrates were evidenced by electronic microscopy (SEM), chemical (ATR-FTIR) and thermal (TGA and DSC) analysis. The results obtained show that the electrospinning technique is an innovative method to coat feminine hygiene products with viable lactobacilli probiotics, in a way to restore the vaginal tract.

44. (223) **DESIGN OF PROBIOTIC CALF FORMULAS BY LOW COST CULTURE MEDIA WITH DIFFERENT SUGAR SOURCES TO OPTIMIZE LIVE BACTERIAL BIOMASS**

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Probiotic formulas for calf with lactic acid bacteria (LAB) strains must contain high numbers of live microorganisms to exert the beneficial effect. Previously, our research group designed a formula in gelatine capsules containing dry bacteria. However, LAB are nutritionally exigent microorganisms, requiring complex culture media to grow, which are very expensive, increasing the cost of production. Then, looking for cheaper culture media for industrial applications is of main importance. The objective of this work was to evaluate different sugar combinations in a base media to reduce the cost of the biomass obtention, growing the LAB at two temperatures. The probiotic strains assayed were: *Lactobacillus johnsonii* CRL1693, *Ligilactobacillus murinus* CRL1695, *Limosilactobacillus mucosae* CRL1696 and *Ligilactobacillus salivarius* CRL1702. 51 different media were assayed with: Glucose (G), Fructose (F), Molasses (M), Pectin (P), Lactose (L) and Corn syrup (CS). The growth was evaluated by optical density (OD_{560nm}) in microplates during 24 h at 30°C and 37°C, calculating growth parameters. CRL1693 showed significant differences in the growth and Lag phase length (LPL) at the two temperatures. The better growth media resulted with G, L and CS; and M+G, L and F. CRL1696 grew in few culture media at both temperatures: LPL and OD did not show significant differences, but with lower growth rate at 30°C. The best combinations at 37°C were: L+G, P and CS. CRL1695 grew in most of the media designed at the two temperatures showing no significant differences. CRL 1702 grew in G, M and L, and in M+G, F in most of the media at the two temperatures, with no significant differences in LPL, but with higher growth rate at 37°C. At 30°C and 37°C, this strain showed good growth in G or M+G. The results indicate that the growth behaviour is dependent of each specific strain, eventhough all the evaluated LAB showed to grow in culture media designed with low-cost-sugar sources, as Molasses and Corn syrup for their scaling up for industrial applications.

45. (299) **DEVELOPMENT OF A NEW VACCINE CANDIDATE AGAINST YELLOW FEVER BASED ON BACULOVIRUS-SURFACE DISPLAY**

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Yellow Fever (YF) is a disease caused by the homonymous flavivirus. There is an excellent attenuated virus vaccine for the prevention of this disease. However, this vaccine cannot be administered to immunocompromised and pregnant individuals. For this reason, development of new alternatives is of the utmost importance. The aim of this work was to generate a new generation vaccine candidate based on a recombinant baculovirus (rBV) that expresses YFV protein E and NS1 on its surface capable of inducing both humoral (protein E) and cellular (NS1) immune responses. To this end, a pBacPak9 vector containing the BV GP64 signal peptide, YFV's prM, E, NS1 open reading frames and the ectodomain of VSV-G was synthetically constructed. This plasmid and the bApGOZA bacmid were co-transfected in High Five cells. Between 5 to 7 days post-transfection, characteristic BV infection signs (polyhedra and cytopathic effect) were observed. Culture supernatant was harvested and used to infect new cultures. When the aforementioned infection signs firstly appeared in the subsequent infection, the supernatant was again collected. This procedure was repeated 3 times. The rBV was recovered from the supernants and used to make a viral stock. Viral genome DNA extraction from the stock was subjected to PCR with specific primers to confirm the incorporation of the target genes in the rBV genome. Protein expression was confirmed by western blot using an anti-E polyclonal antibody (GTX134024, Genetex) using another rBV carrying YFV prM-E genes as a positive control. The stock was then escalated and concentrated by ultracentrifugation at 80.000g for 1:15 hs in a 25%w/w sucrose cushion and the pellet was resuspended in PBS and filtered using a 0.2μm filter. This stock was titrated using the Sf9-GFP transgenic reporter line reaching $10^{5.5}$ virus/ml. In conclusion, we have generated a valid YFV vaccinate candidate to be tested in a murine model.

CARDIOVASCULAR Y RESPIRATORIA

46. (006) **CARDIOVASCULAR CHANGES PRODUCED BY CHRONIC IRON OVERLOAD AND ADMINISTRATION OF VITAMIN IN EXPERIMENTAL METABOLIC SYNDROME.**

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Chronic high-fructose-fat diet (12 weeks) induces metabolic syndrome (MS), conducting to cardiovascular disease. Systolic blood pressure (SBP) was measured in male rats (n=8-10/group) with MS, Iron overload (FE), or antioxidants -vitamins E, C and lipoic-acid- (AoV), alone or with MS (MSFE, MSAoV). After decapitation, systolic and diastolic ventricular function in basal condition and in response to isoproterenol (10^{-9} M) (contractile and lusitropic reserve) were assayed in isolated perfused hearts. Left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (myocardial stiffness) and the relaxation half time (t1/2) were measured. Data was analyzed by Two-way ANOVA and Bonferroni post-test ($p \leq 0.05$). The SBP rose in MS at 4 ($p<0.005$), 8 and 12 weeks ($p<0.0001$) vs basal levels (BL). These increases were not seen when MS received AoV. In rats with control diet (CD), FE rose SBP from week 4 ($p<0.0001$) vs BL, maintaining this increase in weeks 8 and 12 ($p<0.0001$). In MSFE rats SBP was increased vs BL at 8 ($p<0.0001$) and 12 ($p<0.0002$) weeks. Respect to CD, MS increase SBP at 4 ($p<0.0047$), 8 ($p<0.0001$) and 12 ($p<0.025$) week. AoV prevents SBP rise caused by MS from week 8 ($p<0.020$). FE increased SBP in 4 ($p<0.0021$), 8 ($p<0.0009$) and 12 ($p<0.0008$) week vs CD.

MSFE rose SBP vs CD in week 8 ($p<0.0001$). No differences were observed between SMFE and FE at all times. Basal ventricular function did not change between CD and treated groups. Contractile reserve was attenuated in MS and MSFE ($p<0.05$) vs CD. Ventricular relaxation rate was lower in MS, MSFE and MSAoV in response to isoproterenol. Conclusion: MS and FE load alone or in the presence of MS alter the cardiovascular system, inducing a rise in SBP and an impaired ventricular function, evidenced by contractile reserve and ventricular relaxation. AoV counteracts some of these effects, suggesting that proinflammatory condition as FE overload or SM induce cardiovascular damage that can be reverted by AoV.

47. (025) **ASSESSMENT OF CARDIOVASCULAR SAFETY IN MEDIUM PRESSURE HYPERBARIC OXYGENATION THERAPY**

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The "hyperbaric oxygenation therapy" (HBOT) is currently used as an adjunct therapy in many pathologies. Its therapeutic action is based on that hyperoxia at high pressure (2.4 atm) induces vasoconstriction and reduction of inflammation among others effects.
Recently, a more accessible Medium Pressure Hyperbaric Oxygenation therapy" (mHBOT) at 1.4 atm is also used as therapy that resulted equally effective at a lower cost.
However, up to date no studies have been carried out to support the cardiovascular safety of both treatments. For this reason, we studied the cardiovascular safety of mHBOT in rats subjected to a protocol equivalent to that applied in humans.
Male Sprague Dawley rats were submitted to 30 sessions of 60 min in a hypebaric chamber at 1.44 atm. and 100% O_2. Isolated hearts were perfused through aorta at 37ºC, paced at 3 Hz, and exposed to 30 min ischemia (I) followed by 45 min reperfusion (R). Simultaneous mechanical and heat measurements and the heart damaged area (trifeniltetrazolium) were evaluated.
Also aorta rings were superfused and the noradrenaline response was analyzed.
Hearts from mHBOT-treated rats showed an increase in resting pressure (RP) during ischemia ($p<0.05$) but no changes were observed in R.
An improvement ($p<0.05$) in post ischemic contractile recovery was observed in hearts from mHBOT-treated rats (65.4±12.9%) respect to control ones (33.5±6.1%) at 45 min R.
mHBOT did not alter total heat rate (Ht), but an increase ($p<0.05$) in contractile economy during R (120.9±20%) respect to control (53.2±8.9%) at 45 min was observed. Also mHBOT reduced the heart damage area induced by I/R.
Furthermore, arteries from mHBOT-treated rats showed similar response to noradrenaline than controls.
Conclusion: the mHBOT cardioprotects hearts from I/R injury acting as a preconditioning agent. The use of this therapy is safety for the cardiovascular system.

48. (067) **MITOCHONDRIAL DYNAMICS IN CARDIAC TISSUE DURING ACUTE ENDOTOXEMIA**

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Mitochondrial dynamics emerges as a compensatory mechanism in sepsis and endotoxemia, contributing to organ-cellular redox and energy management. In this work, we aimed to elucidate mitochondrial dynamics (fusion, fission, biogenesis and mitophagy) as a fundamental effector of cardiac tissue energy management in an experimental model of endotoxemia. Female Sprague-Dawley rats were subjected to low-grade endotoxemia (ip injection of LPS 0.5 mg kg -1 body weight) and severe endotoxemia (ip injection of LPS 8 mg kg -1 body weight) for 6 or 24 h. TEM analysis after 6 h low-grade endotoxemia showed higher number of mitochondria per cardiac tissue area and a shorter diameter. Also, structures compatible with damaged mitochondria were observed. In severe endotoxemia, highly damaged mitochondrial structures were observed, being these results associated with a 20% decrease in mitochondrial inner membrane potential, suggesting organelle dysfunction. Based on these results, expression of the main proteins involved in mitochondrial processes of fusion (OPA-1), fission (DRP-1), biogenesis (PGC-1α and mtTFA) and mitophagy (Pink-1 and Parkin), were studied. Low-grade endotoxemia exhibited a decrease in DRP-1 expression levels whereas Pink-1 expression was increased, both at 24 h treatment. Severe endotoxemia showed increases in OPA-1 and Pink-1 expression after 6 h treatment. The observed results suggest that the severity of endotoxemia relates to the degree of mitochondrial dysfunction and structural damage, and is linked to changes in mitochondrial dynamics as repairing processes. Our work presents novel results that contribute to elucidate the mechanisms by which endotoxemia, energy management, and mitochondrial architecture interact in cardiac tissue, arising this triade as a target to base future therapeutics for preserving this organ from inflammatory and oxidative damage.

49. (211) **EFFECT OF CARDIAC RENIN ANGIOTENSIN SYSTEM AXIS ON MALE AND FEMALE RATS EXPOSED TO MODERATE ZINC RESTRICCION DURING FETAL AND POSTNATAL LIFE.**

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Introduction: We have demonstrated that zinc deficiency during pregnancy and postnatal life induced cardiovascular alterations in males. Females did not show significant heart alterations.
Objective: Evaluate cardiac renin angiotensin system in adult rats exposed to zinc deficiency during growth.
Methods: Female Wistar rats fed either control (C30ppm) or low zinc (L8ppm) diets from pregnancy to offspring weaning. C male and female offspring continued with control diet (Ccm;Ccf respectively), whereas L male and female offspring fed control (Lcm;Lcf) or low zinc (Llm;Llh) diets. At day 81 we measured in left ventricle: Angiotensin (Ang) II, Ang(1-7) levels by radioimmunoassay, Angiotensin converting enzyme 1(ECA1),ECA2 and AT1 receptor (AT1R) expression by RT-qPCR; AT2R and AT1R expression by western blot and immunohistochemistry. Values are means±SEM, Two-way ANOVA, Bonferroni post-test.
$^{*}p<0.01$vsCcm;$^{§}p<0.01$vsLcm;$^{‡}p<0.01$vsLlm; $^{α}p<0.05$vsLcf;$^{δ}p<0.05$vsCcf,n=6.
Results: Llm and Lcm exhibited increased levels of AT1R mRNA and protein expression, and an increased in AngII(Ccm:4.4±1,2;Llm:12.0±1.9*§;
Lcm:2.6±0.2;Ccf:4.6±0.7;Llf:9.1±0.9^{δα}; Lcf:6.5±2.1 nM/g).
Immunohistochemistry showed similar results in AT1R expression (Ccm:10.0±0.1;Llm:15.2±0.1*;Lcm:14.3±0.5*;Ccf:8.8±0.1;Llf:12.6±0.5^{δα};
Lcf:8.6±0.02 % of positive staining per area) and an increase of AT2R in female rats(Ccm:8.7±0.4;Llm:10.51±0.02;Lcm:11.2±0.3;Cch:15.9±0.2*;Llh:16.53±0.02‡;Lch:15.8±0.4§ % of positive staining per area). No changes were observed in Ang(1-7) content and ACE expression.
Conclusions: Zinc restriction during prenatal and postnatal growth exacerbated the cardiac Ang II-AT1R axis in adult male rats. An adequate zinc diet during postnatal life could not reverse these ef-

fects. This would contribute to morphological and functional cardiac alterations. Females showed higher protected status. These data strengthen the importance of diet optimization to prevent cardiac diseases.

50. (212) **THE BISPHOSPHONATE ALENDRONATE EXERTS A PROTECTIVE ACTION ON VASCULAR RESPONSE INDUCED BY STRESS CONDITIONS.**

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During menopause, women exhibits high prevalence of cardiovascular and bone diseases. Bisphosphonates (BP) represent one of first-line drugs mainly applied for postmenopausal osteoporosis treatment. The aim of this work was to investigate the action of the BP alendronate (ALN) on vascular response to extracellular stress, focusing our attention on alterations in cell proliferation and migration patterns and, on the ability to remodel by angiogenesis. Primary cultures of vascular smooth muscle cells (VSMC) and endothelial cells (EC) isolated from murine aorta were used. In order to induce stress conditions, cells were incubated in a pro-osteogenic medium (OM) or exposed to the pro-inflammatory agent LPS. Using the MTT assay, we showed that 1 μM ALN completely or partially suppressed VSMC proliferation induced by LPS (31%, $p<0.02$) or OM (108%, $p<0.001$), respectively. Employing wound healing assay, we found that the BP exhibited similar cell migration pattern than control group, meanwhile the stressors enhanced cell motility (20%; 25%, LPS; MO, respectively, $p<0.02$). The presence of ALN reversed the stimulation of cell migration induced by LPS or OM. Tube formation assay was used to assess angiogenesis. Total length of the tubes generated from EC seeded on fibrin matrix was quantified. We found that, 1 μM ALN stimulated new capillaries formation (30%, $p<0.05$) in a VEGF dependent manner, since the presence of the VEGF receptor antagonist SU5416, completely abrogated the angiogenic stimulus of the BP. Under stress conditions (LPS or MO), the proangiogenic action elicited by ALN was not affected. We also demonstrated that ALN increases VEGF synthesis by EC (36% vs control, $p<0.05$, ELISA test). In conclusion, the evidence presented suggest that, besides its main action of bone remodeling, ALN could display a protective effect on vascular homeostasis promoting angiogenesis and preserving cell growth and motility, when blood vessels are exposed to environmental stress.

51. (325) **MITOCHONDRIAL H_2O_2 METABOLISM MODIFICATION AS EARLY ADAPTIVE STRESS RESPONSE IN HEART IN DIABETES**

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Background: Insulin signaling is essential for normal mitochondrial function in cardiomyocytes. Hydrogen peroxide (H_2O_2), the main metabolite effective in redox sensing, signaling and regulation, has been described as an insulinomimetic agent. While physiological H_2O_2 steady-state concentrations ($[H_2O_2]_{ss}$) are ~1–10 nM, higher $[H_2O_2]_{ss}$ lead to adaptive stress responses, and supraphysiological $[H_2O_2]_{ss}$ (>100 nM) result in oxidative distress.
Aim: To study heart mitochondrial H_2O_2 metabolism in an early stage of type 1 diabetes.
Methods: Diabetes was induced by Streptozotocin (single dose, 60 mg/kg, ip) in male rats (glycemia at 72 h: 130 ± 5 (C); 415 ± 23 (DM) mg/dl) and animals were sacrificed at day 10. The hearts were removed and mitochondrial function, H_2O_2 metabolism, and lipid peroxidation were evaluated.
Results: State 3 respiration sustained by malate+glutamate (23%) and complex I activity (17%) were reduced in DM rats. Neither the membrane potential nor ATP production were different between groups. Mitochondrial H_2O_2 production was 117% higher in DM rats, and this increase was accompanied by the enhancement in the H_2O_2 detoxification enzymes activities and expressions: catalase (200% and 233%) and glutathione peroxidase (26% and 42%), leading to $[H_2O_2]_{ss}$ ~50 nM. Although $[GSSG+GSH]_{mitochondrial}$ was lower in diabetic rats, there was no difference in GSH/GSSG. Mitochondrial lipid peroxidation, evaluated from 4-HNE expression, was 45% higher in DM than in C animals.
Conclusions: The maintenance of mitochondrial membrane potential, ATP generation and GSH/GSSG suggest the absence of irreversible damage at this early stage of DM 1. The increase in mitochondrial $[H_2O_2]_{ss}$ above the physiological range, but still below 100 nM seems to be part of the adaptive response triggered in cardiomyocytes due to the absence of insulin.

52. (330) **VOLUNTARY WHEEL RUNNING EFFECT IN TRX1-OVEREXPRESSION MICE SUBJECTED TO ISQUEMIA/REPERFUSION INJURY.**

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Exercise training reduces myocardial injury caused by acute ischemia/reperfusion (I/R). Thioredoxin-1 (Trx1) maintains the cellular redox status and decreases the infarct size in I/R injury. However, it is not fully understood its role in exercise mice. The aim was to study if Trx1is involved in the exercise cardioprotection mechanism. Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative mutant (DN) of Trx1 were used, mice were divided in exercise group (E) and sedentary group (S). Mice were placed in cages fitted with running wheels during 4weeks. After the exercise-training period, mice hearts were subjected to 30min of I and 120min of R (Langendorff technique). The assessment of the infarct size was performed using TTC. Also, heart rate variation (ΔHR%) and mice, heart, and soleus weight was measured. Transverse muscle sections of soleus muscles were stained with H&E and the cross-sectional area (CSA) of myofibers were measure. Data were expressed as mean±SEM and $p<0.05$ was considered statistically significant. n= 4 each group.
Training was confirmed by HR variation. The change in body weight at the fourth week of training in transgenic mice was comparable with Wt mice. Soleus weight showed similar values in S groups (Wt:0.42mg/mm±0.01; Trx1:0.44mg/mm±0.04; DN:0.40mg/mm±0.02), but there was a tendency to increase in E groups (Wt: 0,62mg/mm±0,03; Trx1:0.51mg/mm±0.03; DN:0.53mg/mm±0.04). Those results were confirmed by H&E that showed a preliminary increased in CSA in the E groups compare with S groups. Trx1 overexpression reduces infarct size (27.4%±2.2 vs Wt:54.1%±3.2) but this cardioprotection is lost in DN (50.2%±4.0). However, voluntary exercise manages to reduce the infarct size in Wt (26.0%±2.9 vs S-Wt) mice, without showing changes in the Trx1 (26.2%±3.9) and DN mice (45.7%±6.9). In conclusion, we found that Trx1 could be involved in the cardioprotection mediated by exercise.

53. (331) **THE LONG NON-CODING RNA *DAGAR* IS REGULATED BY M^{6}A MODIFICATION AND BOTH PATHWAYS ARE IMPORTANT FOR SMOOTH MUSCLE CELL PLASTICITY**

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Adult vascular smooth muscle cells (SMCs) change between a contractile-differentiated and a proliferative-dedifferentiated phenotype in response to environmental cues constituting a key factor in the onset of cardiovascular diseases. Long non-coding RNAs (lncRNAs) and mRNA modifications, in particular N-6-methyladenosine (m^6A), regulate several physiological and pathological processes, but little is known about these molecules in SMC biology. Our aim was to study both the role of lncRNAs and the m6A machinery during SMC differentiation. By using an *in vitro* model of primary human pulmonary artery SMCs phenotypic modulation and RNA deep sequencing we found several regulated lncRNAs. RT-qPCR and Northern blot confirmed the dramatically increase of a candidate during differentiation, which we refer to as Differentiation And Growth Arrest Related (DAGAR). DAGAR was downregulated in SMCs during tumor necrosis factor (TNF)-induced de-differentiation and in total RNA from pulmonary arteries of patients with COPD compared to non-smoker patients. DAGAR Knockdown by siPools led to differentiation defects and increased SMC proliferation. RNA-Protein affinity purification identified the m^6A machinery bound to DAGAR. Interestingly, DAGAR was enriched in m^6A-RNA immunoprecipitation. Accordingly, we found a marked downregulation of YTHDF proteins during SMC differentiation, which is consistent with DAGAR increase. YTHDF2 immunoprecipitation followed by RNA deep sequencing displayed an enrichment of mRNAs associated to SMC fundamental processes, including smooth muscle myosin heavy chain (MYH11), the most specific SMC marker, and members of the TGFβ, PDGF and VEGF pathways. Remarkably, knockdown of YTHDF2 by siPools induced a significant increase of DAGAR and SMC marker gene expression. We conclude that the lncRNA DAGAR and the m6A reader YTHDF2 contribute to the regulation of SMC plasticity and differentiation programs.

54. (379) **LIPOIC ACID TREATMENT IMPROVES MATERNAL OUTCOME, PLACENTAL DEVELOPMENT AND FETAL GROWTH IN A RAT MODEL OF PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION**

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Superimposed preeclampsia (SPE) occurs in 25-40% of pregnancies affected by chronic hypertension and is associated with poor maternal and perinatal outcomes. Our previous studies showed that pregnant Stroke-prone Spontaneously Hypertensive Rats (SHRSP) develop a SPE phenotype, associated with exaggerate activation of placental oxidative stress pathways. The present study aimed to evaluate the therapeutic potential of lipoic acid (LA), a potent antioxidant, in this model.

Methods: We established timed syngeneic pregnancies using SHRSP and Wistar Kyoto (WKY) females (8-10 weeks old, N=10 animals/day), with vaginal plug detection denoted as gestation day (GD)1. Dams were assigned to four groups: LA-treated WKY / SHRSP, injected 25mg/kg BW LA i.p. on GD1, GD8 and GD12 and control WKY / SHRSP, injected saline with the same protocol. Systolic blood pressure (SBP) profiles were determined using a tail-cuff device. Following collection of blood and 24h urine samples, animals were euthanized and implantation sites isolated for morphological analyses. Fetal and placental weights were recorded on GD20. Statistical analyses were run with ANOVA and Bonferroni post-hoc tests, using Prism v 8.0. Statistical significance was set at $p<0.05$.

Results: LA treatment prevented the pregnancy-dependent SBP increase in SHRSP and decreased significantly maternal proteinuria on GD18. Furthermore, LA significantly increased SHRSP fetal weights and ameliorated the asymmetric growth restriction phenotype, as demonstrated by the decreased cephalization indexes observed on GD20. The placental phenotype was also improved in LA-treated SHRSP, displaying increased numbers of glycogen trophoblasts in the junctional zone on GD14 together with increased placental weights on GD20.

Conclusion: LA treatment prevented development of the maternal syndrome and ameliorated placental function and fetal growth, emerging as a novel tool for prevention of SPE in pregnancies affected by chronic hypertension.

55. (443) ***TRYPANOSOMA CRUZI* INFECTION PROMOTES CELLULAR PROCESSES ASSOCIATED WITH AORTIC STIFFNESS**

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Chagas disease (CD), caused by *Trypanosoma cruzi*, affects 6 million people worldwide. Recently, increased aortic stiffness (IAS) in CD patients classified as indeterminate without cardiac complications was observed by sensitive imaging. IAS is a predictor of cardiovascular disease (CVD) risk. Smooth muscle cells (SMC) phenotypic alteration during vascular injury is associated with reduced elasticity, impaired distensibility, fibrosis, and IAS. The aim of this work was to evaluate SMC alterations associated with IAS during *T. cruzi* infection. Thoracic (Thor), abdominal aorta (Abd), and brachiocephalic artery (BCA) were obtained from both control non-infected (NI), and acute *T. cruzi* infected BALB/c mice (INF). RT-qPCR, immunofluorescence, and FACS revealed alterations related to SMC phenotypic switch from a contractile to a synthetic state in arteries of INF mice, with loss of SMC markers expression such as α-SMA, calponin, transgelin, and Myh11. In addition, we found increased expression of *klf4*, Ki67, and β-catenin which are associated with SMC and macrophage (Mo) proliferation. Recently, it has been demonstrated that SMC can transdifferentiate into Mo*-like* cells. Mo functional phenotype in aortic segments was principally M2-resident-*like* CD206+Arg-1+, despite *T. cruzi* presence. Only Mo in inflammatory foci close to vasa vasorum of Thor from INF mice were CD206+iNOS+ . We used α-SMA+ and F4/80+CD11b+ as SMC and Mo populations, respectively. We found α-SMA+ cells F4/80+CD11b+, and *viseversa*, only in arteries from INF group. Using confocal microscopy, we found cells co-expressing α-SMA+ and the Mo marker CD68 in the media, and in the adventitia of INF mice. Our findings suggest that the infection induces SMC dedifferentiation and transdifferentiation associated with IAS promotion. This knowledge is important to review the integration of indeterminate CD patients on the risk list and comorbidities of CVD, and promote either preventive or therapeutic strategies.

56. (461) **MATHEMATICAL ANALYSIS OF CAROTID ARTERIAL SYSTEM MORPHOLOGY IN PATIENTS WITH VASCULONERVOUS DISEASE USING HIGUCHI ALGORITHM FOR PROGNOSTIC PURPOSES.**

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Cerebrovascular diseases are the second leading cause of death within non-communicable diseases, according to the World Health Organization. The complex system morphology of blood vessels could be studied by mathematical algorithms to obtain fractal parameters: Fractal Dimension (FD) and predictive determination coefficient (R^2) which would indicate the irregular distribution of the vessels in space and their adaptation ability and interaction with the environment, respectively. The aim of this work was to analyze the encephalic carotid arterial system anatomy behavior through Higuchi Algorithm (HA) in patients with neurovascular diseases for prognostic purposes. Observational, cross sectional and descriptive study. Middle Cerebral (MCA), Anterior Cerebral (ACA) and Internal Carotid (ICA) arteries were analyzed in 75 angio-CT images of patients with diagnosed neurovascular pathology, mean age 36.25 years old ± 15.95, 67% women and 33% men, chosen randomly. COREL-Draw, Phillips DICOM Viewer 3.0, Frakout! and Excel spreadsheet were using for HA application. FD and R^2 were determined, values below 0.8 would indicate loss of fractal properties. The results were expressed as median (M) and standard deviation (±). Pearson's correlation coefficient (r) was obtained between FD and R^2 established by arterial vessels studied. Results: FD (MCA): M=0.5±0.33, R^2 (MCA): M=0.88±0.18; FD (ACA): M=0.38±0.23, R^2 (ACA): M=0.76±0.28; FD (ICA): M=0.32±0.25, R^2 (ICA): M=0.64±0.29. Correlation for MCA: r=0.64 ($p<0.0005$), ACA: r=0.81 ($p<0.0001$) and ACI: r=0.75 ($p<0.0001$). Conclusion: HA reveals structural changes and maladjustment in the morphology of arteries studied. HA could evaluate structural changes in patients with neurovascular disease, helping to predict new possible clinical events.

57. (602) **DIFFERENTIAL EFFECTS OF THE ETHANOL ACUTE OR CHRONIC EXPOSURE ON THE EARLY HYPOXIC VENTILATORY RESPONSE (HVR) IN RAT NEONATES**

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Early ethanol exposure disrupts neonatal respiratory patterns and it has been suggested as risk factor associated with the Sudden Infant Death Syndrome. Ambient hypoxia acts as an environmental stressor eliciting breathing adaptations that may be altered by the EtOH exposure. However, the specific effects induce by chronic, acute or the combination of them EtOH intoxication are not clearly understood. In an animal model equivalent to the 3rd human gestational trimester, breathing frequencies and apneas were recorded against an intermittent hypoxic experience as a function of EtOH pre-exposure and/or acute EtOH intoxication. Pups pre-exposed to 0.0 or 2.0g/kg of EtOH (DPs 3-5-7, ig) were evaluated at DP9 in sobriety-0.0g/kg- or under the state of EtOH intoxication-2.0g/kg-. Breathing rates and apneas were recorded through whole body plexismography during 35 minutes [5 min of initial normoxia, followed by 3 episodes of hypoxia (O2 8%) of 5 min, separated by periods of recovery-normoxia of the same duration].
First acute EtOH intoxication diminished the hypoxic ventilatory response (HVR) during the test (p=0.034) relative to it expressed in pups never intoxicated. The prior experience with the drug significantly modified the HVR patterns, as follow: in sobriety, EtOH pre-exposed pups exhibited a depressed HVR relative to vehicle pre-exposed pups. On the contrary, under the state of intoxication, EtOH pre-exposed pups elicited an exacerbated HVR when were defied by hypoxia respect to vehicle pre-exposed pups. With regard to apneas, an increase in the number of apneas was triggered by both, the first acute EtOH intoxication or by the history with the drug in sober pups (p= 0.017). In summary, specific HVR alterations and apneic episodes occurrence were observed in neonates depending on the type of EtOH exposure received (acute or chronic). These results emphasize the complexity of the disruptive EtOH effects upon breathing at this early and critical stage of development.
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ENDOCRINOLOGÍA

58. (003) **GENE EXPRESSION PROFILE DURING PITUITARY DEVELOPMENT IN A TWO-DIMENSIONAL MONOLAYER DIFFERENTIATION PROTOCOL FROM HUMAN INDUCED PLURIPOTENT STEM CELLS**

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Multiple pituitary hormone deficiencies (MPHD) can be caused by mutations in several transcription factor genes in mouse and human, including *Prop1, Pou1f1, Hesx1, Sox2* and other genes involved in early pituitary embryogenesis. In order to study this process of disease development, we aimed to generate a human *in vitro* model of embryonic pituitary as a robust tool for functional testing of genetic variants found in MPHD patients. We hypothesized that combining different stimuli and differentiation protocols *in vitro* [1], [2], it is possible to induce fully differentiated hormone-pituitary cells from human induced pluripotent stem cells (iPSCs). We cultured the iPSCs in the presence of signaling factors involved in pituitary development including Bone Morphogenetic Protein 4 (BMP4), the Smoothened Agonist activator of Sonic Hedgehog pathway (SAG), and Fibroblast Growth Factor 2 (FGF2), for 15 days. During the entire protocol, cell morphology was observed and registered under the microscope, and cellular extracts corresponding to days 0, 4, 7, and 15 of the protocol were collected to assess gene expression by qRT-PCR. We observed an increase in the expression of representative markers of pituitary differentiation (*PITX2, SIX1, HESX1, OTX2*) and a decrease in the expression of pluripotency markers (*NANOG, OCT4*) over the days of treatment compared to control iPSCs (cultured with media only), suggesting that the protocols were effective in their cell specification. An interesting finding was the increase in mRNA levels of *FOXA2* at days 7 and 15 of the protocol, a novel gene in the etiology of MPHD, poorly characterized in human pituitary development and found mutated in one case of MPHD from our Argentinean cohort [3]. This study establishes a new approach to study protein's role in pituitary progenitor cell regulation and offers new candidate genes for MPHD that remain unexplained.
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59. (005) **INFLUENCE OF PRENATAL AND NEONATAL EXPOSURE TO PHTHLATE ON THE SEXUAL BEHAVIOR AND ACTIVITY OF MALE RATS.**

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The endocrine disruptor phthalate (DEHP) has estrogenic and / or anti-androgenic activity. We have previously demonstrated its anxiogenic action in male rats dependent on the androgenic level. **Objective:** to evaluate the effect of DEHP on sexual behavior parameters in adult male rats. **Materials and Methods:** Male rats were exposed to DEHP (n=14) during their intrauterine development and lactation at a dose of 30 mg/kg/d administered in drinking water to the mothers. Controls (C) only received the vehicle (n=13). Between 150 and 180 days of age, the sexual behavior tests were carried out

by placing a sexually virgin male with a young female castrated with estrogen and progestational replacement in a transparent plastic cage, under controlled environment temperature and red light below 20 lux. Continuous filming and recording (BORIS program) were performed during 30 minutes at night (20 PM to 05 AM), to evaluate number of mounts (NM), number of intromission (NI), number of ejaculations (NE), mounting latency (LM), intromission latency (LI), ejaculatory latency (LE). A significance of $p<0.05$ was considered. **Results:** The total NM did not differ between DHEP and C groups. The NI was lower in DEHP compared to C (C: 26.92 ± 0.5 vs DHEP: 25.21 ± 0.5) ($p<0.025$). The NE was lower in DEHP compared to C (C: 1.769 ± 0.12 vs DHEP: 1.071 ± 0.07) ($p<0.0001$). The LM was slower in DEHP compared to C (C: 55.32 ± 2.0 vs DHEP: 96.30 ± 9.0 seconds) ($p<0.001$). The LI was slower in DEHP compared to C (C: 55.32 ± 2.0 vs DHEP: 98.36 ± 8.9 seconds) ($p<0.0006$). LE was slower in DEHP compared to C (C: 823.6 ± 23.5 vs DHEP: 1010.0 ± 42.25 seconds) ($p<0.0009$). **Conclusion:** Exposure to DEHP during the early period of development leads to a modification of sexual behavior in males during their adulthood, evidenced in a delay in the onset of sexual activity and ejaculation time and less sexual activity.

60. (007) **ACTION OF NITRATES PRESENT IN GROUNDWATER WATER ON THE EXPRESSION OF THE NIS TRANSPORTER DURING THE METAMORPHOSIS OF XENOPUS LAEVIS.**

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The exposure of amphibians during their larval stage to thyroid disruptors such as nitrates, produces body morphological changes dependent on thyroid hormone and also thyroid histological alterations. **Objectives:** to evaluate the thyroid disrupting action of nitrates on the expression of the cotransporter NIS. **Materials and method**: Xenopus Laevis larvae (T: 22 ± 2ºC, pH: 7 to 7.8 and light-dark cycle: 12-12hs) immersed in water from: a) filtered net: **Control (C)** (n=6), b) underground water with a nitrate concentration of 83 mg/l (INTI): **Exposed (E)** (n=6) and c) filtered network plus 0.007mg/l of potassium perchlorate: **Positive Control (CP)** (n=6). **Results:** During the 58NF and 60NF metamorphosis stages, it was observed that the level of NIS expression is higher in decreasing order in E>CP>C ($p<0.05$). The values recorded for the means measured in optical density, in stage 58NF were: (C) 680.7 ± 196.9, (E) 1251.2 ± 702.9, (CP) 1059 ± 592.8 and in stage 60NF: (C) 1298.3 ± 195.1, (E) 1794.5 ± 629.1, (CP) 1420.2 ± 1118.7. However, in stage 62NF, a decrease in the expression levels of the NIS transporter was observed, being greater in decreasing order in C>CP>E ($p<0.05$), observing that in some animals it stopped expressing itself. The values recorded for the means measured in optical density were for this stage: (C) 1495.8 ± 1016.9, (E) 49.3 ± 26.2, (CP) 129.9 ± 93.1. **Conclusion:** during the metamorphosis of amphibians exposed to the e ndocrine disrupting action of nitrates transported by groundwater, the expression of the NIS transporter is quantitatively affected in critical stages of metamorphosis such as prometamorphosis and climax. This being a possible compensation mechanism triggered by the alteration of the correct metamorphosis process caused by hypothyroximemia, which generates the interference exerted by nitrates in the uptake of iodine in the larvae.

61. (009) **DIFFERENCES IN SEXUAL DEVELOPMENT (DSD) AND PRENATAL GROWTH. ANALYSIS OF THE PREVALENCE OF BEING BORN SMALL FOR GESTATIONAL AGE (SGA) AND ITS CORRELATION WITH BIOCHEMICAL AND PHENOTYPIC CHARACTERIZATION (IN 46 XY, DSD)**

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Context: DSD are a group of rare and heterogeneous congenital conditions in which the development of chromosomal, gonadal, or anatomic sex is atypical. Specific molecular diagnosis is identified in only about 40-50% of cases. Associated conditions, specially being born SGA, have been reported with a higher prevalence than expected for general population. The relationship between the presence of genital abnormalities and intrauterine growth restriction is unknown.
Objective: to analyze the prevalence of being born SGA in a cohort of DSD patients evaluated in a single tertiary pediatric center between 2000-2021, and its relationship with karyotype, molecular diagnosis, and clinical phenotype.
Methods: DSD patients were classified according to karyotype. External genital score (EGS), gestational age (GA), birth weight (BW), length (BL), and molecular diagnosis were evaluated. BW and/or BL standard deviation scores were calculated according to gestational age (Intergrowth21).
Results: A total of 556 DSD patients were available: 187 (33%) DSD 46,XY, 207 (37%) DSD 46,XX, and 162 (29%) chromosomal DSD. Molecular diagnosis was achieved in 64% (258/403) of 46,XY and 46,XX DSD patients. SGA was found in 25.4% of DSD 46,XY with a higher frequency in those without molecular diagnosis, and with apparently normal testicular function (Fisher test $p<0.05$). EGS did not correlate neither with GA nor BW (p ns).
Conclusions: The frequency of being born SGA in 46,XY DSD patients without molecular diagnosis and with no specific disorders of undermasculinization was higher than reported in Latin-American population (9%). Factors involved in early embryonic growth and development, and in gonadal differentiation, could mediate the association between being born SGA and DSD in humans. Further studies to clarify etiological diagnosis are needed.

62. (055) **INSULIN RECEPTOR EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS AS AN EARLY BIOMARKER FOR GLUCOSE METABOLISM AND ADIPOSITY IN MOTHER-CHILD DYADS**

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Rationale: early detection and prevention of diabetes and obesity is needed. Peripheral-blood-mononuclear-cells (PBMC) are a source of molecular biomarkers reflecting metabolic status.
Objective: to search associations between anthropometric and glucose homeostasis variables, including insulin receptor (InsR) gene expression in PBMC, in mothers with pregestational normal-weight (NW, n=31) and overweight/obesity (OW/OB, n=31) at 10 days postpartum and their children at 6 months of age.
Findings: glucose, insulin, HOMA index and milk insulin did not differ between NW and OW/OB mothers. InsR gene expression was lower in OW/OB mothers and negatively correlated with pregestational body mass index (BMI) ($r=-0.51$, $p=0.011$) and with children zBMI at 10d ($r=-0.40$, $p=0.049$). Anthropometric variables, insulin and InsR did not differ between children from NW or OW/OB mothers. In mothers and children, InsR negatively correlated with adiposity ($r=-0.69$, $p<0.001$ and $r=-0.74$, $p=0.009$, respectively), whereas insulin positively correlated with adiposity ($r=0.35$, $p=0.006$ and $r=0.59$, $p=0.016$, respectively).
Conclusions: In the early postpartum, InsR gene expression in PBMC is a sensitive biomarker of glucose metabolism in mothers with OW/OB without insulin resistance. Adiposity correlated moderately with plasma insulin and strongly with InsR. PBMC are useful for early detection of metabolic disorders.

63. (096) **ROLE OF PITUITARY ACTIVINS AS INHIBITORS OF LACTOTROPH FUNCTION AND PROLACTINOMA DEVELOPMENT**

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A prepuberal ovariectomy (OVX) prevents pituitary hyperplasia in mouse models of prolactinoma indicating the participation of ovarian factors in tumor development. However, a hormone replacement does not restore tumor growth suggesting the participation of other ovarian factors. Pituitary activins inhibit lactotroph function, and activin expression is reduced in prolactinomas. We demonstrated that the loss of gonadal inhibins after OVX improves pituitary activin expression, it could be involved in the inhibition of prolactinoma growth. In the present work, we analyzed if the OVX also recover activin receptor expression and biological function in lactotrophs. We used female mice overexpressing the β subunit of the human chorionic gonadotrophin (hCGβ+) as a model of prolactinoma.
Our results show that the expression of activins, activin-receptors (ActRIIA, ActRIIB, Acvr1B by RTqPCR), and pp38 (double IHC, as a signal of activin biological activity in lactotrophs) are decreased in prolactinomas from hCGβ+ females, concomitant with an increase in Pit1 expression. But surprisingly the protein expression (IHC) of activin receptors, did not decrease in prolactinomas. We observed high expression in lactotrophs from transgenic females but mainly in the cytoplasm, contrary to the plasma membrane localization in lactotrophs from wt pituitaries. A prepuberal OVX prevented all these alterations in the pituitary activin system, even changes in receptors localization.
We conclude that the decrease in pituitary activin expression and biological activity found in hCGβ+ female pituitaries is involved in prolactinoma development. The loss of gonadal inhibins after an OVX recovers activin inhibitory function on lactotrophs preventing prolactinoma growth. Alterations in receptor localization could involve specific proteins (ARIPs) which interact with activin receptors type II and down-regulate their activity by controlling mechanisms of endocytosis (study in progress).

64. (108) **IMPACT OF PHYSICAL AND FUNCTIONAL INTERACTION OF DOPAMINE D2 AND BRADYKININ B2 RECEPTORS. PRELIMINAR STUDY IN HUMAN PITUITARY ADENOMAS**

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Most prolactinomas are effectively treated with dopamine D2 receptors (D2R) agonists. Nevertheless, a subset (~20%) became resistant to the treatment and require extirpation. The molecular mechanisms underlying the escape from dopamine inhibition include alterations in D2R signalling. It has been reported that bradykinin B2 receptor (B2R) is highly expressed in human prolactinomas and can heteromerize with D2R, abolishing G_i signalling of D2R. In the present study, we proposed to assess 1-the physical and 2- the functional interaction of D2R-B2R, and 3- to determine whether those receptors interact in human pituitary adenomas. **1-** By using the NanoBiT protein-protein interaction assay (NB-assay): 1a- we validated the formation of BR2-D2R measuring NanoLuciferase (NL) activity in HEK293T cell line transiently transfected with human D2R and B2R fused to one of the two inactive fragments of the split NL. 1b- we studied the coupling of B2R to G proteins in cells transiently transfected with B2R and G_i, G_s, $G_{12/13}$ or G_q, fused to inactive fragments of the split NL. We found that B2R recruits G_q and G_i after stimulation with B2R specific agonist. **2-** We measured the Ca^{2+} mobilization by Fluo4-NW Assay in HEK293T transiently transfected with B2R alone or in combination with D2R. B2R agonist (100nM) increased intracellular Ca^{2+} in cells expressing B2R alone or B2R-D2R. D2R agonist or antagonist did not alter B2R signal suggesting that the coupling of protein G_q to B2R is not altered in the B2R-D2R heteromer. **3-** B2R-D2R interaction was measured in membrane extracts of human pituitary adenomas by AlphaLisa assay. We found B2R-D2R complexes in prolactinomas and non-secreting tumours, but not in mixed tumours (GH+PRL). We hypothesized that D2R-B2R dimerization is increased in prolactinomas, disturbing D2R signalling by prevailing the D2R-B2R coupling to G_q, promoting resistance to D2R agonists in patients with resistant prolactinomas.

65. (144) **FILAMIN A ALTERS SECRETION, PROLIFERATION AND CELL MORPHOLOGY OF PITUITARY TUMOURS CELLS**

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Filamin A (FLNA) is a promiscuous protein, with a multiplicity of functions, being its canonical role an acting binding protein which is related to cytoskeleton rearrangement, but also having non canonical roles as a scaffold protein related to signal transduction or exerting gene expression regulation.
The role of FLNA expression and function in tumours is described as dual, having found to promote or inhibited tumour progression in a tissue and subcellular localization dependent manner. Despite the progress being made in the past years, FLNA impact in prolactin-secreting pituitary tumours remains elusive, possibly due to the variety of factors that modulate its final action.
This work aims to determine the impact of FLNA expression levels in global cellular processes in the tumoral somatolactotropic cell line GH3.
Transfected GH3 cells for FLNA overexpression (GH3F+) were used. FLNA expression and prolactin secretion levels was analysed by Western blot, FLNA subcellular localization by indirect immunofluorescence (IFI), viability by clonogenic assay, cell cycle progression by flow cytometry, Ki67 index by Immunocitochemistry (ICC), and cellular morphology was performed by flow cytometry analysis and optical microscopy. The statistical analysis used was ANOVA-Tukey.
FLNA localization was found in the nucleus and cytoplasm in GH3 and GH3F+. FLNA overexpression significantly decreased cellular viability (23% less colonies formed) and proliferation capacity (25% less Ki67-labelled cells). Morphological analysis showed double number of pleomorphic cells, longer cellular protrusions and a decrease in granularity and size in GH3F+. Furthermore, PRL secretion was decreased in GH3F+.
These results show that FLNA has a significant global impact in the survival and function of tumoral PRL secreting cells, inhibiting proliferation and secretion, placing this protein as a potential therapeutic target to prolactinomas resistant to conventional treatment.

66. (149) **P53 AND P21 ACTIVATION INDUCED BY THE PRO-OXIDANT EFFECTS OF 17B-ESTRADIOL IN NORMAL AND TUMORAL PITUITARY CELLS**

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It is well known the estrogen effects on pituitary cell growth. Previously we demonstrated the activation of Nrf2 antioxidant pathway in

response to DNA damage triggered by 17β-estradiol (E2). Here we analyzed the impact of E2 on p53 and p21 activation and the damage response in pituitary cells.
The experimental pituitary tumour was induced by subcutaneous implantation of silastic capsules with estradiol benzoate (30mg) for 10 days (E10) in adult male Wistar rats. Control group was implanted with empty capsules. Then, normal and tumoral pituitaries were collected, with cells being cultured and exposed to E2 (1-10-100nM) for 15, 30 and 60min. The p53 and p21 levels were determined by western blot. To analyze the cell population involved in the oxidative damage response activation, PRL or GH/p-Nrf2 expression was evaluated by immunofluorescence. Statistical analysis: ANOVA-Fischer ($p<0.05$).
In tumoral cells, a significant increase in p53 protein was detected at the cytoplasmic level after 15 and 30min of E2 (1nM) treatment. Under higher doses (10-100nM), this response was observed after 30 and 60min. At nuclear level, only increases in p53 was detected at 15min, regardless the dose. The p21 expression showed a similar profile in both subcellular compartments, with significant increases after 15 and 30min of E2 exposure (1nM) and after 30 and 60min after treatment with higher doses. In normal cells, no significant changes were observed in both p21 and p53 expression. The lactotroph tumoral cell number expressing p-Nrf2 significantly increased after 30min compared to high doses of E2. No changes were detected in the expression of p-Nrf2 in somatotroph cells.
In tumoral cells, the pro-oxidant effects induced by E2 trigger the p53 and p21 activation in order to repair DNA damage through the stabilization of Nrf2. This response would mainly have an impact on PRL tumoral cells. These mechanisms could guarantee the cell viability, thus regulating pituitary tumour development.

67. (219) **DXM EFFECT ON WHITENING OF RAT RETROPERITONEAL ADIPOSE TISSUE**

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We previously reported that Dexamethasone (Dxm) inhibited thermogenic process in retroperitoneal Adipose Tissue (RPAT); however, the Dxm effect on conversion from beige into white adipocytes after cold exposure (whitening) remains unknown. Here, we examine if Dxm inhibitory effect on thermogenesis was through mitophagy activation, favoring WAT whitening. First, male rats were divided into four groups: control (CTR) and Dxm (sc injected 0,03mg/Kg/d, DXM) housed at RT for 7 days; and CTR and DXM housed at 4°C for 7 days (CTR-C and DXM-C). RPAT pads were dissected and processed for quantification of genes involved in thermogenic and mitophagic processes (qRT-PCR). Using 2-way ANOVA for statistical analysis, we found that Dxm inhibited the expression of different thermogenic markers (ucp-1, pgc1a and dio2, Interaction $p<0,05$) and increased pink1 and atg12 expression under cold exposure (Interaction $p<0.05$). To evaluate if Dxm modify the whitening process, CTR rats were exposed 7 days at cold temperature and later housed for 2 days at RT, and injected or not with Dxm (DW2d and CW2d, respectively). RPAT was processed for ucp1 and pink-1 quantification. Result showed that ucp1 levels from CW2d and DW2d decreased ($p<0.001$, vs CTR-C), while pink levels from DW2d increased ($p<0.01$, vs CTR-C and CW2d). We also studied the effect of Dxm in *in vitro* differentiated adipocytes. For this purpose, beige adipocytes were incubated or not with 0,25μM Dxm for 48hs (DXM and CTR cells, respectively). In the last 4hs a subset of CTR and DXM cells were incubated with forskolin (10μM, CTR-FSK and DXM-FSK). Cells were then processed to quantify pink-1 and atg12 mRNA levels. We found that DXM increased the expression of both mitophagic markers ($Dxm p<0.05$). Overall, here we described for the first time that WAT browning inhibition by Dxm could be due to an increase in mitophagy-related genes expression, favoring the whitening of WAT. PICT 2019-2787, PICT 2017-2038, PICT 2017-2314

68. (344) **EFFECTS OF PREPUBERTAL CASTRATION ON WHITE ADIPOSE TISSUE THERMOGENESIS**

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Beige adipocytes dissipate energy as heat through the action of uncoupling protein-1 (UCP-1). Cold exposure or β3 adrenergic agonist treatment stimulates beige adipocyte generation. Our aim was to study the effect of prepubertal ovariectomy (OVX) on Retroperitoneal Adipose Tissue (RPAT) pad thermogenesis using *in vivo* and *in vitro* models. On age 27 days, rats were randomly split into two groups, sham-ovariectomized (SHX) and OVX-pair fed (OVX-PF). On age 60-day old, one half of rats were kept at 4°C (C) during 7 days, while the other half was housed at room temperature (RT). Food intake and body weight (BW) were daily registered. At sacrifice, RPAT pads were excised and weighed for the analyses of UCP-1, Pgc-1α, β3AR and DIO-2 gene expressions. Isolated adipocyte precursor cells (APCs), from both groups, were induced to differentiate and examined 6 days after 10 μM forskolin (FSK) addition or not. Later on, APCs from SHX RT animals were cultured in either basal conditions (B, medium alone) or in medium containing E2 (either in the absence or presence of FSK). Gene expression analyses of UCP-1, Pgc-1α and resistin were performed in cultured cells. BW, RPAT mass and circulating triglycerides levels were affected, with lower levels found in OVX-PF C rats. Nevertheless, when RPAT gene expression levels were analyzed, we found that while UCP-1 and DIO-2 diminished that of Pgc-1α enhanced in OVX-PF C group; conversely, β3AR did not vary. Similar data were found in cells from RT groups incubated with FSK. Indeed, a decrease in UCP-1 gene expression and an increase in that of Pgc-1α were observed. In order to find a possible explanation, we noticed that E2 addition to APCs from SHX-RT cells showed a significant increase in both UCP-1 and Pgc-1α expression levels when stimulated with FSK. Considering both, *in vivo* and *in vitro* results, it can be concluded that RPAT pad-derived cells thermogenesis appears to be inhibited by prepuberal OVX (PICT2017-2334 and 2017-2314).

69. (396) **REGULATION OF PITUITARY TUMORS PROLIFERATION INDUCED FOR THE FGF2/FGFR1 PATHWAY**

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The growth factors and their receptors dysregulation could lead to abnormal growth and progression of pituitary tumors. Previously, we demonstrated that the expression of Fibroblast Growth Factor 2 (FGF2) increased in experimental prolactinomas development, suggesting its participation in tumor progression. However, the molecular mechanisms that generate this effect still now unknown. The aim was to determine the role of FGF2, FGF receptor 1 (FGFR1) and MEK-ERK1/2 pathway in the pituitary tumor cells proliferation. Somatolactotroph (GH3) and corticotroph (ATt20) pituitary tumor cell lines were stimulated with FGF2 (10 and 100 ng/mL). The expression of FGFR1 was evaluated by western blot. Cell viability was determined by MTT assay after FGF2 stimulation for 24-48h in medium with or without 10% serum. Proliferative response was analyzed by BrdU uptake for 24h and ERK1/2 phosphorylation by western blot after FGF2 stimulus for 30min. Additionally, the MEK inhibitor PD 98059 (50uM) was used. Statistics: ANOVA-Post-test: Tukey. The FGFR1 expression was higher in GH3 than in ATt20. FGF2 induced a significant increase in cell viability in GH3 at 24 and 48h in both doses, while in ATt20 cells, the cell viability increase ($p> 0.05$) was only observed after FGF2 (100 ng/mL) stimulation for 48h. Considering that FGF2 effect in GH3 was higher, we continue working on this cell line. The expression levels of ERK1/2 phosphorylated increased after FGF2 (10 and 100 ng/mL) stimulus for 30min ($p<0.05$ vs control). Cell viability and BrdU uptake was significantly higher in the cultures treated with FGF2 to both doses in presence of 10% serum, effect that was reverted with PD98059 co-incubation. These findings show that FGF2/FGFR1/ERK1/2 pathway partici-

pates in the increase of proliferation in GH3 lactosomatotroph tumor cells, which present greater expression of FGFR1, effect that was enhanced in serum medium which would be represent part tumor microenvironment in the tissue.

70. (400) **GUT MICROBIOTA ALTERATION IN DIABETIC MICE EXPOSED TO CHRONIC STRESS.**

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Gut microbiota is the group of microorganism (commensal, symbiotic and pathogenic) that we find in our gut. It participates in multiple functions and an association between unbalanced microbiota and several diseases, including diabetes, has been reported. Type 1 diabetes (T1D) is characterized by impaired insulin secretion and it has been recognized the contribution of psychosocial factors in T1D. The aim of the present study is to characterize microbiota alterations in diabetic mice exposed to chronic stress. To induced diabetes, we treated male BALB/c mice with multiple low doses of streptozotocin (stz) and then, the animals were subject to chronic mild stress (CMS) by a daily application of different mild stressors. Fecal samples were collected and genomic DNA was extracted. 16s total bacteria, 16s Bacteriodetes and 16s Firmicultes (most abundant component of the microbiota) were measured by qPCR using specific primers. After 13 weeks of CMS, glycemic levels in diabetic and diabetic + CMS were elevated ($p<0.05$). No significant changes in 16s Bacteroidetes/16s total bacteria and 16s Firmicutes/16s total bacteria were detected but a significant CMS effect was found in 16s Bacteroidetes/16sFirmicutes (Two-way ANOVA, $p<0.05$). Also significant correlations were found between glycemia levels vs 16s Firmicutes/16s total bacteria (Pearson correlation r=-0.63, $p<0.05$) and glycemia levels vs 16s Bacteroidetes/16s Firmicutes (Pearson correlation r=-0.57, $p<0.05$). These results suggest gut microbiota is altered by CMS.

71. (421) **THE PRIMARY CORPORA LUTEA ARE THE MAIN FORCE IN RESTORING THE STEROIDOGENIC CAPACITY IN THE OVARY OF MID-PREGNANT VIZCACHAS (*LAGOSTOMUS MAXIMUS*, RODENTIA)**

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Shortly after beginning the five month-length pregnancy, a natural decline in serum progesterone (P4) triggers reactivation of the hypothalamus-hypophysis-ovary (HHO) axis in vizcachas. Accessory corpora lutea (aCL) developed after this event has been proposed to ensure a successful pregnancy by restoring P4 levels. In this work, we compared the steroidogenic input of primary CL (pCL) vs aCL by histological and morphometric analysis, and by immunohistochemical analysis of the luteinization markers: Star, Cyp19, 3β-HSD, VEGF, FSHR, and LHR in early- (EP), mid- (MP) and term-pregnant females (TP). Non-pregnant ovulated females (NP) were considered as the CL-starting point control group. In EP, the ovaries showed only pCL, whose reactivity levels for the markers mentioned above were significantly lower than those exhibited by the NP group ($p<0.05$, n=5). This is tied to the reported P4 decline at early stages of pregnancy of vizcachas. Once HHO reactivation occurred, the amount of aCL counted in ovaries of the PM group was slightly higher than that of pCL. In addition, luteal cells of both pCL and aCL showed histological characteristics compatible with secretory activity. Towards TP, the structure of both CLs was disorganized, and the luteal cells lost their secretory features, all of which agrees with the reported drop in P4 that precedes parturition. Both CLs showed a significant increased reactivity for Star, Cyp19, 3β-HSD and VEGF in PM. Yet, the luteal area of the pCL was significantly higher than that of aCL ($p<0.05$, n=5). Based on our results, we propose that the LH surge derived from HHO reactivation during mid-pregnancy targets pCL and boost the steroidogenesis. Since antral follicles express LHR, they also respond to the LH stimuli and become luteinized. As its area is about one sixth that of the pCL, aCL surely contribute, but it is pCL that is the main force that restores P4 levels from mid-pregnancy of vizcachas.

72. (464) **ROLE OF ESTRADIOL ON PITUITARY GNRH TRANSCRIPTION PATHWAY FOR LH EXPRESSION IN *LAGOSTOMUS MAXIMUS*.**

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The massive release of pituitary luteinizing hormone (LH) triggers the ovulatory event. This release is regulated by the combined action of gonadotropin-releasing hormone (GnRH) and the steroid hormones, estradiol (E2) and progesterone (P4). At gonadotrophs, GnRH activates a transcriptional pathway that regulates the expression of EGR1 and SF1, both essential transcription factors required for LHβ promoter activation. The plains vizcachas have shown reproductive axis activity during gestation with changes in hormone levels around mid-pregnancy. The aim of this work was to determine the role of E2 in the pituitary expression of Sf-1 and Egr-1 involved in GnRH transcriptional activation pathway for LH expression. Different *in vivo* and *ex vivo* approaches were developed (N=4/group): 1- Non- pregnant females were ovariectomized (OVX) and treated two days with low (OVX-2) or five days with high (OVX-5) doses of E2 (5ug/kg or 15ug/kg, respectively). Sham and OVX animals were used as control groups. 2- Pituitaries of non-pregnant females were probed in a pulsatile assay under GnRH or GnRH+E2 supplementation. 3- Pituitaries of non-pregnant females were probed in a pulsatile assay under different conditions: a) CTL, b) PPT (ERα agonist) + CYCLO (ERβ antagonist), c) MPP (ERα antagonist) + WAY (ERβ agonist), d) MPP+CYCLO. LH release was measured by RIA, whereas pituitary GnRHR, Egr-1 and Sf-1 expression was studied by Western Blot. We observed that E2 induced significant changes ($p<0.05$). E2 negatively modulated the expression of Sf-1 and Egr-1, accompanied by a concordant decrease of LH released. This result was reverted when pituitaries were treated with both ER antagonists. In addition, high LH released levels were determined exclusively by ERα agonist supplementation. These results show that E2 regulates LH release acting throughout Egr-1 and Sf-1 and suggest ERα as the main involved E2 receptor (Fundación Científica Felipe Fiorellino, PIP110/14, PICT1281/2014).

73. (480) **FOLLICULOSTALLATE CELLS AND LACTOSOMATOTROPHS INTERACTION CAN MODULATE TUMOR PROPERTIES *IN VITRO*.**

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Folliculostellate cells (FSC) are a non-endocrine, heterogeneous cell population of the anterior pituitary that interact with endocrine populations through their long cytoplasmatic processes and by paracrine signaling. The purpose of this work was to study how these cells interact with lactosomatotrophs and how this interaction may impact on tumor features as proliferation, angiogenesis and hormone production. We used a FS cell line (TtT/GF) and a lactosomatroph tumoral cell line (GH3). To study the effect of released factors, we cultured each cell line in complete media and then changed to low serum media (LSM) for 12 hours. This conditioned media (CM) or LSM alone (Control) were used as treatments. Additionally, to study cell-cell interaction, we grew cells alone or in combination in a 8:1 proportion (GH3:TtTGF). When GH3 cells were treated with FSCs' CM, cell viability (MTT assay, $p<0.05$) and cell proliferation (FC, $p<0.05$) were increased. Furthermore, and increase in S phase

of the cell cycle was observed (FC PI staining, p<0.01). No differences were found in *Prl* or *Gh* synthesis (RTqPCR, ns) or angiogenic factors release (ELISA, ns). When FSC were cultured with GH3's CM, we observed an increase in cell viability (MTT assay, p<0.05) and proliferation (CF, p<0.05). However, a decrease in VEGF production (ELISA, p<0.05) and *TNF*α synthesis (RTqPCR, p<0.05) was observed. In coculture experiments, an increased number of GH3 cells respect to isolated GH3 cells was found (FC, p<0.001). Nevertheless, a decrease in the percentage of GH3 cells in S phase (FC PI staining, p<0.05) as an increase in late apoptosis was determined (FC AnnexinV-PI, p<0.05). *Gh* synthesis was not modulated but *Prl* was increased in cocultured GH3 cells respect to isolated GH3 cells (RTqPCR, p<0.01). Our results suggest that both CFS and GH3 cells can release mitogenic factors, that tumor cells can modulate FS function and that a complex regulation exists when both cells grow together and interact.

74. (483) **PITUITARY PROLACTIN RECEPTOR FUNCTION REVEALED BY ITS CRE/LOXP MEDIATED DELETION IN LACTOTROPES**

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Prolactin has critical functions during pregnancy and lactation, but the wide distribution of the prolactin receptor (PRLR) suggests potential metabolic actions acting on different tissues. In most tissues, prolactin has a proliferative effect, but it inhibits proliferation in primary cultures of lactotropes. Furthermore, *Prlr-/-* mice develop prolactinomas, suggesting an antiproliferative role in the pituitary. In order to evaluate this effect, we generated a transgenic mouse model with specific deletion of *Prlr* in lactotropes (lacPrlrKO) using the Cre/loxP technology.

We verified a decrease in the mRNA expression of the *Prlr* in pituitary glands of female lacPrlrKO, and its physiological inactivation by lower immunoreactivity of pSTAT5 in the pituitary after prolactin stimulation. Even though pituitary size was unaltered, prolactin levels in lacPrlrKO females were increased at 2 months of age. We next evaluated the prolactin releasing capacity of haloperidol (D2R antagonist) and found that lacPrlrKO females have increased dopaminergic tone at 6 and not at 2 months. We can therefore infer that at an early stage the absence of pituitary PRLR induces prolactin release and this prolactin may act on hypothalamic PRLR to favor the dopaminergic control of prolactin, demonstrating that the hypothalamic PRLR predominates over the pituitary PRLR, justifying the lack of prolactinoma generation in our model. Furthermore, we observed that lacPrlrKO females have similar body weight and basal glucose levels compared to the controls at an early age, but at 6 months they have an improved glucose tolerance.

Our results uncover an inhibitory function of the pituitary PRLR on prolactin release, using a unique transgenic mouse model with cell specific deletion of the PRLR, highlighting not only the pituitary PRLR function, but also the lasting effects of early but transient high prolactin levels on the regulation of the pancreas.

75. (484) **FOXO3A EXPRESSION VARIES DURING MAMMARY GLAND TISSUE REMODELING AND WOULD BE PARACRINALY MODULATED BY GNRH IN FEMALE VIZCACHAS (*LAGOSTOMUS MAXIMUS*)**

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FoxO3a belongs to the Forkhead box class O (FoxO) transcription factor family which modulates many metabolic processes, ranging from apoptosis, cell cycle progression, stress resistance to fine endocrine regulation of reproductive organs. For the latter, it has been reported that GnRH transactivates genes critical for gonadal function through another member of the FoxO family: FoxO1. Since we have recently demonstrated local expression and paracrine action of GnRH in mammary glands (MG) of pregnant vizcachas, we aimed to investigate FoxO3a expression throughout pregnancy and its relationship with GnRH by immunohistochemistry. In addition, FoxO3 expression levels were assessed in MG explants of term-pregnant females stimulated with a GnRH analogue. FoxO3a reactivity was detected in all the analyzed stages (early-, mid- and term-pregnant, non-pregnant, and lactating), always in the secretory epithelium of the MG. However, both the percentage of FoxO3a immunoreactive area (%IR) and its immunoreactive optic density were significantly higher in early- and non-pregnant than those of the other groups (p<0.05 and p<0.0005 respectively, n=5 per group, one-way ANOVA). Moreover, mainly nuclear localization was detected for those with the highest reactivity, whereas in the other groups it was translocated to the cytoplasm. Given that phosphorylation of FoxO3a induces its inactivation and translocation to the cytoplasm, these results would indicate that FoxO3a expression profile is strongly tied to the levels of cell proliferation and tissue remodeling of the MG throughout the pregnancy of the vizcacha. When incubated in the presence of GnRH, the mammary explants exhibited a significant increase in the FoxO3a %IR (p<0.05, n=3, t-test), which pinpoints this transcription factor as a mediator of the MG remodeling mediated by GnRH. This work contributes to elucidate the role of GnRH as an active modulator of the MG remodeling dynamics in vizcachas.

76. (496) **MAMMARY GLAND FOXO3 IS DOWNREGULATED IN A HYPERPROLACTINEMIC ENVIRONMENT**

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Forkhead box class O (FoxO) transcription factor family modulates a large number of metabolic processes. Particularly, Foxo3a regulates cell cycle progression, retaining the tissue dynamics in a quiescence stage. Highly proliferative breast cancers have reported a marked down-regulation of Foxo3a. Since prolactin (PRL) is one of the main modulators of mammary gland (MG) tissue remodeling, our objective was to study the expression of Foxo3a in MG under a hyperprolactinemic condition. For this, females from three hyperprolactinemic animal models were evaluated: dopamine type 2 receptor (D2R) knockout mice (KO); mice over-expressing the βsubunit of the human chorionic gonadotrophin (hCGB+), and vizcachas with sulpiride-induced hyperprolactinemia. MG from wild-type mouse models and vehicle-treated vizcachas were used as controls. Immunohistochemistry assays revealed nuclear localization of Foxo3a in MG from the three models, indicating transactivation activity of this transcription factor. In addition, all three models showed the same pattern: a marked decrease in Foxo3a expression in MG from hyperprolactinemic females compared to their controls. Moreover, the percentage of Foxo3a immunoreactive area (%IR) was significantly lower in MG from hyperprolactinemic mice than in controls (p<0.05, n=4 per group, t-test). A similar tendency, but not significant, was observed in MG from hyperprolactinemic vizcachas compared to the controls. Given that PRL has a known role in inducing proliferation and differentiation of MG, it is expected to limit the expression of markers that operates in the opposite direction of tissue growth, such as Foxo3a. When PRL levels are deregulated, such an imbalance would incline the tissue towards excessive proliferation, favored by the low expression of Foxo3, as has been reported in mammary tumors. Our data contribute to understand the modulation of Foxo3a MG expression and to elucidating its participation in MG remodeling.

77. (502) **HEART LIPID PROFILING IN RATS POSTNATAL HYPOTHYROIDISM**

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In a previous study, we demonstrated that heart mitochondrial function is altered during thyroid disorder in rats. Decreased thyroid hormone level would probably be a hormonal environment that promotes changes in mitochondrial NO bioavailability modulating oxygen consumption and cell respiration. Alterations in the structure and/or content of phospholipid are responsible for mitochondrial dysfunction in a variety of pathological settings, including hypothyroidism. The aim of the present work was to determine whether postnatal hypothyroidism alters lipid profiling in rat heart. Male Sprague–Dawley rats weighing approximately 50 g were used in this study and were randomly assigned to one of the groups: euthyroid rats (eut, received SC injections of 0.9 NaCl (0.1 ml/100 g body weight)) and hypothyroid rats (hypo, received 0.02% methimazole in drinking water for 60 days). Cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylcholine (PC) were isolated and identified by TLC and the quantification was for Bartlett's technique. The results of the present study showed that hypothyroid animals had a higher total lipid content than euthyroid rats (nmol/protein mg, hypo:1132*±135* vs eut:558±219,p<0,005n:5). Furthermore, content of CL, PE, PA and PC were higher in hypothyroid rats compared with eut group. Content (nmol/protein mg) of CL (hypo:119±36 vs.eut:61±19,p<0,05n:5), PE (hypo:528±42 vs.eut:205±146,p<0,005n:5), PA (hypo:210±36 vs.eut:120±27,p<0,005n:5) and PC (hypo:274±76 vs.eut:173±35,p<0,05n:5). Results are mean ± SEM, unpaired t-test was used as statistical analysis (SPSS 23 version). Our results suggest that alterations mainly in cardiolipin level could contribute to cardiac mitochondrial dysfunction, together with the decrease in complex I activity of the respiratory chain of hypothyroid animals. The above can justify the modifications in contractility, heart relaxation time and cardiac output that are observed in hypothyroid rats.

78. (521) **OVARIAN AMH PRODUCTION IS TRANSIENTLY AFFECTED IN PUBERTAL AND PREPUBERTAL GIRLS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA AND NON-HODGKIN LYMPHOMA RECEIVING CHEMOTHERAPY: A PROSPECTIVE, LONGITUDINAL STUDY.**

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Introduction: Improvements in the treatment of oncological disease have increased survival, with the consequent concern about the long-term effects that childhood chemotherapy may have on ovarian function. AMH constitutes an indirect, reliable biomarker of the ovarian reserve.
Aim: To evaluate small ovarian follicle status in girls and adolescents with hematologic malignancies during and after treatment.
Methods: Prospective cohort study in children with Acute Lymphoblastic Leukaemia (ALL) or Non-Hodgkin lymphoma (NHL). Serum AMH was measured at diagnosis, during chemotherapy and until 3 years after treatment. Secondarily, FSH levels were analysed. Results were interpreted according to age or pubertal stage as appropriate and expressed as median (range) or percentage.
Results: Twenty-three girls aged 7.3 yr (1-15.7) were included; 15 were prepubertal and 8 pubertal at diagnosis. 83% were diagnosed with ALL, 32% classified as high risk, and 17% had NHL. Total follow-up was 4.7 yr (3-5.1).
AMH was low (<3rd centile) in 20 patients (86.9%) at some point during treatment. In 4 prepubertal girls AMH was low since diagnosis. In the others, a marked decrease was observed within the first year of treatment, being the difference between baseline AMH and at 6 and 9 months of treatment significant
(P 0.0149, P 0.0198).
Fifteen out 20 patients (75%) recovered normal serum AMH; 4 of the 5 girls who did not recover AMH had AMH <25th centile before treatment and a diagnosis of high-risk ALL or NHL, receiving more aggressive chemotherapy.
Mild increased FSH was seen in 5 pubertal girls.
Conclusion: These preliminary results suggest that most girls with ALL or NHL suffered a transient dysfunction of the ovarian follicles during chemotherapy, with recovery in a large majority of them in the long-term. Most of the girls with persistently low AMH had received more aggressive chemotherapy.

79. (547) **VITAMIN D STATUS AND GLYCEMIC REGULATION INDICES IN HYPOTHYROID PREGNANT**

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INTRODUCTION. Vitamin D deficiency is prevalent in worldwide. This vitamin deficiency has been linked with metabolic diseases. Gestational diabetes mellitus (GDM) is associated with numerous complications for mother and offspring. Hypothyroidism is a highly prevalent pathology related to metabolic disorders and complications in mother and child.
OBJECTIVE. To determinate status of vitamin D and its relationship with glycemic regulation indices in hypothyroid pregnant in second trimester of gestation.
MATERIAL AND METHODS. This study included 305 pregnancy, hypothyroid (n=185) and euthyroid (n=120) were seen second (24-28 weeks) trimesters. Each participant performed a 75 g oral glucose tolerance test. Levels of vitamin d (25OHD), insulin, Thyrotropin, free thyroxine, antithyroid antibodies and insulin were measured by chemiluminescence (ACCESS 2- Beckman Coulter). Insulin resistance (HOMA-IR) was estimated from insulin and blood glucose measurements. Mean differences were compared using the Student t-test, Chi², and Pearson correlation, with significance level set at p<0.05. Serum 25OHD concentration (ng /mL) stratified as sufficient (> 30), insufficiency (20-30) and deficiency (< 19).
RESULTS. The mean age was 29 (SD: 5). Mean level of vitamin D was lower in hypothyroid pregnant compared to the control (euthyroid), p <0.05. Regarding the 25OHD status in hypothyroid vs. euthyroid (%) it was observed: 33 vs. 46 sufficient, 52 vs. 44 insufficiency and 15 vs. 10 deficiency respectively. Three per cent of the euthyroid and 5% of the hypothyroid women presented GDM, significantly higher in patients with 25OHD levels <30 ng / ml. Lower levels of 25OHD were associated with higher insulin resistance (r = -0.07; p <0.05).
CONCLUSION. Most of the pregnant showed decreased levels of 25OHD. Our results suggest that low levels of vitamin D and hipothyroidism are associated with the presence of GDM and insulin resistance in these women.

80. (568) **THYROID STATUS IN MIDDLE AND LATE ADOLESCENCE**

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INTRODUCTION. According to WHO adolescence is defined as the

period of growth between 10 and 19 years extended to 21 years. The prevalence of primary, clinical (CHT) and especially subclinical (SHT) hypothyroidism in children-adolescent is less than 2% with a low incidence of hyperthyroidism (HyT). These pathologies have a profound impact on growth, maturation, pubertal development, adult height and have been linked to pro-atherogenic metabolic abnormalities. **OBJECTIVE**. Study the frequency of thyroid dysfunction in adolescents treated in a public institution. **MATERIALS AND METHODS**. We evaluated 134 adolescents of both sexes who attended the laboratory for 6 months. The demographic and hormonal data were collected from the Laboratory Informatic System. Serum thyrotrophin (TSH) and free thyroxine (FT4) were dosed by chemiluminescence in ARCHITECT. Results are expressed as mean (X) ± standard error of mean (SEM), ranges, parametric t-test, significance p <0.05 (GraphPadPRISM 8.0.1). Stages of adolescence (age) Middle Adolescence (MA): 14 to 16, Late (LA): 17 to 21; SHT, CHT and HyT according to consensus. **RESULTS**. The group included 34 MA and 100 LA, 81% female and 19% male. In MA males 35% vs 13% in LA, were similar distribution of female between groups. TSH (μIU /mL) and FT4 (ng/dL) in MA 3.36 ± 0.33 (0.89 to 8.10) and 0.99 ± 0.09 (0.88 to 1.19), in LA 3.65 ± 7.75 (0.006 to 77.91) and 1.05 ± 0.11 (0.85 to 1.41) respectively. The frequency of thyroid dysfunction was 16.2%: SHT 13.5% (5% MA, 8.5% LA), CHT 0.7% (male LA), HyT 2.0% (Graves Basedow, subclinical hyperthyroidism). The female-male ratio was MA 5/2 and LA 15/1. **CONCLUSION**. In our group, the frequency of hypothyroidism was higher than the prevalence reported in the literature, respecting the patterns by sex. Our results support the intervention of the endocrinologist for diagnosis, follow-up and eventual treatment in the suspected illness and/or TSH level altered.

FARMACOCINÉTICA

81. (124) **COMPARACIÓN DE LA FARMACOCINÉTICA DE AMPICILINA SÓDICA EN LLAMAS (*LAMA GLAMA*) ADMINISTRADA POR VÍA INTRAMUSCULAR EN DIFERENTES SITIOS DE APLICACIÓN**

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Las enfermedades infecciosas en la llama (*Lama glama*) repercuten negativamente en la producción y en la preservación de las especies silvestres. La ampicilina es un antibiótico betalactámico activo contra las bacterias que producen las enfermedades más comunes en el ámbito veterinario. Existen pocos productos aprobados para su uso en llamas y la mayoría de los tratamientos antibacterianos se aplican empíricamente y extrapolando de otras especies emparentadas (ovejas, cabras, vacas), siendo escasos los estudios farmacocinéticos de antibióticos en camélidos. Tanto la formulación como la vía de administración pueden modificar el perfil farmacocinético y con ello la eficacia clínica. El objetivo del presente trabajo fue comparar la farmacocinética de la ampicilina sódica administrada por vía intramuscular (im) en dos sitios de aplicación, músculo semitendinoso (ST) y músculos sublumbares (SL), en llamas.
Las concentraciones plasmáticas se determinaron mediante el método microbiológico, utilizando *Bacillus subtilis* ATCC 6633 como microorganismo patrón. La curva fue validada en plasma para linealidad (r^2: 0,99), exactitud (>90%) y precisión (6.33%) para concentraciones entre 100 y 0,09 μg/ml. Los resultados fueron analizados utilizando Graph Pad Prism, Excel y WinNonlin. Los límites de cuantificación y de detección del método fueron de 0,09 μg/ml. Los parámetros farmacocinéticos fueron: $C_{máx}$: 35,89±9,31 y 26,69±14,21; $T_{máx}$: 0,19±0,07 y 0,62±0,47; $t_{½}$: 0,66±0,14 y 1,24±0,38 y TMRinf: 0,68±0,09 y 1,38±0,31; T>CIM para 0,5 μg/ml: 3,51±0,31 y 5,58±1,26, para la administración en ST y SL, respectivamente.
Se encontraron diferencias significativas en $t_{1/2}$, TMR_{inf}, y T>CIM relacionadas con el sitio de aplicación, siendo mayores los valores para la aplicación en SL. Sin embargo, no se requiere realizar modificaciones en la posología para la ampicilina sódica cuando se administre a dosis de 20 mg/kg por vía im cada 6 u 8 h cuando los microorganismos presenten CIM 0,5 μg/ml.

82. (287) **SUSTAINED TREATMENT WITH FENBENDAZOLE INDUCES CYTOCHROME P450 ENZYME ACTIVITIES IN SWINE**

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The anthelmintic fenbendazole (FBZ), a benzymidazole (BZD) drug, is used to control gastrointestinal parasites in swine production. This compound is commercially available as a powder to be mixed with food for oral administration in pigs for 7-10 days. BZD-containing drugs possess the ability to significantly induce certain cytochrome P450 (CYP) isozymes in different species, particularly those belonging to the CYP1A family. This work aimed to evaluate *in vitro* the effect FBZ sustained administration on CYP1A-dependent enzyme activities in pig liver. Eleven (11) piglets were divided in two groups: five (5) animals remained untreated and used as controls; six (6) animals were treated with a FBZ commercial powder mixed with food. The drug concentration in food was 0.01% and animals were fed *ad libitum* for 10 days. Animals were euthanized for preparation of liver microsomes. Two CYP 1A-dependent enzyme activities, namely 7-ethoxuresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MROD) were assayed in a spectrofluorometer. FBZ and its S-oxygenated metabolites, oxfendazole (OFZ) and fenbendazole sulphone (FBZSO2), were detected in the systemic circulation of treated piglets. Mean plasma AUCs (μg.day/mL) were 0.28±0.08 (FBZ), 4.10±0.58 (OFZ) and 4.56±1.01 (FBZSO2). The parent drug FBZ represented around the 46% (4.66±1.59 μg/g) of the total anthelmintic molecules in the liver, followed by OFZ (3.11±1.06 μg/g, 31%) and the inactive FBZSO2 (2.30±0.99 μg/mL, 23%). In liver microsomes from treated animals, both EROD and MROD enzyme activities increased 24.5-fold (p=0.003) and 17.2-fold (p=0.0006), respectively. The sustained administration of FBZ caused the induction of the CYP1A-dependent metabolism in pig liver. This fact may affect the metabolic fate of FBZ itself but also of other foreign compounds such as aflatoxin B1 present in certain pig foodstuffs.

83. (429) **EFFECT OF DIFFERENT ORGAPHOSPHATES ON THE HEPATIC OXIDATIVE METABOLISM BY MIXED FUNCTION OXIDASES IN CATTLE.**

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Organophosphates (OPs) are widely used for crop protection in agriculture and for the control of ectoparasites in animal husbandry. The sustained use of these chemical compounds increases the risk of environmental contamination and/or alteration of different physiological cellular functions in farm animals. A number of OPs are substrates of hepatic mixed function oxidases, such as those belonging to the cytochrome P450 (CYP) and flavin-containing monooxygenase (FMO) families of enzymes. In addition, these xenobiotics may also affect enzyme function by induction or inhibition of their catalytic activities. This work aimed to evaluate *in vitro* the effect of the following OPs: chlorpyrifos (CPF), ethion (ETN), diazinon (DZN) and dichlorvos (DCV) on CYP- and FMO-dependent enzyme activities in cattle liver. Bovine (n=4) liver microsomes were incubated (10 min at 37°C in aerobiosis) in the absence (control assays) and in presence of each OP compound under study at 1, 10 and 100 μM (final concentrations). Five CYP- or FMO-dependent catalytic activities were assayed by spectrofluorimetric or HPLC methods: 7-ethoxyresorufin O-deethylase (EROD, for CYP1A1), methoxyresorufin O-demethylase (MROD, for CYP1A2), benzyloxyresorufin O-debenzylase (BROD, for CYP2B), testosterone 6-beta hydroxylase (for CYP3A) and benzydamine N-oxidase (for FMO). Only the CYP3A-dependent hepatic metabolism was significantly affected by the presence of

ETN and DZN. ETN, at 10 μM and 100 μM, inhibited ($p<0.01$) testosterone 6-beta hydroxylase activity (76% and 81%, respectively) in cattle liver microsomes. Similar results were obtained in presence of equimolar concentrations of DZN (74% and 93% at 10 μM and 100 μM, respectively; $p<0.01$). Both ETN and DZN would potentially interfere with the pattern of the hepatic metabolism of relevant CYP3A substrates pharmacologically relevant in bovine medicine, such as tiamulin, macrolide antibiotics and the ionophore monensin.

84. (448) **MEROPENEN INHIBITS THE CYTOCHROME P450 (CYP) 3A-DEPENDENT BIOTRANSFORMATION OF THE IMMUNOSUPPRESSIVE TACROLIMUS IN HUMAN LIVER MICROSOMES.**

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Tacrolimus (TAC), a immunosuppressive drug used in solid organ transplantation, is metabolized by CYP3A4 and 3A5. The simultaneous administration of TAC and meropenem (MEP) in pediatric kidney transplant patients may led to a significant increase in plasma concentrations of TAC. We hypothesized that this negative pharmacokinetic interaction is due to the inhibition of the CYP3A4-mediated biotransformation of TAC by MEP, particularly in those individuals lacking the expression of CYP3A5. The aim of this study was to evaluate *in vitro* the potential metabolic interaction between TAC and MEP. Human liver microsomes were prepared with discard liver samples obtained from healthy donors (n=2) and individuals subjected to tumor resection (n=2). The specific CYP3A-dependent enzyme activity, testosterone 6-beta hydroxylase, was assayed in the absence (control) and in presence of TAC (5 and 20 μM), MEP (10 μM) and the combinations of TAC and MEP. TAC, incubated at 5 and 20 μM, inhibited ($p<0.05$) the CYP3A-mediated 6-beta hydroxylation of testosterone (18±13% and 51±16%, respectively). This finding may confirm the high affinity of CYP3A4 for TAC. MEP, at 10 μM, did not affect this enzyme reaction. After co-incubations of TAC and MEP, testosterone 6-beta hydroxylase activities resembled those observed when TAC was incubated alone. In control assays, rates of TAC metabolism were 30±20 and 120±40 pmol/min.mg of microsomal protein, respectively. MEP, at 10 μM, significantly inhibited ($p<0.05$) the hepatic biotransformation of TAC; rates (pmol/min.mg) of TAC metabolism (at 5 and 20 μM) were 20±10 (43±24% inhibition) and 70±50 (49±23% inhibition), respectively. These preliminary results show a metabolic interaction between TAC and MRP on CYP3A-dependent metabolism in human liver. The enhancement of the systemic availability of TAC observed *in vivo* in the co-administration with MEP would be due to the inhibition of the CYP3A4-dependent biotransformation of the immunosuppressive drug.

Farmacognosia - Farmacobotánica

85. (091) **ANTIANGIOGENIC ACTIVITY OF THE ALKALOID SKIMMIANINE ISOLATED FROM *ZANTHOXYLUM COCO*.**

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The term angiogenesis refers to the development of new blood vessels from the preexisting vasculature. Under physiological conditions, this process is strictly regulated being focal and self-limited in time. Nevertheless, disbalances between the biochemical signals that regulate angiogenesis may occur, resulting in a chronic neovascularization that takes part in a large number of diseases including neoplastic transformation, rheumatoid arthritis, psoriasis, and different ocular conditions. In this context, the development of new agents capable of downregulating pathological angiogenesis become relevant in the field of drug discovery.

The flora from Argentina stands out among the different sources of new bioactive molecules. In previous studies conducted by our research team, the ethanol extract of *Zanthoxylum coco* showed a remarkable antiangiogenic effect. Therefore, this species was submitted to the bioassay guided isolation of its active principle. This process involved the alternation of different chromatographic techniques with the evaluation of the antiangiogenic activity in terms of the tube formation assay. One compound identified by diverse spectroscopic techniques as the alkaloid skimmianine was isolated. This molecule significantly inhibited tube formation even at 12.5 mg/mL. HPLC analysis showed that this compound is one of the major constituents of the ethanol extract of *Z. coco*. No toxic effect against peripheral blood mononuclear cells, used as model of normal cells, was observed. Additionally, the compound did not affect the integrity of the erythrocyte membrane. Pharmacokinetic and drug-likeness parameters were evaluated by SwissADMET online tool.

The obtained results support the potential of the flora from Argentina as a source of new small molecules capable of downregulating neovascularization and position this naturally occurring alkaloid as a promising lead for the development of new analogs with improved antiangiogenic activity.

86. (194) **INHIBITION OF LIPID PEROXIDATION BY *CANNABIS SATIVA* AND *LARREA DIVARICATA* EXTRACTS AND THEIR COMBINATION**

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Oxidative stress, through lipid peroxidation, affects central nervous system altering cognitive functioning during epilepsy. *Cannabis sativa* L. (Cannabaceae) is a medicinal plant used as anticonvulsant, being cannabidiol (CBD) its main anticonvulsant agent. *Larrea divaricata* Cav. (Zygophylliaceae) is an autochthonous plant with antioxidant activity. The aim of this work was to study the synergistic effect of an ethanolic extract of *C. sativa* (CSR) and an aqueous extract of *L. divaricata* (LE) on inhibition of lipid peroxidation to improve the therapeutic outcomes. The participation of CBD and nordihydroguaiaretic acid (NDGA) was evaluated.

CBD and NGDA were identified and quantified by HPLC-UV. Antioxidant activity was determined in an egg yolk phospholipid peroxidation model. A combination index (CI) was calculated to investigate the interaction between extracts. Results were expressed as inhibitory concentration 50 (IC50) or as mean g% p/p ± SEM of two or three assays made in triplicate.

Quantification of CBD: 23.1 g% p/p. Quantification of NDGA: 1.56 g% p/p. Inhibition of lipid peroxidation: IC50 drugs alone: CSR: 30.5±3.0 μg/ml; LE: 630.95 ± 63 μg/ml; CBD: 10.2±1 μg/ml. IC50 of better combinations: CSR + LE 500 μg/ml: 2.45±0.1 μg/ml ($p< 0.0001$); CBD+ LE 500 μg/ml: 2.18 ± 0.2 μg/ml ($p< 0.0001$). CI of better combinations: CSR 10 μg/ml/LE 500 μg/ml: 0.16 (strong synergism); CSR 3 μg/ml/LE 500 μg/ml: 0.36 (significative synergism).

Conclusions: CSR and LE presented inhibitory activity. CBD was involved in CSR activity. NDGA showed a very low activity. The association of extracts showed strong, significative or week synergism

and even antagonism depending on the concentrations associated. Results allow in a future their association at some concentrations to increase antioxidant effects.

87. (321) **POLYPHENOLS FROM ANDEAN POTATO INDUCE CITOTOXICITY IN GLIOBLASTOMA CELLS BY MODIFING THE REDOX STATUS**

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Andean potatoes (*Solanum tuberosum* ssp. *andigena*) are a good source of dietary polyphenols, such as chlorogenic acid and anthocyanins. This study aimed to analyze the cytotoxic activity of polyphenols from Andean potato var. Santa María on glioblastoma cells. In order to test this, we first assayed the cell viability by incubating different concentrations of polyphenol extracts with human glioblastoma LN-229 cells. We observed that polyphenols induced changes in the morphology of the cells and reduced the viability in a concentration-dependent manner. Then, we calculated the CC_{50} (50% cytotoxic concentration) of total polyphenols extract and proceeded to investigate how the cells dye. First, we treated the cells with the CC_{50} for 4 hr and measured the intracellular reactive oxygen species (ROS) using the probe H_2DCFDA. At the beginning of treatment, the ROS levels decreased compared to control, but after 2 hr, they increased, suggesting that the polyphenols altered the redox homeostasis in glioblastoma cells. To analyze what happens in the mitochondria, we determined the potential mitochondrial membrane with Rhodamine 123. After 3 hr of treatment, we observed a significant decrease, confirming that polyphenols would induce a dysfunction in the mitochondria that contributes to increased ROS levels. Finally, we performed a DAPI staining of cells' nuclei and visualized them with fluorescence microscopy, observing significant alterations in treated cells such as bright nuclear condensation and, in some cases, fragmented nucleus. However, we checked the genomic DNA fragmentation in agarose gel, and we determined that polyphenols produced a slight reduction in genomic DNA size with a lack of oligonucleosomal fragments, suggesting the activation of a mechanism of death caspase-independent. These findings demonstrated that polyphenols from Andean potato var. Santa María would be a good source of bioactive compounds with anti-glioblastoma activity that impacts human health.

88. (339) **HISTOPATHOLOGICAL EVALUATION OF THE EFFECT OF CARROT FIBER ON THE STOMACH OF RATS**.

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Dietary fiber intake elicits a wide range of physiologic effects, not just locally in gastrointestinal tract, but systemically. These changes can then alter the physiology of the body's other nutrient management and detoxification organs, such as the liver and kidneys. Nevertheless, establishing the source of origin, type, and dose of dietary fiber inclusion is importance to obtain the above-noted benefits. A study was conducted to investigate the effect of carrot fiber isolated on stomach histomorphology, in rats. The fibers were obtained from discards from carrot production. Twelve conventional Wistar rats were fed fibre-free or fibre supplemented diets (90 days), and their stomach were examined by optical microscopic. Fixed tissue samples were processed, and embedded in paraffin. Sections of 5 - 6 µm thick, were cut using a rotary microtome. Slides were routinely stained with Hematoxilyn & Eosin. Postmortem alterations such as gland dilatations with epithelial elongation and dysplasia in the mucosa of the stomach were observed, in supplemented rats. These results indicate that carrot fiber may have an effect on rat stomach, which may have health implications.

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89. (187) **REPLACING GLUCOSE MEDIA WITH GALACTOSE TO EVALUATE MITOCHONDRIAL TOXICITY OF IMIQUIMOD.**

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The off-label use of imiquimod (IQ) for hemangioma treatment has shown clinical benefits. We have previously reported a selective direct IQ-cytotoxic effect on transformed H5V endothelial cells (EC) (hemangioma model) vs normal 1G11 EC. We observed a severe imbalance in antioxidant defense and apoptosis in H5V but not in 1G11. To further address this issue, we studied the possibility of IQ being a mitochondrial toxicant. H5V and 1G11 cells were treated with IQ (0-50 μg/mL) for 2, 4, 12 or 24 h and analyzed for reactive oxygen species (ROS) with $DCFH_2$-DA probe and mitochondrial stress by MitoTracker™ Red CMXRos fluorescence. Viability assays were performed using the standard culture medium with 5.5 mM glucose (regular) or media containing 25 mM glucose (high) or 25 mM galactose (depleted). IQ treatment increased ROS level in H5V after 2 h (35-60%; $p<0.05$) but in 1G11 only at 4 h (50%; $p<0.05$). Mitochondrial membrane potential in H5V cells was affected after 4 and 12 h treatment, revealed by a decreased in MitoTracker fluorescence (≈50%; $p<0.05$). In contrast, 1G11 cells were unaffected and only presented a significant 30%-decrease in fluorescence after 12 h with 50 μg/mL IQ ($p<0.05$). Cells grown in a high glucose medium can adapt to a glycolytic phenotype. By assessing the effect of IQ in this medium, both cell lines became significantly less affected than with the regular culture medium. On the contrary, by forcing cells to respiration with galactose instead of glucose-containing medium, IQ treatment enhanced cell death in both cell lines, being fully cytotoxic for H5V ($p<0.05$) but leaving ≈32% 1G11 cells still alive at the highest IQ concentrations.
These results provide more evidences about the higher susceptibility of transformed EC to IQ, where an early ROS production and mitochondrial dysfunction drove H5V cells to death. By shifting cells towards diminished respiration in absence of glucose, we proved IQ acts as a mitochondrial toxicant in both EC lines.

90. (249) **BEHAVIORAL AND MOLECULAR BASES OF THE ANTHELMINTIC ACTIVITY OF ESSENTIAL OILS EXPLORED IN THE NEMATODE *CAENORHABDITIS ELEGANS***

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Control of helminth infections in both human and veterinary medicine currently relies mainly on chemotherapy, but acquisition of resistance is an increasing problem that leads to the urgent need of discovery of novel drugs. *C. elegans* has demonstrated to be a model system for the discovery of new anthelmintics and for characterizing their mechanisms of action and resistance. Essential oils (EOs) are natural products produced by aromatic plants. We perform paralysis assays of wild-type and mutant *C. elegans* strain to identify EOs with potential anthelmintic activities, reveal the active components, their target sites, and mechanisms of action. We found that EOs belonging to different orders produce rapid paralysis of *C. elegans* with EC50 values between 0.02-2 % EOs. All EOs tested also inhibited egg hatching, a property related to anthelmintic ability. Thus, EOs mediate both rapid and long-term anthelmintic effects. We examined anthelmintic properties of terpenoids and phenylpropenes and determined that all compounds tested produce both paralysis and egg-hatching inhibition. By testing mutant worms, we identified the muscle L-AChR and GABA receptors as EOs and trans-cinnamaldehyde (TC, phenylpropene) targets. Thus, by mod-

ulating two receptors with key roles in worm motility, these EOs emerge as novel sources of anthelmintic compounds. To unequivocally confirm that these receptors are targets of TC and to describe the mechanism by which they affect these receptors, we performed whole-cell and single-channel recordings from *C. elegans* muscle cells. Electrophysiological recordings at the single-channel level revealed that TC reduces L-AChR channel activity without affecting channel properties. The results are compatible with the action of these drugs as allosteric inhibitors. It is hoped that this work can update the recent progress on natural nematicide discoveries and provide new ideas for the design and mechanism of action studies of anthelmintics.

91. (284) **DEVELOPMENT AND ASSESSMENT OF THE COENCAPSULATION OF CARVEDILOL AND CURCUMIN IN A NANOMICELLAR DISPERSION SYSTEM IN AN EXPERIMENTAL MODEL OF HYPERTENSION.**

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Carvedilol is a third-generation ß-blocker with pleiotropic effects, including antioxidant, anti-inflammatory and anti-apoptotic effects. Curcumin is a phenolic compound belonging to turmeric, with a beneficial effect on blood pressure. The objective of the present work was the development and assessment of the pharmacokinetic (PK) and pharmacodynamic (PD) profile of the coencapsulation of carvedilol and curcumin in nanomicellar dispersions of Soluplus in spontaneously hypertensive rats (SHR).

Nanomicellar dispersions of 10% w/v Soluplus containing carvedilol 3 mg/ml, curcumin 2 mg/ml , or both were prepared using the solvent evaporation technique. Plasma pharmacokinetics and hemodynamic response after oral administration of carvedilol 9 mg/kg, curcumin 6 mg/kg or carvedilol/curcumin 9/6 mg/kg were assessed in 20 male SHR rats after carotid artery cannulation.

Results: Plasma curcumin levels were comparable after coadministration of carvedilol/curcumin or curcumin only. Coencapsulation did not modify the PK profile of carvedilol. Curcumin oral administration did not induce changes in blood pressure and heart rate. Coencapsulation resulted in a greater MAP reduction when compared with carvedilol (-28,5±4,0 % vs –12,1±3,1%, $p<0.05$). HR reduction was comparable after oral administration of carvedilol or coencapsulation.

Conclusions: Coencapsulation of curcumin with carvedilol in nanomicellar dispersions potentiates the antihypertensive effect of carvedilol without modifying the bradycardic responser nor the pharmacokinetic profile. These results suggest that coencapsulation of carvedilol and curcumin represents a potential pharmacodynamic synergistic combination for the management of arterial hypertension.

92. (305) **MOD-1 RECEPTOR AS A NOVEL DRUG TARGET FOR ANTHELMINTIC THERAPY**

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Caenorhabditis elegans (Nematoda) contains a homomeric 5HT-gated chloride channel, MOD-1, that belongs to the Cys-loop receptor family and modulates locomotor behavior. Although it binds 5-HT, MOD-1 is not present in vertebrates, and it therefore emerges as a possible anthelmintic target. We deciphered MOD-1 pharmacological properties and searched for novel modulators with potential anthelmintic activity by performing patch-clamp recordings from mammalian cells heterologously expressing MOD-1 and locomotor activity assays in *C. elegans*. Whole-cell recordings showed that MOD-1 desensitizes slowly and recovers from desensitization with a time constant of about 1 s. Compared to the vertebrate 5-HT_3A receptor, dose-response curves were similar for 5-HT but very different for the orthosteric agonists tryptamine and 2-Me-5HT. The anthelmintic drugs ivermectin (IVM), levamisole, and piperazine (PZE), which are agonists of other Cys-loop receptors, did not activate MOD-1. However, IVM produced a slight and irreversible inhibition and PZE produced a profound and reversible inhibition of MOD-1 currents elicited by 5-HT. The analysis indicated that PZE is a non-competitive antagonist of MOD-1, revealing a novel function of this drug. To relate the molecular effects to behavioral actions of these compounds, we performed locomotor activity assays in *C. elegans*. We found that 5-HT produces rapid and reversible paralysis of wild-type (WT) worms while MOD-1 mutants are partially resistant under similar conditions, thus indicating that MOD-1 is the main 5-HT target in this type of assays. Additional assays using drug combinations in WT and mutant strains confirmed the inhibition of MOD-1 activity by IVM and PZE. The elucidation of the molecular pharmacology of MOD-1 enhances our knowledge of function and drug selectivity of Cys-loop receptors and contributes to determine its potential as a novel target for anthelmintic therapy.

93. (390) **RATIONAL SEARCH FOR G PROTEIN-COUPLED RECEPTOR KINASE 5 INHIBITORS UTILIZING DOKING-BASED VIRTUAL SCREENING**

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G protein-coupled receptor (GPCR) kinase 5 proved to be overexpressed in failing hearts causing increased desensitization of beta-adrenergic receptors (βARs), deficit in cardiac contractility and failure progression. It has two main domains: Regulator of G protein Signaling (RGS) homology domain (RH), and protein kinase domain (KD). The mechanism of GPCRs phosphorylation by GRK5 requires the disruption of an ionic lock in RH/KD interface, leading to a more stable complex with the GPCR that enhances catalytic properties of the kinase.

To obtain GRK5 inhibitors we performed a docking-based virtual screening (VS) using pdb ID 4TND to search within Enamine Advanced Collection for compounds able to bind to RH/KD interface, intending to strengthen ionic lock and avoid the catalytically competent conformation to be reached. We obtained a list of hits ordered by docking energy, ranging from -10.4 to -9.7. Using Protein Ligand Interaction Profiler tool, we evaluated non-covalent interactions between GRK5 and predicted docking poses, and observed several hydrophobic interactions and hydrogen bonds joining residues from RH and KD. Also, interesting interactions such as salt bridges, halogen bonds and π-cation were found. Accordingly, we chose 15 compounds to be purchased and evaluated in biological activity assays, for what we setted up FRET-based determinations to quantify real-time intracellular cAMP. HEKT Epac-SH187 cells co-transfected with GRK5 and β1AR or β2AR were stimulated with 10μM isoproterenol (Iso) and AUC (area under curve) values of 10min response were determined in FlexStation3 at 37°C. AUCs were reduced from 244.4±24 to 112.5±7.4 for β1AR and from 138.7±10 to 64.73±3.45 for β2AR ($p<0.05$) by GRK5 overexpression (confirmed by Western Blot).

Both computer aided identification of potential GRK5 ligands and obtention of a methodology for screening these compounds will allow us to identify candidate inhibitors of GRK5 in the search of new cardioprotective drugs.

94. (476) **BIOACTIVITY OF ALPHA-HALOACRYLATES OF METYL BETA-SUBSTITUTED SYNTHETIZED *DE NOVO* IN AN *IN VITRO* MODEL OF MAST CELL DEGRANULATION**

INDUCED BY A PRO-INFLAMMATORY STIMULUS

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Objective: To evaluate the effect of 11 new synthetic drugs with α,β-unsaturated carbonyl bioactive groups in an in vitro model of mast cell degranulation induced by a pro-inflammatory stimulus.
Materials and Methods: Adult male Wistar rats (n=205) were used. Animals were sacrificed by CO_2 inhalation. Mast cells were obtained by peritoneal lavage and purified in discontinuous gradient of Percoll's solution. Cell purity was assessed by staining with 0.2 % toluidine blue. Mast cell viability was assessed with 0.1 % trypan blue. Compound 48/80 (0.1 mg/ml) was used as a mast cell degranulator and pro-inflammatory stimulus. Different concentrations of the 11 synthetic drugs (10 to 320 µM), as well as different incubation times were used. In both incubation solutions and remaining cells the release of β-hexosaminidase was quantified spectrophotometrically as a marker of mast cell activation. The percentage of β-hexosaminidase release and EC50 were calculated for each drug. Mast cell morphology was evaluated by light microscopy after incubations. Statistical analysis: ANOVA-1/Tukey-Kramer.
Results : Enzymatic release under basal conditions showed values below 10 %. Compound 48/80 significantly stimulated the release of β-hexosaminidase from mast cells ($P<0.0001$). Of all the synthetic drugs tested, (*Z*)-2-bromo-3-(furan-3-yl) methyl acrylate and (*E*)-2-bromo-3-(3-bromophenyl) methyl acrylate inhibited enzymatic release, at concentrations of 320 µM for the first drug ($P<0.0001$), and 160 µM ($P<0.001$) and 320 µM ($P<0.0001$) for the second drug. The percentage of cell vitality was not affected. The biochemical results were consistent with the morphological ones.
Conclusions: The new synthetic drugs (*Z*)-2-bromo-3-(furan-3-yl) methyl acrylate and (*E*)-2-bromo-3-(3-bromophenyl) methyl acrylate inhibit mast cell degranulation without affecting cell viability. The implications of these results are relevant as a basis for the development of new anti-inflammatory and mast cell stabilizing drugs.

95. (486) **SILDENAFIL DUAL EFFECTS ON MEMORY SUPPORT THE SEARCH FOR NEW DERIVATIVES WITH RESTRICTED ACCESS TO THE BRAIN**

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Sildenafil (SILD) is a drug widely used in clinical practice for its inhibitory effects on phosphodiesterase type 5 (PDE-5), mainly used for peripheral pathologies. Although, it crosses the blood brain barrier. Previous results indicated that systemic acute SILD administration facilitated hippocampal (HP) long-term potentiation, a synaptic plasticity phenomenon that underlies some types of learning and memory processes. **Objectives:** to evaluate the effects of SILD on acquisition of HP-dependent memories, and to identify the structure activity relationships driving SILD interaction with PDE-5 and to further search for derivatives with higher hydrophilicity able to restrict its passage to the brain while maintaining or improving their inhibitory activity. **Material and methods:** male Wistar rats were administered with an acute SILD dose 2 h before exposure to novel object recognition (NOR) test, modified Y-maze, step-down and contextual fear conditioning. Twenty-four hours later the memory expression was evaluated. Also, hydrophilic SILD derivatives were identified by *in silico* methods. **Results:** SILD enhanced the % of freezing after weak fear conditioning (unpaired t-test) and the latency to step-down (Mann Whitney test). Surprisingly, it reduced novel object (unpaired t-test) and novel arm exploration (two-way ANOVA) when compared to controls. On the other hand, molecular docking identified SILD-PDE-5 pharmacophoric contacts, with some hydrophilic derivatives of SILD already described in the bibliography being docked within the PDE-5 binding site. **Conclusions:** these results revealed that SILD divergently contribute to HP-dependent memory formation, probably depending on the stimulus nature and participation of other brain structures. Furthermore, these results support the experimental evaluation of SILD derivatives that could avoid these and other possible unwanted central effects, but promising maintenance of their inhibitory power on PDE-5 at the peripheral level.

96. (494) **THE RH DOMAIN OF GRK2 IS INVOLVED IN ENDOTHELIN RECEPTOR DESENSITIZATION**

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Endothelin-1 (ET-1) is a potent vasoconstrictor and proinflammatory peptide implicated in the pathophysiology of diabetes mellitus and hypertension via activation of the endothelin receptor A (ETA) and B (ETB). Both G protein-coupled receptors (GPCRs) couple to Gq and PLC signalling pathway. Previous reports suggested that ET receptors are desensitized by GPCRs kinase 2 (GRK2) but independently of its kinase activity and that negative regulation by ET-1 of insulin signalling may involve GRK2 and heterologous desensitization of insulin receptor. The purpose of this study was to determine molecular mechanisms underlying negative regulation of ET-1 response with particular attention to the possible involvement of RH domain of GRK2 as a first step to elucidate insulin resistance mediated by ET-1. To do that, we characterized Ca2+ response and desensitization to ET-1 in HEK293 cells transfected with GRK2 variants containing different GRK2 domains or mutants.
We found that after 15 min of pre-treatment, cells are completely desensitized to ET-1 stimulation $p<0.05$. Although none of the variants of GRK2 could revert this phenomenon, a 20% higher response to ET-1 was obtained after knocking down GRK2 with an antisense construct and a slighter difference between desensitized and control response $p<0.05$. Moreover, when cells are transfected with the RH domain of GRK2 the response to ET-1 decreased in a 19%. Similar results were obtained after pharmacological inhibition of the kinase or RH activities of GRK2.
These findings suggest that desensitization of ET receptors after exposure to ET-1 involves the RH domain of GRK2 as was proved by RH domain overexpression and pharmacological inhibition. Considering that it has been proposed that desensitization of ETA may lead to cross-desensitization of insulin receptor, these findings point to the RH domain of GRK2 as a potential target for overcoming insulin resistance that should be further investigated.

97. (574) **SKF96365 IS A SELECTIVE INHIBITOR OF CA^{2+} RELEASE-ACTIVATED CA^{2+} CHANNELS (CRAC CHANNELS) RATHER THAN A TRPC CHANNEL-SELECTIVE INHIBITOR**

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Background and puropose: SKF-96365 (SKF) has been broadly used as a tool to discern TRPC participation in the Store Operated Ca^{2+} Entry (SOCE) phenomenon, being generally accepted that SKF96365 is a TRPC channel-selective inhibitor and is sold as such (Sigma Aldrich cat #S7809, Tocris cat #1147, Alomone cat #S-175). However, SKF was also used as a non-selective blocker of SOCE. Considering that the effect of SKF96365 on the SOCE mechanism remains controversial and taking advantage of the TRPC 7KO MEFs, we hypothesized that SK96365 blocks SOCE (or ROCE) by blocking ORAI1-based channels instead of inhibiting TRPC-based channels.
Experimental approach: TRPC 7KO MEF cells, a unique cell model in which the seven TRPC are absent, were used to elucidate the effect of SKF 96365 on native ORAI1-based channels. In addition

TRPC 7KO and ORAI1 KO MEFs were transfected with ORAI1 plus STIM1 to constitute CRAC channels by heterologous expression.. TRPC-based channels were test for SKF sensitivity in HEK293 in which TRPC6 was stably expressed and Ca^{2+} influx was activated with OAG. SKF effect was always studied by changes in Tg-activated Ca^{2+} influx monitored by FUR2-AM method and the expression level of TRPC, ORAI and STIM were analyzed by RT-PCR.
Key results: We found Tg-evoked SOCE was nearly 50% suppressed by 5μM SKF in TRPC 7KO MEF cells. In addition, the increment in Tg-evoked SOCE produced by the transfection of ORAI1 in ORAI1 KO MEFs and ORAI1 plus STIM1 in TRPC 7KO MEFs was completely prevented by 5μM SKF. OAG-activated Ca^{2+} entry in HEK293 stably transfected with TRPC6 was insensitive at ORAI1 overexpression and GSK7975A treatment in contrast to TRPC7KO MEFs in which Tg-activated Ca2+ influx was sensitive to GSK7975A. Finally, we showed TRPC-based Ca^{2+} influx in HEK293-TRPC6 was barely reduced by 10 μM SKF and 50 μM dose was needed to block OAG-activated Ca^{2+} influx.
Conclusions and implications: In conclusion, we report convincing evidence showing that SKF96365 acts as a blocker of CRAC channels in MEF cells having no TRPC. In addition, SKF showed higher potency to block CRAC channels compared to its potency against TRPC channels. This finding suggests we should be in cautious when analyze results where SKF is used as a pharmacologic agent to asses TRPC channels activity.

FARMACOLOGÍA CARDIOVASCULAR Y RENAL

98. (076) **COMPARISON BETWEEN NEBIVOLOL AND ATENOLOL EFFECTS ON CALORIMETRICAL AND MECHANICAL RECOVERY OF RAT HEARTS EXPOSED TO ISCHEMIA REPERFUSION**

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Previously, we showed that nebivolol (Nbv), a third-generation β-blocker, was cardioprotective by releasing nitric oxide (NO) in hyperthyroid (HypT) and euthyroid (EuT) rat hearts when perfused before severe ischemia-reperfusion (sI/R). Oral Nbv also prevented cardiac stunning in EuT hearts more than in HypT rats. However, in hypothyroid hearts NO production reduced cardioprotection. For understanding the differences in oral Nbv effects between EuT and HypT hearts, we have a) compared the effects of Nbv with those of atenolol (Atl), a selective β1-blocker, and b) assessed the role of NO in oral-Nbv treated EuT and HypT rats, during sI/R. Rats became HypT by s.c. daily injected 20 µg/kg T3 for 15 days. EuT and HypT rats were treated with 20 mg/kg Nbv daily administered in drinking water during 7 days. Other EuT group was similarly treated with 30 mg/kg/day Atl. Isolated perfused ventricles inside a calorimeter were exposed to sI/R (30 min I/ 45 min R). Left intraventricular pressure (P, mmHg) and total heat rate (Ht, mW/g) were measured. In Nbv-treated hearts the NO-synthases were blocked by perfusing L-NAME. Results: Atl improved PICR (postischemic contractile recovery) in EuT to 64.5±3.2% of pre-I (vs 14.2±2.5 % in EuT-C, $p<0.05$) and P/Ht (muscle economy) to 3.1±0.7 mmHg.g.mW^{-1}(vs 1.0±0.4 in EuT-C, $p<0.05$) but increased diastolic contracture during R (ΔLVEDP) to 32.0±6.9 mmHg ($p<0.05$). In EuT, L-NAME strongly decreased PICR to 18.2±0.9 % ($p<0.05$ vs 78.8±10.6% in EuT-Nbv) and P/Ht to 1.3±0.2 mmHg.g.mW^{-1} ($p<0.05$ vs 5.32±0.87), with high ΔLVEDP (31.3±5.3 mmHg). In HypT, L-NAME prevented the poor cardioprotection of Nbv with higher ΔLVEDP. Conclusions: a) Atl was less cardioprotective than Nbv in EuT, so β1-blockade explains part of Nbv beneficial effect; b) the additive Nbv cardioprotection is related to NO, which could induce vasodilation or direct myocardial effect; c) the low Nbv cardioprotection in HypT rats seems related to a reduced NO production. UNLP-X-795

99. (100) **THE STUNNING CONSEQUENT TO ISCHEMIA AND REPERFUSION IS REDUCED BY PERFUSING DRONEDARONE IN ISOLATED RAT HEARTS, WITH INCREASE IN MUSCLE ECONOMY**

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In previous works we have showed that amiodarone, a class-III antiarrhythmic improved the postischemic cardiac recovery when administered daily during one week via oral, but it was not cardioprotective when it was perfused in the isolated hearts before ischemia and reperfusion (I/R) in euthyroid rats (Bayley et al.- AAFE 2020; Bayley et al. SAFE 2019). Since dronedarone is another class-III antiarrhythmic without iodine in structure, the aim of this work was to evaluate the differences with amiodarone, by studying the effect of perfusing dronedarone before the I/R protocol in rat hearts. Isolated cardiac ventricles from Wistar rats were quickly perfused and stimulated at 3 Hz, introduced inside a calorimeter at 37°C, and then stabilized and simultaneously measured the signals of left intraventricular pressure (LVP) and heat rate, before and during a protocol of I/R (30 min I/45 min R). There were calculated the maximal LVP in contraction (P, mmHg), total heat rate (Ht, mW/g), diastolic pressure (LVEDP), contraction/relaxation rates and times, and muscle economy (P/Ht, in mmHg/mW.g). Dronedarone (Dnd) was perfused at 1 µg/ml, during 20 min before I/R (n = 5) and compared to non-treated rat hearts (control, n = 5). Results: Dnd improved the post-ischemic contractile recovery (PICR) up to 62.6 ± 7.9% of initial P ($p<0.01$ vs. 11.8 ± 5.3% in control) and P/Ht to 86.2 ± 18.3% ($p<0.01$ vs 18.9 ± 8.5% in control), both values at the end of R. During I/R, Dnd group increased the ΔLVEDP, but improved the relaxation times at the start of reperfusion. Conclusions: a) perfusion of Dnd reduced the stunning consequent to ischemia and reperfusion, having the advantage over amiodarone, b) Dnd improved both, contractility and muscle economy during reperfusion, but it did not reduce the diastolic contracture during I/R (UNLP- X795).

100. (116) **MYOCARDIAL STUNNING BY ISCHEMIA AND REPERFUSION IS ATTENUATED BY AGEING IN MALE RATS**

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Clinically, young women have a lower incidence of cardiovascular disease than men, but differences disappear when estrogens decline. Estrogens have cardioprotective mechanisms, but effects and mechanisms of testosterone on ischemia/reperfusion (I/R) injury are contradictory. Previous results showed that aged female rat hearts had less postischemic contractile (PICR) and muscle economy (MER) recoveries than young female rat hearts (SAFE 2016). In this work, we studied the influence of ageing on the mechano-energetics of male rat hearts in two post-ischemic stunning models: moderate (I/Rm) and severe (I/Rs). Young (YM, n=10) and aged (AgM, n=12) male rat hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, mmHg) and total heat rate (Ht, mW/g) and calculated the maximal contractile (P) and diastolic (LVEDP) values, and muscle economy (P/Ht). Results: Before I, ageing reduced P (YM: 84.6±6.8 vs AgM: 51.9±5.4 mmHg, $p< 0.05$) but increased the contractile energy expenditure (Ht/P: 0.21±0.03 vs 0.09±0.03 mW/mmHg.g), without changing Ht ($p>0.15$). Surprisingly, in I/Rm PICR was higher in AgM than in YM (77.4±12.9 vs

45.3±6.2% of pre-I), as well as MER (100.3±12.5 vs 57.2±8.1%). The same comparisons were obtained in I/Rs: PICR and MER were higher in AgM than in YM (PICR: 78.5±11.5 vs 14.5±2.4%; MER: 94±8 vs 19.8±5.4%). Since accumulation of adipose tissue in AgM could raise aromatase and circulating estradiol levels, thus contributing to protection in I/R, we evaluated whether cardioprotection was related to NO production, as one of estrogenic pathways. However, perfusion of the NOS blocker L-NAME 30 uM before I/Rs did not reduce PICR nor MER in AgM hearts (75±6 and 100±2%). Conclusions: unlike females, in males the ageing plays a cardioprotective role in I/R. Aged male rat hearts recovered contractility and economy more than younger male and aged female ones. However, AgM cardioprotection was not due to NO production (X-795 UNLP).

FARMACOLOGÍA CLÍNICA

101. (013) **PHARMACOLOGICAL SENSITIVITY CHARACTERIZATION TO ANTHRACYCLINE COMPOUNDS IN PRIMARY CELL LINES DERIVED FROM CENTRAL NERVOUS SYSTEM DISSEMINATED RETINOBLASTOMA**

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Metastatic disease can be up to 50% of retinoblastoma (RB) cases in low-income countries and despite aggressive treatment, patients with central nervous system (CNS) involvement is still fatal. Patients with high-risk histopathological features and orbital RB receive intensive i.v chemotherapy including doxorubicin to prevent fatal CNS dissemination. Other anthracycline, idarubicin, is less cardiotoxic, more lipophilic favoring penetration across the blood–brain barrier, and presents the active metabolite idarubicinol favoring antitumor activity in CNS. Idarubicin use in middle-income countries is limited by restricted commercial availability and high costs. Our aim was to compare the pharmacological sensitivity of both anthracyclines in cell lines derived from CNS disseminated patients.
We compared the growth-inhibitory activity of both anthracyclines in two primary cell lines derived from CNS dissemination (HPG-CSF-1, HGP-CSF-2) established at our hospital and 1 primary cell line from an intraocular tumor enucleated upfront (HPG-RBT-12L) used as control. Cells were exposed to increasing concentrations of drugs and cell viability was assessed using MTT. EC50 was calculated.
Idarubicin EC50 was 7- to 8-fold lower than the EC50 of doxorubicin in HPG-CSF-1 and HPG-CSF-2. This difference was 1.7-fold in HPG-12L showing low and similar sensitivity to both anthracyclines. Assuming a proportional increase in drug exposure with the dose, this result may imply that doxorubicin dosage should be increased 7 to 8 times to obtain a comparable activity in high-risk patients. However, these dosages would exceed the maximum recommended cumulative dose related to doxorubicin associated cardiotoxicity. Based on our results of *in vitro* chemosensitivity, equivalence in dosages associated with cardiotoxicity, and favorable biological characteristics of idarubicin it continues to be the anthracycline of choice in disseminated RB.

102. (024) **ESTABLISHMENT AND CHARACTERIZATION OF A MODEL OF RESISTANCE TO TOPOTECAN. IMPLICATIONS IN RETINOBLASTOMA TREATMENT**

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During the last decades, retinoblastoma (Rb) treatments have changed to local delivery of chemotherapy greatly improving ocular preservation rates. Nevertheless, some eyes develop relapse or recurrence disease probably associated with acquired pharmacological resistance. Our aim was to establish a topotecan-resistant cell subtype (HSJD-RBT-7-TP) as a tool for studying the pharmacological sensitivity to the most common chemotherapy agents and identifying alternative treatments that circumvent resistance.
A primary cell culture was established from the tumor biopsy of an upfront enucleated patient with intraocular Rb. The parental cell line (HSJD-RBT-7) was exposed to three weekly doses of topotecan equivalent to the IC50. Once HSJD-RBT-7-TP cells were established, we determined the sensitivity to topotecan, melphalan and carboplatin. Alternative treatments assessed for cytotoxicity in HSJD-RBT-7-TP included 3-weekly doses of high-dose (IC90) topotecan, digoxin exposure and metronomic topotecan. Cell viability was determined using the MTT assay.
Mean (range) topotecan IC50 in HSJD-RBT-7-TP cells was 8,1 nM (6,8-9,3), 3-fold higher than that in HSJD-RBT-7 ($p<0.05$). Melphalan and carboplatin IC50 in the HSJD-RBT-7-TP cell was 2,5-fold and 1,8-fold higher than in HSJD-RBT-7,respectibily ($p<0.05$). Resistance was not achieved using high-doses of topotecan. Both cell lines (HSJD-RBT-7 and HSJD-RBT-7-TP) showed similar IC50 to digoxin. Metronomic topotecan schedule resulting in 3-fold lower IC50 in HSJD-RBT-7-TP.
Topotecan-resistant cells subtype showed a significant decrease in drug sensitivity. Cross-resistance to frequently used drugs was observed, with a probable clinical implication consequence. High doses of topotecan, digoxin or metronomic topotecan, are three alternatives treatment that circumvent topotecan resistance in Rb cell lines.

103. (203) **RESTING AND STIMULATED SALIVARY FLOW IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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Objective: To determine the periodontal status and salivary flow in patients with rheumatoid arthritis (RA) and to compare them with those of the general population. Also, to study the RA clinical parameters in relation to salivation.
Methods: An observational, cross sectional study was carried out, which included consecutive RA patients according to ACR/EULAR 2010 criteria and a control group of persons from the general population without a known inflammatory rheumatic disease. RA clinical parameters (erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), disease activity (DAS28), rheumatoid factor (RF) were considered. Periodontitis was evaluated according to the American Academy of Periodontology (1999) and chronic periodontitis was assessed by full mouth periapical radiographic examination, periodontal probing depth, clinical attachment level and bleeding index. Resting and stimulated saliva (RSF, SSF) was collected during 5 minutes and expressed in mL/min. Results were expressed as mean±SD. A value of $p<0.05$ was considered statistically significant.
Results: A total of 195 subjects were included, 103 patients with RA and 92 controls, with no significant differences in sex (females/

males=6) and age (48±12 years). Salivary flow was lower in RA group: RSF 0.2±0.1 vs 0.5±0.2 (p<0.001) and SSF 0.7±0.3 vs 1.1±0.3 (p<0.001). Also, hyposalivation (RSF£0.15) was more frequent in RA group: 27.2 vs 6.5 % (p<0.001). There was no association between salivary flow and several characteristics of RA patients: disease duration, ESR, CRP, RF, erosions, nodules and DAS28. RA group had higher prevalence of severe periodontitis: 16.0 vs 4.4 % (p<0.001). However, there was no association between salivary flows and the periodontal status.
Conclusion: RA patients had reduced resting and stimulated salivary flow. There was no association between salivation capacity and RA severity. There was also no association with the presence of periodontitis of any degree.

104. (231) **PRELIMINARY RESULTS ON SAFETY AND EFFECTIVENESS OF AN IMMUNOBIOLOGICAL DRUG IN COVID-19**

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Passive immunotherapy with high antibody titer shows benefit in hospitalized patients with COVID-19 within 72 h of symptoms onset[1]. An immunobiological treatment based on the concentration of equine F(ab')2 fragments with anti-SARS-CoV-2 titer greater than 1/5120 was developed. This is the preliminary report of the phase 2/3 trial in the first 20 patients treated, whose objective was to evaluate the efficacy and safety. Primary endpoint was to obtain a decrease in time needed to clinical improvement (ClinicalTrials.gov NCT04913779). Methods: This was a double-blind, randomized, placebo-controlled trial to assess efficacy and safety of the immunobiological treatment in hospitalized adult patients with COVID-19 pneumonia (OMS stage 3, 4 or 5). Results: The 20 initial patients (10 treatment / 10 control) were aged 44±14 (18-73) years and 6 (30%) were women. All had symptoms for up to 48 hours and a positive PCR for SARS-CoV-2. The WHO Scale was 4 (55%) or 5 (45%). All patients had COVID pneumonia with decreased oxygen saturation causing admission in the general ward, without any criteria for intensive care. There were no baseline differences between the treatment groups (symptoms, severity, saturation, anthropometry, lung involvement, laboratory). On third day of treatment there was an improvement (P=0.02) in arterial saturation (95±1.6 Vs 93±2.5%) with significant increasing differences over time between treatments (day 8: 97±0.1 Vs 94±0.3%). The mean time of hospitalization was 13±2.5 Vs 14±0.8 days (P=0.08) and the time to clinical improvement was 2±0.5 Vs 3±0.9 days (P = 0.047) in patients with initial 5 WHO category, with no differences in patients who started with WHO stage 4. No adverse reactions or intercurrences were detected. Discussion: Although the initial number of patients included is small, the results show therapeutic benefits without adverse reactions in individuals with WHO category 5 at admission of recently-onset covid pneumonia (<72 hours).
1. Libster R et al. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. N Engl J Med. 2021;384(7):610-618.

105. (503) **PRESCRIPTIVE PROFILE OF DRUGS USED FOR THE TREATMENT OF TYPE 2 DIABETES IN MEMBERS BELONGING TO A SOCIAL SECURITY INSTITUTE OF CORRIENTES, 2021**

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The diagnosis of type II diabetes (DBT2) has increased exponentially in recent years, in the same way, new groups of antidiabetic drugs (ADT) also appeared, some of them still without showing a decrease in long-term morbidity and mortality. Objective: to characterize ADT drug prescriptions in outpatients affiliated to a Social Security Institute. Cross-sectional descriptive observational study; of drug use for the treatment of DBT2. All prescriptions made in requests for long-term treatment plans for seven months (January-July) were analyzed. The variables analyzed were: sex, age, prescribed medications; loaded into an Excel spreadsheet, subsequently analyzed using descriptive statistics. Of a total of 444 plans, 128 were observed with ADT prescriptions (29%), corresponding to 230 drugs; 62.5% for males. Average age: 58 years; range: 36 to 94 years. The following prescriptions were observed: biguanides as a single drug (107), in association with fixed doses with incretins(3); incretins(46) sulphonylureas(24), glyphlocins as a single drug(22) in association with fixed doses(1), glitazones; (2); fast-acting secretagogues(1), insulins(11), 5 times together with metformin. In order of frequency, the most prescribed drugs were: metformin (n = 110), glimepiride (20), sitagliptin (19), empagliflozin (15). The drug most frequently prescribed was metformin, coinciding with current guidelines that suggest it as a first-line drug for the treatment of DBT2. Having observed a high prescription of incretins and glyphlocins, the need arises to evaluate their rationality by inquiring about comorbidities and metabolic response, since according to them it is decided which drug should be used in the therapeutic scheme of a patient with DBT2.

FISIOLOGÍA CELULAR

106. (176) **PRENATAL PROTEIN MALNUTRITION INDUCES PREMATURE SENESCENCE IN MOUSE EMBRYONIC FIBROBLASTS THROUGH ALTERATION OF EPIGENETIC MECHANISMS**

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Nutrition during early life have a strong impact on organism development. An inadequate nutrition results in defective organ structures and elicit long-term effects leading to premature aging. Cellular senescence is an irreversible growth arrest triggered by exogenous or endogenous stress and may be an important factor that contributes to the aging phenotype. The aim of this work was to study the effect of protein malnutrition during pregnancy and its relationship with the establishment of the senescence in mouse embryonic fibroblasts (MEFs). CF-1 female mice were fed with a normal protein diet (NP 20%) or a low protein diet (LP 8%) from a week before mating until MEFs extraction (13.5-day-old embryos). The statistical analysis was performed by *t*-tests and 2-way ANOVA; p values lower than 0.05 were considered statistically different. MEF from LP embryos showed a large and flat morphology, premature cell cycle arrest and reduced cell viability at lower passage than MEF from NP embryos, indicating an early induction of different cellular senescence markers (p<0.05). The oxidative stress and antioxidant capacity was evaluated by measuring ROS production in presence or absence of a ROS inductor. We found higher ROS basal levels and reduced antioxidant capacity in MEF from LP embryos (p<0.05). Also, LP MEFs showed a downregulation of the antioxidant enzymes catalase and superoxide dismutase. These results coincided with a downregulation of Sirtuin 3 and Sirtuin 7 in LP MEFs (p<0.05), both usually repressed in senescence and involved in ROS-induced injury and DNA damage response, respectively. Finally, the expression of senescence-associated microRNAs was studied and we found an upregulation of miR-26a in LP MEFs (p<0.05).
We propose that MEF from LP embryos undergo premature senescence which would be caused by an increased oxidative stress and seems to be epigenetically regulated by Sirt-3, Sirt-7 and miR-26a.

107. (188) **ANALISYS OF LONG NON-CODING RNA TRAN-**

SCRIBED FROM TELOMERES DURING ADIPOCYTE DIFFERENTIATION

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Long non-coding RNAs transcribed from telomeres, known as TERRA (telomeric repeat containing RNA), are associated with telomere and genome stability. Oxidative stress damages biomolecules and telomeres are particularly prone to be damaged, thus it is relevant their protection to avoid telomere dysfunction. We have previously shown that the level of TERRAs increased in response to oxidative stress as a mechanism of telomere protection. However, ROS have proven to be signalling molecules, and in this regard, we demonstrated that physiologic increase of ROS in brown adipose tissue of mice exposed to cold also triggered the induction of TERRAs. Since it is well known that ROS increase during adipocyte differentiation, we hypothesized that the expression level of TERRAs is induced during adipogenesis to protect telomeres and possibly having other potential functions to be unveiled. Our preliminary results showed that TERRAs were induced during adipogenesis. In order to test telomere integrity during adipocyte differentiation, we analysed telomeres in 3T3-L1 preadipocytes prior and post-induction of differentiation by IIF labelling TRF1, a component of the sheltering complex in telomeres. We observed a punctate pattern of TRF1 staining that was conserved throughout the process of differentiation. In addition, we analysed DNA damage foci by IIF by labelling gH2AX, the histone variant H2AX phosphorylated on Serine 139 that is found at sites of DNA breaks. We observed a slight increase in gH2AX foci as adipogenesis proceeded, possibly due to physiologic ROS production. No colocalization was observed between gH2AX and TRF1 indicating no telomere-associated DNA damage foci (TAF) formation during adipogenesis, indicating the maintenance of telome integrity. In summary, we found that TERRAs were induced during the process of adipocyte differentiation possibly to protect telomers.

108. (385) **5' TIRNA GLY-GCC AS A POTENTIAL NEW BIOMARKER FOR ASTHMA PHENOTYPING**

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The heterogeneous nature of asthma has been understood for decades, but this feature of the disease is becoming increasingly important in the era of specific biologic therapy. Unfortunately, accurate biomarkers for asthma phenotyping remain an unmet medical need. tRNA-derived fragments have been proposed as markers of different diseases and among them, tiRNA are of particular interest since they are produced under stress conditions. To test whether tiARNs can be used to evaluate asthma severity, 5' tiRNA Glu-CTC and 5' tiRNA Gly-GCC, two abundant tiRNAs in saliva, were quantified in sputum samples of patients (n=42, 13 healthy controls and 29 asthmatics, 13 of which are severe cases). Their levels were assessed both in cells obtained from the sample (mainly leukocytes) and in sputum supernatants. We found that 5' tiRNA Gly-GCC levels were significantly elevated only in supernatants from patients with severe asthma. This lack of correlation with what is observed in sputum cells suggests that tiRNAs could be secreted by the bronchial epithelium.
Similar results were obtained when we discriminated patients according to sputum inflammatory phenotype, where extracellular 5' tiRNAGly-GCC showed increased levels in eosinophilic infiltrates, a hallmark of severe patients. Interestingly, levels of this tiRNA were significantly correlated with the expression of angiogenin (ANG) in circulating leukocytes. ANG is the enzyme responsible for tiRNA production through specific cleavage of tRNAs in their anticodon loop. High expression of ANG in blood samples from severe and eosinophilic patients indicates that this pathway is activated in asthma. In line with these findings, levels of 5' tiRNA Gly-GCC and ANG are positively correlated with production of pro-inflammatory cytokines such as IL-1, IL-6 and IL-8 in sputum. In sum, our results contribute to shed light on the role of tiARNs in the pathogenesis of asthma and suggest the potential use of 5'tiRNA-Gly-GCC for its phenotyping.

109. (583) **FRACTAL MATHEMATICAL MODELS FOR THE ANALYSIS OF FEMORAL GROWTH AND DEVELOPMENT IN FEMALE RABBITS OF THE NEW ZEALAND LINE**

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Endochondral growth progresses from the proliferation, maturation and hypertrophy of chondrocytes, organized in the ossification centers, to the mineralization of the cartilage matrix to form bone tissue. It begins during fetal life and ends at the end of early adulthood. Cartilage, as a complex system, is constituted by self-similar parts, which possess self-organizing capacity and fractal rhythm. Fractality can be analyzed through algorithms that determine the Fractal Dimension(DF) and the Predictive Determination Coefficient(R^2), which would indicate the irregular distribution of chondrocytes in space and their capacity of adaptation and interaction with the environment, respectively. The growth and development of the femur was analyzed through the fractal study of the chondrocytic lineage, by means of the Box Counting Algorithm (ABC), in different vital stages. The units of analysis were 12 New Zealand 2 weeks female rabbits. Femoral samples were obtained from the 1st to the 6th month of life. The samples were decalcified, fixed and dehydrated, and longitudinal sections of the diaphyses were made and stained with hematoxylin eosin. ABC was applied to digitized images of the femur histological sections with FrakOut! software and the DF and R^2 of chondrocyte cellularity (CC) were determined. The results were expressed as Median(M) and Standard Deviation (±) of DF and R^2 of CC. Pearson's correlation coefficient between DF(CC) and age in months was obtained. Results: DF(CC) was M= 1.66 ± 0.06, while R^2(CC)= 0.99 ± 0. The correlation between DF(CC) and age was obtained: r=(-)0.85 ($p < 0.001$). Conclusions: R2 values indicate extensive, sustained interaction of cells with each other and with the interstitium, with the ability to accommodate possible variations in the environment. Fractal studies confirmed clear decrease in chondrocytic cellularity during growth and development.

GASTROENTEROLOGÍA

110. (027) **STUDY OF MRP2 IMPAIRMENT INDUCED BY CHOLESTATIC AGENTS IN HEPARG CELL LINE**

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Human HepaRG is an *in vitro* alternative to animal models for biliary excretion studies, due to functional bile canaliculi formation and expression of main canalicular transporters, like multidrug resistance-associated protein 2 (Mrp2). HepaRG differentiation toward biliary and hepatocyte phenotypes requires a culture medium containing fetal bovine serum (FBS) and differentiating agent dimethyl sulfoxide (DMSO) to promote polarization.
AIMS:To evaluate hepatocyte markers expression and Mrp2 localization in response to culture media supplemented with local FBS, and to study Mrp2 activity impairment by cholestatic agents.
METHOD:Proliferation: cells were cultured 2 weeks (manufacturer´s protocol) in medium supplemented with 10% FBS Internegocios S.A.(Bs. As.). Differentiation: cells were cultured 2 additional weeks increasing DMSO concentrations (0.5, 1, 1.7%). Extraction of total mRNA was performed using Trizol following RT-qPCR. Mrp2 and zonula occludens 1 (ZO1) localization were analyzed by confocal immunofluorescence. Cells were treated with estradiol 17ß-d-glucu-

ronide (E17G 200 μM) and taurolithocholate (TLC 2.5 μM) 20 min and Mrp2 activity was evaluated by secretion of fluorescent methylfluorescein (GMF).
RESULTS:HepaRG differentiation was verified by hepatocyte transcript levels at day 28 (fold change (FC) to day 6 proliferation) of aldolase FC=83, cytochrome P450 3A4 FC=47, organic anion transporting polypeptide 2B1 FC=5 and Mrp2 FC=3. Confocal images of differentiated cells showed polarized ZO1 hepatocyte-like distribution and canalicular localization of Mrp2. Mrp2-mediated transport of GMF was decreased (% Control) by E17G (43±8*) and TLC (47±3*) *p<0.05 vs Control, n=3.
CONCLUSION:HepaRG cultured with local FBS and gradually increasing DMSO induced differentiation showing canalicular localization of Mrp2. Functional studies with E17G and TLC induced a decrease in Mrp2 activity, supporting that HepaRG under described conditions are useful for cholestatic studies.

111. (071) **EFFECT OF YERBA MATE (*ILEX PARAGUARIENSIS*) ON THE RESTORATION OF ETHANOL-INDUCED GASTRIC MUCOSA DAMAGE IN RATS**

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There is a popular belief that the consumption of yerba mate (YM) causes symptoms of gastric distress (heartburn or gastritis). However, there is no scientific evidence in this regard. In previous experiments, we have evaluated the effect of chronic consumption of YM infusions on the gastric mucosa without finding signs of damage. We wonder how the previous state of the mucosa would influence the referred symptoms. The objective of this work was to study the effect of the ingestion of an infusion of YM on the restoration of the gastric mucosa of rats with a previous gastric injury due to ethanol consumption.
Thirty-two fasted rats were administered 1 mL of 60% ethanol by orogastric tube to produce damage and then a 10% ethanol solution was supplied *ad libitum* (E). In addition, a group of control animals (C) received only water and another, YM infusion (25 g/500 ml, 70 °C, 5 min), (n = 8 each). After 7 days, animals were sacrificed (n = 8 each). E was discontinued in the remaining animals of group E (n = 24), they were separated and given water (E+W) or YM (E+YM). Euthanasia was performed at day 3 and 7, the stomachs were processed for histological evaluation of macro and microscopic lesions and the levels of lipoperoxidation were determined in homogenates of the mucosa.
Histopathological analysis: C and YM showed a mucosa with normal characteristics both macro and microscopically. Group E evidenced loss of folds, edema and congestion. Microscopically, it presented slight distortion of the glandular lumens, disruption of the epithelial surface and mild edema and congestion. After the suspension of E, both E+W and E+YM macroscopically showed a mucosa with normal characteristics both at day 3 and 7. Microscopically, at day 3, both groups presented mild edema and congestion, and completely normalizing after day 7 of suspension of E.
Evaluation of oxidative damage (mean ± SD): C: 0.26 ± 0.04 nmolTBARS / mg protein, YM: 0.17±0.05*, E:0.35±0.09, ANOVA, Tuckey post test, *p <0.05 vs group E). Post-sacrifice: no differences were observed at day 3 or 7.
We conclude that the administration of an infusion of YM did not affect the restoration of ethanol-induced gastric mucosa damage in rats. The presence of lower levels of lipoperoxidation in the group treated with YM could indicate a beneficial effect that cannot be observed histologically with the times used.

112. (143) **TAUROLITHOCHOLATE CONTRIBUTES TO MRP2 ACTIVITY IMPAIRMENT BY INDUCING A TRANSIENT ROS PRODUCTION**

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Previous studies in different models of cholestasis showed that reactive oxygen species (ROS)/oxidative stress mediate the internalization of the hepatocanalicular transporters, such as multidrug resistance-associated protein 2 (Mrp2). Taurolithocholate (TLC) is known to be the most pro-oxidative bile salt. However, there is no direct evidence that ROS production derived from TLC action is a mechanism involved in cholestasis pathogenesis. Herein, we evaluated a possible role of ROS in the TLC- induced impairment of Mrp2 activity.
Methodology: ROS production was measured by the 2´,7´-dichlorofluorescin-diacetate (DCFH-DA) assay in primary culture rat hepatocytes. Cells were exposed to TLC (2.5 μM) at different times (5, 10, 15 and 20 min) followed by incubation with DCFH-DA (5 µM). On the other hand, isolated rat hepatocyte couplets (IRHC) were co-treated with TLC (2.5 μM) and antioxidants: vitamin C (VitC 100 μM) or mannitol (Man 60 mM) for 20 min. To analyze the TLC-induced ROS involvement in Mrp2 activity impairment, functional studies were carried out by assessing the canalicular vacuolar accumulation of its substrate glutathione methylfluorescein (GMF).
Results: (% of Control±SEM; n=3-5): TLC increased intracellular ROS production in hepatocytes, reaching the maximum peak at 5 min (133±7a) and rapidly returning to control levels at 10 min (102±4). This transient production suggests the participation of ROS as signaling molecules. Pre-treatment of IRCH with both antioxidants prevented TLC- induced impairment of canalicular accumulation of GMF: TLC (67±6a), TLC+VitC (92±3b), TLC+Man (94±7b), pointing out ROS as possible modulators of Mrp2 internalization. ap<0.05 vs Control, bp<0.05 vs TLC.
Conclusion: TLC treatment induced transient rise in ROS levels which could be a key signaling component that contributes to the altered Mrp2 function found in cholestasis.

113. (156) **NADPH OXIDASE PARTICIPATE IN THE CHOLESTATIC ALTERATIONS INDUCED BY ESTRADIOL 17 BETA-D-GLUCURONIDE DOWNSTREAM MEK-ERK 1/2 KINASES**

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We have previously demonstrated that NADPH oxidase (NOX)-generated reactive oxygen species (ROS) are involved in the impairment of canalicular secretion induced by estradiol 17β-D-glucuronide (E17G) [*Physiological Mini Reviews* 12 (Special Edition): 019, 2019], and that NOX is in the same pathway that MEK-ERK ½ [*Medicina (Bs. As.)*, 80 (supl V): 67, 2020]. Now, we intended to locate NOX into this pathway. To achieve this goal, we employed two different methodological approaches in primary-cultured rat hepatocytes (PCH) treated with E17G. First, we evaluated the ability of the NOX inhibitor apocynin (Apo) to prevent ERK 1/2 activation by measuring by western blotting (WB) its degree of phosphorylation. Additionally, we tested the possibility that ERK 1/2 inhibition with the MEK inhibitor PD98059 (PD) can avoid the ROS increase induced by E17G, assessed fluorometrically by the 2',7'-dichlorofluorescin-diacetate assay, which correlates dichlorofluorescin (DCF) fluorescence intensity with intracellular ROS concentration. E17G (200 μM, 20 min) increased by 54±14% (154±14, p<0.05 vs control, n=3) the amount of p-ERK 1/2, while pre-incubation of PCH with Apo (100 μM, 30 min) showed the same degree of ERK 1/2 phosphorylation, indicating that NOX activation is not necessary for E17G-induced ERK 1/2 activation. Furthermore, pre-incubation of PCH with PD (5 μM, 15 min) exhibited the same complete inhibitory effect as Apo on the elevation of intracellular ROS induced by E17G (200 μM, 15 min) [DCF intensity (% of control), Mean±SEM:E17G=140±10%*, E17G+A-

po=95±3%#, E17G+PD=85±7%#; *p<0.05 vs control, #p<0.05 vs E17G, n=3-5], thus suggesting that MEK-ERK 1/2 activation by E17G is a prerequisite for NOX activation and intracellular ROS elevation. These findings locate NOX downstream MEK-ERK 1/2 in the E17G-induced, ROS-mediated pathway that contributes to the impairment of canalicular transport in hepatocytes.

114. (159) **ANTICHOLESTATIC MECHANISMS OF OBETICHOLIC ACID (OCA) IN LIPOPLYSACARIDE (LPS)-INDUCED CHOLESTASIS IN THE RAT**

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Sepsis-induced cholestasis is due the release of inflammatory cytokines induced by LPS from Gram (-) bacteria, which impair expression/localization of hepatocellular transporters. There is no therapy for this condition. OCA is a potent FXR agonist used to treat human inflammatory chronic cholestasis. We ascertained here its anticholestatic mechanisms in LPS-induced cholestasis.

Methods: Male Wistar rats were randomized in Control, OCA (20 mg/Kg/day, i.p., 6 days), LPS (6.5 mg/Kg, i.p., last 2 days) and OCA+LPS groups. Then, we assessed serum alkaline phosphatase (ALP), a surrogate of bile salt (BS) hepatic accumulation, and taurocholate-stimulated BS output (BSO). *mRNA/protein* levels of Bsep and Mrp3 (apical and sinusoidal BS efflux pumps, respectively) and Ntcp (BS uptake carrier) were assessed by either or both Real time PCR and Western blot. Bsep localization was assessed by immunohistochemistry followed by confocal microscopy and image analysis. The inflammatory cytokines TNF-α and IL-1β were measured in serum by ELISA.

Results: (*p<0.05 *vs.* control; #p<0.05 *vs.* LPS). OCA reduced ALP (U/L) in LPS-treated rats (193 ± 18# *vs.* 338 ± 72*), and improved BSO (nmol/g liver) (1274 ± 152 *vs.* 896 ± 86). This was due to an increase in the % of Bsep in the apical membrane (104 ± 13# *vs.* 52 ± 9*), a finding confirmed by image analysis of confocal images (p<0.001). OCA also halted the elevations (pg/ml) of TNF-α (266 ± 56*# *vs.* 860 ± 153*) and IL-1β (13 ± 2# *vs.* 52 ± 8*). Neither the drop in Ntcp (46 ± 11* *vs.* 52 ± 9*) nor the increase in Mrp3 (279 ± 29* *vs.* 299 ± 60*) expression induced by LPS (% of control) was affected.

Conclusions: LPS impairs BSO, leading to BS accumulation (cholestasis). OCA prevented this by improving apical Bsep localization and the subsequent BSO; the adaptive BS urinary elimination afforded by the impaired uptake and the increased sinusoidal efflux of BSs was maintained. Counteraction of cytokine elevations may be crucial for OCA beneficial effects.

115. (162) **OXIDATIVE STRESS IS INVOLVED IN THE IMPAIRMENT OF MRP2 ACTIVITY INDUCED BY IL-1ß IN RAT**

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The inflammatory cytokine IL-1ß is increased in inflammatory diseases that are associated with cholestasis. IL-1ß activates signaling pathways that lead to endocytosis of canalicular transporters such as Mrp2. IL-1ß generates reactive oxygen species (ROS) and these species are known to act as signal promoting canalicular transporters endocytosis.

Aim: To evaluate the possible role of ROS as signaling mediators in Mrp2 internalization in the cholestasis by IL-1ß using the isolated rat hepatocyte couplet (IRHC) model.

Methodology: To prevent the formation of ROS, IRHCs were pre-incubated with the antioxidants vitamin C (1 mM), mannitol (60 mM) for 15 min, followed by addition of cholestatic agent IL-1ß (10 ng/ml) for 20min. Then, they were exposed to chloromethylfluorescein diacetate (2.5 μM, 15 min), converted intracellularly into glutathione methylfluorescein (GMF), Mrp2 substrate. Finally, Mrp2 activity was estimated by the percentage of IRHCs that accumulated GMF in the canalicular vesicle (cVA).

Results: (% of control ± SE): IL-1ß significantly reduced Mrp2 activity (cVA: IL-1ß=65±5%a). This was prevented by vitamin C (IL-1ß+VitC=101±5%b) and mannitol (IL- 1ß +M=100±10b). a different from control, b different from IL. P <0.05. (n = 4).

Conclusion: These results suggest a central role of ROS formation in the IL-1ß-induced impairment of Mrp2 activity.

116. (246) **SPARC (SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE) CAN ACTS AS A "DAMAGE-ASSOCIATED MOLECULAR PATTERNS" TRIGGERING INFLAMMASOME ACTIVATION IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)**

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Nonalcoholic steatohepatitis (NASH) is a progressive and inflammatory form of NAFLD. Inflammation and hepatocyte damage distinguish NASH from simple fatty liver (steatosis). NLRP3 inflammasome, which sense damage signals and acts as a driver of innate sterile inflammation via activation of caspase1 (casp1) and release of proinflammatory cytokines including IL1β, have a central role in NAFLD. SPARC is an extracellular protein expressed in response to injury. We previously demonstrated that SPARC absence reduce NASH in a murine diet-induced obesity model. The present study aimed to assess the role of SPARC in the initiation of inflammasome-mediated immune response that can lead to NAFLD progression.

We performed a bioinformatic analysis of available RNAseq data from NAFLD patients. Patients were classified according histopathological NAFLD activity score. NAFLD patients increased SPARC expression, while NLRP3 inflammasome-related gene were increased mainly in NASH patients. Positively correlation between SPARC, IL1β, NLRP3 and casp1 were shown. Ontology analysis revealed that genes that positively correlate with SPARC in NASH were involved in innate immune pathways. In a murine diet-induced NAFLD model $SPARC^{+/+}$ and $SPARC^{-/-}$ mice were fed with high fat diet for 12 (steatosis) or 20 weeks (NASH), we observed that the absence of SPARC attenuated NLRP3 inflammasome-related gene expression. *In vitro* studies on primary hepatocyte cultures from $SPARC^{-/-}$mice, showed decrease expression of IL1β and casp1 in response to free fatty acid. Macrophage cell line, primary Kupffer and hepatocyte cultures were incubated with free fatty acids and SPARC to assess effect on inflammasome-related gene expression, IL1β secretion and casp1 activity. SPARC increased IL1β and casp1 expression and secretion. SPARC alone or in combination with fatty acid trigger NLRP3 inflammasome activation. Our results suggest a key role of SPARC as a damage-associated molecular patterns in NAFLD progression.

117. (247) **THE ROLE OF AQUAPORIN-1 IN CHOLANGIOCARCINOMA PROGRESSION**

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Background and aims cholangiocarcinoma (CCA) is a heterogeneous group of malignancies arising from cholangiocytes with very poor prognosis. It is essential to investigate the molecular pathogenesis of CCA to identify new potential therapeutic targets. During the last decades, the association between the aquaporin-1 (AQP1) water-channel expression and tumor behavior has been investigated in several cancer types but in CCA, remains unknown. Our aim was to begin studying the role of AQP1 in CCA progression. **Materials and methods:** bioinformatic analysis was performed to determine correlationsbetween AQP1 and overall survival in human intrahepatic CCA samples. AQP1 KO HuCCT1 CCA cells were generated by using the CRISPR/Cas9 system. Cell proliferation and migration studies were performed by using Incucyte live-cell imaging system. We also performed an unbiased discovering experiment using Next-generation sequencing technique (RNAseq), comparing wild-type and AQP1 KO CCA cells. Epithelial-mesenchymal transition (EMT), receptors and molecular signaling pathways were evaluated by RT-qPCR, Western-Blot and confocal immunofluorescence microscopy. **Results:** there was a positive correlation between AQP1 and overall survival in human CCA. AQP1 KO significantly induced proliferation and migration of CCA cells. RNAseq showed a total of 2702 differentially expressed genes upon AQP1 KO in HuCCT1 cells. The Ingenuity Pathway Analysis showed cancer, cellular movement, and cellular growth and proliferation as the top affected Diseases and Biological functions. AQP1 silencing was associated with an EMT phenotype, i.e., downregulation of epithelial (e.g., E-cadherin) and upregulation of mesenchymal (e.g., Vimentin) markers, and showed differential activation of intracellular pathways involved in tumor progression (i.e., IGF2/IR/IGF1R and Hippo). **Conclusion**: Our data suggest that AQP1plays an important role driving CCA progression, especially, in the dissemination of the tumor cells.

118. (404) **GLYCOSYLATION AS A TARGETED THERAPY FOR HEPATOCELLULAR CARCINOMA**

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Reaching efficacious drug delivery to target tissues represents a major obstacle in the current treatment of solid malignancies. The aim of this work was to develop a specific targeting for hepatocellular carcinoma (HCC) therapy.
The therapeutic efficacy of enzymatically synthetized rutinosyl-4-methylumbelliferone (4MUR), which expose rhamnose as a terminal unit, was analyzed *in vitro*. 4MUR showed a significant anti-proliferative effect on liver tumoral cells (Huh7 IC_{50} = 488 μM), as compared to non-tumoral cells (IHH IC_{50} >2000 μM) and hepatic stellate cells (CFSC-2G IC_{50} >2000 μM) in a dose-dependent manner ($p<0.05$) .The mechanistic bases of 4MUR selective target to liver tumor cells was evaluated by the interaction with asialoglycoprotein receptor (ASGPR), the expression of hyaluronan synthases (HAS2 or HAS3) and hyaluronan deposition. We demonstrated that ASGPR are overexpressed in liver tumoral cells and 4MUR is incorporated via interaction with these receptors. 4MUR-treatment decreased the levels of HAS2 and HAS3 and the cytoplasmic deposition of hyaluronan. Finally, to determinate 4MUR effect on CFSC-2G activation, the expression of the profibrotic-related genes α-SMA, TGF-β, COLI A2, TIMP-1 and the antifibrogenic gene MMP-2 was evaluated. We found that these genes were upregulated, while MMP-2 was not affect.
Altogether these insights provide evidence that 4MUR is a potential antitumoral drug that presents targeting capability, without damaging non-tumoral cells.

119. (534) **IMPACT OF ELDERLY DONORS IN LIVER TRANSPLANT OUTCOME**

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Elderly donors are on the increase due to the urgent need of organs for transplant procedures. Currently the impact of donor age on liver transplantation is subject of debate. Particularly, there is no published data about the use of organs from elderly donors in our country. *Objective:* Studying the impact of donor age in the clinical outcome of liver transplant (LTx) patients at the Hospital Universitario Fundación Favaloro. *Methodology:* A retrospective cohort study of 493 LTx (period 2009 -2020) in HUFF was performed. Patients were grouped into different categories based on donor age, ≥18-39 (Young, Y), 40-59 (Adults, A), ≥60 years (Elderly, E). *Results:* After applying the exclusion criteria, a total of 279 donors were included (94 Y, 136 A and 49 E). Kaplan-Meier analysis revealed reduced survival rate of adults and elderly patients who were transplanted using E livers in contrast with the younger grafts (log-rank.test, $p<0.05$). The postoperative liver tests were analyzed during the first week post-Tx. Levels of both hepatocellular (ALT, AST, ALP) and cholestatic (TBIL) markers were lower ($p<0.05$) in patients with E livers (n=9) compared with the A group (n=14). To study the effect of donor allograft age on recipient's alloimmune responses, the frequency of acute cellular rejection (ACR) during the first-year post-Tx was determined by analyzing the histopathological reports in Y (n=27), A (n=32) and E (n=27) donor groups. Increasing donor age was associated with an increased rate of ACR ($p>0.05$, ns). Histopathological diagnosis of ACR was found in 30% of E, 25% of A and 15% of Y donor group. In addition, E recipients presented a lower rate of ACR compared with A recipients (36% *vs.* 11%; significant relationship between ACR and Recipient group was detected, $p<0.05$), independently of graft age (AD-AR (44%) vs AD-ER (6%), $p<0.05$; YD-AR (20%) vs YD-ER (8%), $p>0.05$; ED-AR (45%) vs ED-ER (19%), $p>0.05$). *Conclusion:* Our results indicate that the use of E donors was associated with reduced long-term survival rate, increased rate of ACR during the first year post-Tx; despite having lower initial hepatocellular and cholestatic injury than younger donors.

120. (549) ***HELICOBACTER PYLORI* DIAGNOSIS, SERUM GHRELIN AND PEPSINOGEN LEVELS AS POTENTIAL NON-INVASIVE BIOMARKERS OF GASTRIC PATHOLOGY**

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The search for biochemical markers of gastric pathology has been a focus of research during last years. Ghrelin is an orexigenic hormone mostly synthetized in the stomach, which we demonstrated to be lower in serum from *Helicobacter pylori* infected patients. Serum pepsinogen I (PGI) and II (PGII) have been associated with the topography of gastric pathology. To achieve our general aim of finding potential biomarkers of gastric pathology, the objective of this study was to determine serum concentrations of ghrelin and pepsinogens I and II in *H. pylori* infected and uninfected patients with different types of gastric pathology. The protocol included samples from dyspeptic adults (18-70y) referred for an upper gastrointestinal en-

doscopy to the Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo". Histopathology and *H. pylori* diagnosis were evaluated from gastric biopsies. Serum ghrelin concentration, PGI and PGII were measured by ELISA. Kruskal-Wallis and Mann Whitney tests were applied. Thirty-five individuals were included to date, 77.1% female, with an age of 40.2±13.0y. *H. pylori* prevalence was 71.4% (CI95% 54.9-83.7%). Median ghrelin levels were 396.0 pg/ml (IQR 315.0-501.0); PGI, 45.1 ng/ml (IQR 29.5-55.7); PGII, 3.3 ng/ml (IQR 2.0-6.0); PGI/PGII 11.0 (8.0-16.7). PGI and PGII were significantly higher for *H. pylori* infected compared to non-infected patients (*P*=0.014 and *P*=0.0001, respectively); however, PGI/PGII ratio did not differ between both groups (*P*=0.14). Serum PGII differed significantly between normal mucosa, chronic inactive and active gastritis of the antrum and corpus (*P*=0.003 and *P*=0.049, respectively), being higher in the presence of gastric pathology. These preliminary results suggest that the measured biochemical variables might be useful as potential biomarkers of gastric pathology; further analysis after inclusion of the total sample size for this protocol is needed to confirm these findings.

121. (582) **BENEFICIAL PROPERTIES OF PASSIFLORA CAERULEA ON INTESTINE IN STRESS MODEL INDUCED BY IMMOBILIZATION IN RATS**

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Irritable bowel syndrome (IBS) is a prevalent gastrointestinal disorder that has been related with psychological factors. There is growing evidence that IBS or IBS-like symptoms could be a prodrome before diagnosis of Inflammatory Bowel Disease or can be observed in IBD patients in remission. Its treatment is challenging and use of alternative medicines, especially herbal therapies is increasing. In this sense, *Passiflora caerulea* (PC), a native plant that previously showed to possess beneficial properties in preclinical models related to Inflammatory Bowel Disease was studied. Male Sprague Dawley rats weighing 210 –230 g were used. Four groups of animals (n=6) were treated with PC (250 and 500 mg/Kg), clonazepam (Clo) 1 mg/Kg or water during four days (S). In the fifth day these groups were restrained using a plastic rodent restrainer. Control group (C) were not subjected to stress. Immediately after 6 hours, blood was collected and colon and ileon were removed for posterior analysis. Exposure to acute immobilization produced an increase in plasmatic corticosterone level (S: 487.4±193.8 ng/mL, C: 118.2±28.9) and a slight increase in blood glucose. These effects were reduced by treatment with PC (250 mg/Kg: 195.5±26.5, 500 mg/Kg: 74.25±14.4) and reference drug (Clo: 244.0±20.1). Redox status expressed as GSH:GSSG ratio was increased in ileon and colon in S groups, meanwhile the extract significantly reduced this effect only in ileon. Histopalogical analysis showed that goblet cells were depleted by immobilization in ileon and colon and both doses of extract in ileon and only higher dose in colon ameliorated these effects. Western blot analysis showed that P53 and caspase-3 proteins were increased in ileon and colon of stressed animals, meanwhile PC reduced both parameters in both portions of intestine. These observations suggested that PC could represent a promising treatment to improve gastrointestinal health in stressful conditions.

GENÉTICA

122. (037) **GJB2 AND GJB6 GENETIC VARIANT CURATION IN A NON-SYNDROMIC HEARING LOSS COHORT FROM ARGENTINA**

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Hereditary hearing impairment affects 1-500 newborn children. It is characterized by the large number of genes involved (more than 100) and its phenotype heterogeneity. Despite the wide genetic variety of hearing impairment, the most commonly mutated genes in severe to profound autosomal recessive non-syndromic hearing loss are *GJB2* and *GJB6*, accounting for nearly 50% of the cases in most populations around the Mediterranean Sea. Molecular diagnosis enables proper genetic counseling and medical prognosis to patients. Therefore, correct interpretation of the phenotypic consequences of genetic variants is crucial in genetic diagnosis, since discrepancies in sequence variant interpretation and classification has been reported to lead to serious impact in patient health maintenance.In this study we aimed to identify the genetic causes of hearing loss and performed a manual genetic variant curation following the American College of Medical Genetics and Genomics/Association for Molecular Pathology ACMG/AMP standards and hearing-loss-gene-specific criteria of the ClinGen Hearing Loss Expert Panel.A total of 600 patients were studied for genetic variants in GJB2 and *GJB6* genes by Sanger Sequencing technique and Multiplex Gap-PCR, respectively.Overall, 48 different sequence variants were detected in our cohort of patients, being the c.35delG the most common causative variant identified. Besides, more than 50% of sequence variants were reclassified from their previous categorization in ClinVar after careful manual analysis. These results provide an accurately analysed and interpreted set of variants to be taken into account by clinicians and the scientific community, and hence, aid the precise genetic counseling to patients.

123. (184) **ANALYSIS OF TP53 ABERRATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

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Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world. *TP53* (*Tumor protein P53*) gene deletions and mutations constitute important prognostic and predictive factors associated to poor outcome in CLL, with relevance in clinical decisions. We evaluated *TP53* alterations in 218 CLL patients (131 men; mean age: 64.9 years, range: 35-87 years; RAI clinical stages: 0: 36%; I-II: 47.1%; III-IV: 16.9%), to analyze their frequency, distribution and association with prognostic factors of the disease. PCR and bidirectional Sanger sequencing to evaluate IGHV (*immunoglobulin heavy variable region*) and *TP53* mutational status were used. Cytogenetic and FISH (*Fluorescence in situ hybridization*) analysis were performed. The study was approved by the Local Ethics Committee. All individuals gave their informed consent. Twenty-nine patients (13.3%) had *TP53* aberrations. Deletion 17p13 [del(17p)] was observed in 26 cases (12%); 3 patients showed only *TP53* mutations (*TP53*-M), and 10 cases had *TP53* deletion and mutation. Two deletions and two insertions (3 frameshifts) (26.7%), and 11 point mutations (73.4%) (1 at the splicing site and 10 missense), were found; 86.6% variants were located in the DNA-binding domain (exons 4-8). Cases with *TP53*-M showed significantly higher mean percentages of leukemic cells with del(17p) (33.5%) than those with unmutated *TP53* (12%) (p=0.016). *TP53*-M patients showed association with unmutated IGHV (87.5% cases) and chromosome alterations (88.9%), including complex karyotypes. Similar clinical behavior between cases with del(17p)

and those with *TP53*-M was found. To our knowledge, this is the first evaluation of *TP53* aberrations in a large series of CLL patients in our country. Our findings show high genomic instability in these cases, provide information about the molecular heterogeneity of this disease, and reinforce the importance to evaluate *TP53* aberrations to optimize patient stratification and outcome.

124. (185) **METABARCODING OF HUMAN GUT BACTERIAL COMMUNITIES IN INFLAMMATORY BOWEL DISEASE**

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BACKGROUND: Globally, Inflammatory Bowel Disease (IBD) is the most common form of intestinal inflammation associated with a deregulated immune-system response to commensal microbiota in a genetically susceptible host. IBD includes ulcerative colitis (UC) and Crohn disease (CD). In the present study we aim to describe the unknown gut microbiota of IBD-patients in comparison with healthy individuals of the Argentine population in order to find novel IBD biomarkers in our region. METHODS: We evaluated 40 non-IBD-controls, 20 UC-patients and 14 CD-patients from the metropolitan area of Buenos Aires, Argentina. Fecal DNA was extracted and hypervariable regions V3-V4 of the bacterial 16SR-gene were sequenced using a MiSeq-Platform. Sequences were analyzed with the QIIME2 environment. Differential functional pathways were evaluated using PICRUSt. RESULTS: Beta diversity was significantly different between groups (UniFrac distances PERMANOVA p-value <0.05). In UC-patients we found no significant differences in alpha diversity compared to CD and non-IBD-control. However, differences in alpha diversity were found in CD compared to controls (Shannon q=0.04). The genus *Paraprevotella* was found to be overrepresented in UC compared to CD. Our analyzes also indicate that the genus *Lactobacillus* has a higher representation in the CD group while the genus *Fusicantenibacter* is more represented in the controls. Finally, functional pathways were analyzed finding that CD has different metabolic capabilities compared to the control group. CONCLUSIONS: Overall, our study provides new knowledge on the gut microbiota composition of our population, allowing the association of local changes in gut microbial diversity in UC and CD. These novel findings would enable personalized therapies development through the use of metagenomic profiles of the Argentine population.

125. (238) **FINE-TUNING THE MOLECULAR APPROACH TO DIAGNOSE CHILDREN WITH MITOCHONDRIAL DNA DELETION SYNDROMES**

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Single large-scale mitochondrial DNA deletions are associated with 3 overlapping phenotypes: chronic progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (SKS) and Pearson's syndrome (PS).
OBJECTIVES: To describe the optimization of a molecular diagnostic strategy for mitochondrial DNA (mtDNA) deletion syndromes (DS). To perform the clinical-molecular characterization of 7 patients with mtDNA DS.
PATIENTS AND METHODS: Clinical data from 7 patients diagnosed with DS were analyzed. The "common deletion" (CD) in mtDNA (m.4977del) was determined by Long-PCR. Other deletions and the level of heteroplasmy were studied by Multiplex Ligation-dependent Probe Amplification (MLPA). The breakpoints of deletions were determined by Sanger sequencing.
RESULTS: 3 patients with SKS, 2 PEO and 2 PS were identified (age range: 0.5- 11 years). The most frequent clinical manifestations were PEO and short stature. All patients presented high lactic acid levels in plasma/urine. The 4 available muscle biopsies (MB) showed red-ragged fibers. Molecular studies showed the CD in 3 patients with SKS. Larger deletions (6,927-7,436bp), with a heteroplasmy range of 25-78%, were found in the other 4 cases. In 3 patients, the deletion was detected in peripheral blood (PB) and in 4 of them in MB. The m.8637_16072del deletion in a patient with PS was detected by MLPA in PB and further studied in PB, buccal mucosa and urine samples by long-PCR. Type-1 deletions associated with repetitive sequences were detected in 6 patients and 3 deletions had not been previously reported.
CONCLUSIONS: The severity of the disease showed no relation with the size or type of the deletion in this group of patients. The implementation of Long-PCR and MLPA allowed the diagnosis of patients with DS in non-invasive samples, leaving MB as a 2nd-line-study. A thorough clinical characterization and these molecular studies are key steps for reaching the definitive diagnosis of patients with mtDNA DS.

126. (322) **ANALYSIS OF A SPLICING VARIANT IN *DMD* AND THE IMPACT AT mRNA LEVEL**

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Dystrophinopathies are rare progressive X-linked muscular diseases caused by mutations in *DMD.* Duchenne muscular dystrophy (DMD) is the most common and represents the most severe form. Although most of the alterations that occur in the *DMD* gene affect coding areas, ~ 7% are found in splicing regions. It is difficult to predict the effect that these variants will have on the splicing process.
We present the case of an 8-year-old boy with dystrophinopathy, biopsy with preserved dystrophin and a NGS study where the probably pathogenic variant *DMD*:c.4519-2A> C (exon 33) was identified. Our aim was to analyze the consequence of the splicing acceptor site variant at the mRNA level.
Muscle mRNA was extracted and cDNA was synthesized. Long-range and nested PCRs of exons flanking the variant were performed and sequenced by Sanger.
Two isoforms of different molecular weight were observed. The most abundant with the highest molecular weight (A) had a 14bp deletion, while the less abundant with lower molecular weight (B) had a exon 33 complete deletion. The sequencing allowed the prediction that A would produce a frameshift (FS) with the appearance of a premature translation codon, leading to the absence of dystrophin protein. On the other hand, it is expected that FS will not be produced in B and a shorter dystrophin protein will be generated. This finding agrees with immunohistochemistry where DYS 1, 2 and 3 domains were preserved. The presence of isoform B, which product would generate a shorter but partially functional dystrophin, would explain the milder condition observed in the patient.
In conclusion, the mRNA study allowed corroborating the deleterious impact of the variant at the splicing level, allowing the correlation between the splicing alteration, the dystrophin isoforms, the biopsy result, and the patient's clinical manifestations.

127. (323) **DIFFERENTIAL MOLECULAR ALGORITHM FOR DUCHENNE MUSCULAR DYSTROPHY. COMPARATIVE STUDY OF LATIN AMERICA MUTATION FREQUENCIES WITH THERAPEUTIC TARGET**

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Dystrophinopathies cover a spectrum of rare progressive X-linked muscle diseases, arising from *DMD* mutations. They are among the most common neuromuscular diseases, being Duchenne Muscular Dystrophy (DMD) the most severe form. Despite that there is still no cure for these diseases, advances are being made for the development of DMD therapies. Some are already approved: exon skipping and premature stop codon read-through (Ataluren). We aimed to characterize the mutational spectrum of *DMD* in an Argentinian cohort, to identify candidates for available treatments and, to conduct a comparative analysis of the Latin American (LA) frequencies of mutations amenable for available DMD therapies.
We studied 400 patients with dystrophinopathy, implementing a diagnostic algorithm including: MLPA/PCR/Sanger/Exome/in-silico panels and bioinformatics. We performed a meta-analysis of LA's metrics for DMD available therapies. The algorithm resulted effective for achieving differential diagnosis, reaching a 97% detection rate. Therefore, 371 patients with genetic confirmation of dystrophinopathy resulted candidates for corticosteroid treatment, 20 were eligible for exon skipping of exon 51, 21 for exon 53, 12 for exon 45 and another 70 for Ataluren. We determined that 87.5% of DMD patients will restore the reading frame with the skipping of only one exon. According to the meta-analysis, only 4 LA countries (Argentina, Brazil, Colombia and Mexico) complete the molecular algorithm for dystrophinopathies. In conclusion, this manuscript describes the theragnosis carried out in Argentinian dystrophinopathy patients. The implemented molecular algorithm proved to be efficient for the achievement of differential diagnosis, which plays a crucial role in patient management, determination of standard of care and genetic counselling. Finally, this work contributes with the international efforts to characterize the frequencies and variants of LA, pillars of drug development and theragnosis.

128. (336) **LOW PENETRANCE RETINOBLASTOMA FAMILIES**

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Retinoblastoma (RB), the most common eye pediatric neoplasm, results from biallelic inactivation of *RB1* tumor suppressor gene. Predisposition to RB is transmitted as an autosomal dominant trait with 90% penetrance. However, some RB families included unaffected carriers indicating low penetrance (LP). Our aim was to study the families with LP retinoblastoma and detect the asymptomatic carriers. *RB1* mutations were identified by sequencing, MLPA assay and haplotype analysis. We reviewed the analyses of 18 families, 15 of them segregated with high-penetrance (HP) mutations and 3 with low-penetrance (LP) mutations. In addition, 4 families with sporadic RB patients included unaffected mutation-carrier relatives. The HP families included 5 unilateral, 26 bilateral patients and 2 unaffected carriers, the mean diseased eye ratio (DER) was 1.74. The LP families included 27 germline carriers of which 8 were unilateral, 3 bilateral, 1 had retinoma, 1 was a mutation mosaic and 14 were unaffected, the mean DER was 0.54. The parental origin of mutant allele was documented in 20 germline carriers: 15 carriers received the mutation from their father and 5 from their mother. Seven carriers of father´s mutant allele were affected, 7 carriers were unaffected and 1 developed a retinoma. Four carriers of mother´s mutation developed RB and 1 was unaffected. The mutations identified in LP families were: 2 in promoter, 2 missense, 1 in splicing site and 1 *RB1* gene deletion. These mutations cause less pRB protein or less functional pRB or decrease cell viability and tumor development. Our results show that the survey of 18 RB families and sporadic RB cases revealed 7 families with 15 unaffected mutation carriers, what indicates a LP of RB. Detection of unaffected carriers is of utmost importance to assess the risk to their offspring. The parental origin of LP mutations defines the penetrance of hereditary RB and allows to delve into molecular bases of LP retinoblastoma.

129. (410) **MOLECULAR CHARACTERIZATION OF PEDIATRIC ARGENTINEAN PATIENTS WITH MITOCHONDRIAL DISEASES USING NEXT-GENERATION SEQUENCING OF NUCLEAR DNA GENES**

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Mitochondrial diseases (MD) are a group of disorders caused by pathogenic variants in mitochondrial (mtDNA) or nuclear DNA (nDNA) that primarily affect oxidative phosphorylation.
Aims: to perform the molecular characterization of a pediatric patient cohort with clinical diagnosis of MD, by Next-generation Sequencing (NGS) of nDNA genes.
Methods: We included 35 patients based on clinical, biochemical, neuroimaging and pathologic findings from a total of 119 patients with suspected MD and negative for the "common" deletion and frequent mtDNA variants. nDNA variants were studied using a MD-customized NGS capture panel (24); or by exome sequencing (11).
Results: nDNA pathogenic variants were identified in 13 unrelated patients (37%, panel=6, exome=7), and our custom panel gave a diagnostic yield of 25%. Six patients with Leigh syndrome had variants in *SURF1* (3), *PDHA1* (2) or *COQ4* (1); three patients with mitochondrial hepatopathy in *POLG* (2) or *MPV17* (1); one patient with cavitating leukoencephalopathy in *NDUFS1*; one patient with myoclonic epilepsy and tubulopathy in *PC*; one patient with myopathy in *TK2*, and one patient with optic atrophy plus in *OPA1*. Ten out of the 23 variants found were *novel*. Seven were truncating (3 SNV, 3 indels and 1 deep intronic variant) and the rest were missense. In most cases the variants were biallelic, except for *MPV17* (homozygous), and *PDHA1* (hemizygous, one mosaic).
Conclusions: The diagnostic yield obtained highlights the advantages of an NGS panel approach for MD. While the exome seems more efficient, it is worth noting that all the variants were found in genes that are included in our panel and would have also been detected with it. Remarkably, the deep intronic variant in *OPA1* was found by Sanger sequencing after an exhausting literature review of the missense variant detected by NGS, emphasizing the limitations of panels and exomes and the relevance of an interdisciplinary work.

130. (467) **EARLY-ONSET MUSCULAR DYSTROPHIES: MOLECULAR CHARACTERIZATION IN ARGENTINIAN PEDIATRIC PATIENTS**

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Muscular dystrophies (MD) are genetically and clinically heterogeneous hereditary disorders characterized by progressive degeneration and weakness of skeletal muscle. MD are classified according to the age of onset, muscle involvement and clinical features. More than 70 genes related to MD have been identified. **The aim** of this study is to describe the molecular findings in a pediatric cohort followed-up by the neuromuscular interdisciplinary team of our hospital. **Patients and methods**: we included 46 patients with clinical and histopathological features compatible with early-onset MD. A customized NGS panel for 84 genes related to neuromuscular diseases was designed. Bioinformatics' tools were applied to detect copy number variations (CNVs). **Results:** molecular analysis

revealed pathogenic or probably pathogenic variants in 36 patients (78,3%), including *LAMA2* (n=12), *COL6A1* (n=7), *COL6A3* (n=5), *DMD* (n=4), *LMNA* (n=2), *CAPN3* (n=1), *FKRP* (n=1), *FHL1* (n=1), *GMPPB* (n=1), *POMGNT1* (n=1) and *SGCG* (n=1) genes. We identified 13 novel variants in 8 genes, among them c.4992_4996del (p.(Gln1666Cysfs*2)) and c.6145A>T (p.(Lys2049*)) in the *LAMA2* gene accounted for 25% of the pathogenic alleles detected in this gene. CNV analysis allowed us to detect 3 patients with deletions of exons 3–4 of the *LAMA2* gene and 1 female patient with a large deletion in the *DMD* gene. A patient with *LAMA2* variants presented duplication of the *DMD*, *FHL1* and *MTM1* genes (located on the X chromosome), suggesting the coexistence of Klinefelter syndrome (47,XXY). In addition, we detected a splice site variant in the *DMD* gene in a patient previously diagnosed with Dravet syndrome and high CK. **Conclusion:** the customized NGS panel showed a high diagnostic yield for early-onset muscular dystrophies. The proportion of large deletions detected in our cohort (7,7% of the pathogenic alleles) highlight the importance of including CNV analysis for NGS data.

131. (555) **TARGETED NEXT GENERATION SEQUENCING FOR THE DIAGNOSIS OF INBORN ERRORS OF METABOLISM IN A PEDIATRIC REFERENCE CENTER**

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Introduction: Inborn errors of metabolism (IEMs) comprise heterogeneous and rare monogenic disorders with a variety of overlapping or unspecific clinical phenotypes. Diagnosis of an IEM is supported by clinical suspicion and biochemical studies and is confirmed by molecular analysis. Next generation sequencing (NGS) is a valuable tool, allowing the analysis of various genes simultaneously. This minimizes the turnaround time, which is crucial for some IEMs. The aim of the study was to evaluate the performance of targeted NGS in confirming the diagnosis of IEMs in a clinical setting. Materials and Methods: 124 patients with clinical and biochemical suspicion of IEM were analyzed between July 2019 and August 2021 with a custom NGS panel of 87 genes. Bioinformatics tools were applied to detect and prioritize small variants, and to predict copy number variations. Results: Global diagnosis rate was 73%, but when dividing patients according to their previous studies, in those with a defined biochemical and clinical suspicion the diagnostic yield was 90%, meanwhile when previous findings were not conclusive, it was 26% ($p < 0.0001$). A total of 113 variants related to the clinical suspicion of the patients in 31 genes were identified: 42% were absent from population and disease-based databases; nevertheless, 75% of them could be classified as pathogenic or probably pathogenic, according to the ACMG consensus criteria. Only 12 variants were identified in more than one patient. Conclusions: NGS technology applied to this cohort of patients provided a high diagnostic rate and a better knowledge of the molecular basis of IEMs in our population. It also evidenced the high allelic heterogeneity and the low representation of local variants in genetic databases. A genetic diagnosis is determinant for the treatment of these patients. The optimal use of targeted NGS is achieved when clinical and biochemical characterization of the patients allows identification of candidate genes.

132. (579) **ROLE OF VARIANTS OF *ABCG2* GENE IN THE TRIGGERING OF PORPHYRIA CUTANEA TARDA IN HIV-INFECTED INDIVIDUALS**

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Porphyria Cutanea Tarda (PCT) is caused by a deficiency in Uroporphyrinogen decarboxylase; there are 2 main types of PCT: hereditary and acquired. Xenobiotics, alcohol, abuse drugs, hormones and hepatotropic viruses are the main triggering factors of this hepatic disease. In our country, 16% of PCT patients are HIV infected individuals. Genetic variants affect the expression of the *ABCG2* transporter, altering the efflux of drugs and heme; NM_004827.3:c.34G>A, and NM_004827.3:c.421C>A are present in a high frequency. Previously, the influence of *ABCB1* genetic variants, a transporter of the same family as *ABCG2*, in the onset of PCT in HIV carriers was reported. The aim was to evaluate the role of the NM_004827.3:c.421C>A (rs2231142) variant of *ABCG2* gene in the association PCT-HIV. A population of control (n=33), HIV (n=33), PCT (n=35) and PCT-HIV (n=42) individuals was studied. Genotyping was done by PCR-RFLP. The non-wild type allele A was in a very low frequency in all the groups. In PCT-HIV, the frequency of A (0.16) was higher than PCT (0.07, $p<0.001$) and HIV (0.06, $p<0.001$) values. When genotypic frequencies were analyzed, higher values for heterozygosis SNV (CA) in PCT-HIV group (40%, $p<0.05$) than PCT (26%) and HIV (21%) were found;all of them significantly higher than Controls (CA=4.35%). The AA genotype (2%) was only found in PCT-HIV group. These results, suggest that NM_004827.3:c.421C>A variant in the *ABCG2* gene could be related to PCT/HIV association. The analysis of NM_004827.3:c.34G>A SNV, will allow us to establish the presence or absence of risk haplotypes in the manifestation of PCT associated or not with HIV infection. The results of this analysis, together with those previously obtained for *ABCB1* drug transporter gene variants, will enable us to further conclude about a risk haplotype for PCT triggering. Due to the multifactorial nature of porphyria onset, they are of great value to understand the susceptibility to develop PCT.

133. (607) **EXPLORING NANOMETER UNIVERSES TO DECIPHER THE EMISSARIES OF THE TUMOR**

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Extracellular vesicles (EVs) participate in tumor-stroma crosstalk within the tumor microenvironment (TME) contributing to tumor promotion. Thyroid cancer is the most prevalent endocrine malignancy, and the relevance of fibroblasts (Fb) and EVs in the TME is beginning to be elucidated. Using an in vitro model, we demonstrated that Fb-thyroid tumor cell co-culture induced the activity of matrix metalloproteinase-2 (MMP2), and that the EVs obtained from these cultures stimulated the secretion and activation of MMP2 by normal Fb. Given this background, we hypothesized that the interaction of thyroid tumor cells with Fb would promote modifications in the EVs' protein cargo, which would influence their communication with the TME components. Besides, EVs would enable the study of potential biomarkers for the diagnosis and monitoring of the evolution in cancer patients. The bioinformatic analysis of EV-proteomic data identified 1977 proteins; 98% of EV-protein cargo and 97 out of 100-most frequent proteins in Vesiclepedia database were identified in our samples, thus confirming the identity and purity of the EVs samples analyzed. Furthermore, we demonstrated that EVs released from Fb-thyroid tumor cell co-culture expressed a proteomic profile related with extracellular matrix (ECM) remodeling. Specifically, the crosstalk between Fb and a papillary thyroid cancer cell line (TPC-1) enhanced the functionality of EVs for collagen degradation. Consistently, MMP2 and its related proteins, detected in EVs, allowed to discriminate between EVs from tumoral and non-tumoral contexts. Finally, the proteins HRAS and MAMDC2 that were found to be enriched in EVs from Fb-TPC-1 co-culture, constitute interesting targets for further studies as cancer biomarkers. In conclusion, EVs produced in the thyroid tumor milieu participate in ECM remodeling,

promoting the synthesis and local activation of MMPs, facilitating ECM-degradation as well as tumor aggressiveness and progression.

HEMATOLOGÍA

134. (097) **TRANSCRIPTIONAL AND PROTEOMIC ANALYSES OF REDOX ENVIOREMENT IN BETA-THALASSEMIA TRAIT**

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β-Thalassemia trait (BTT) is a heterogeneous group of genetic defects leading to decreased β -globin production, ineffective erythropoiesis and oxidative stress. Reactive oxygen species production and oxidative environment have an impact on all blood cell lineages. The aim was to evaluate, at transcriptional and proteomic levels, the pro-oxidative and anti-oxidative status in BTT patients. A descriptive study was performed with 66 subjects (40 apparently healthy and 26 with BTT). Real-time PCR was used for gene expression analyses of transcription factors forkhead homeobox typeO (FOXO3a) and nuclear factor erythroid2-related factor-2 (NRF2); antioxidant enzymes: catalase (CAT), peroxiredoxin-2 (PRX-2), superoxide dismutase (SOD); and cytokines TNF-α, IL-6. Quantitative mass spectrometry was performed on cytosol erythrocyte membranes depleted of hemoglobin. Bioinformatic analysis was performed with Perseus, BlastKoala and Proteome Discoverer V1.4 programs.
Relative expression of NRF2 was 4.7-fold higher in BTT than in control group, whereas FOXO3a expression was similar in both. Transcriptional expression of SOD, PRDX2 and proinflammatory cytokines were significantly upregulated in BTT compared to controls (p<0,005). Proteomic study showed significant difference in abundances of oxidative stress and inflammation markers such as lipoxygenase15 (ALOX15), poly-C-binding protein 1/2 (PCBP 1/2), P40/P67 subunit of NADPH oxidase and 70 kilodalton heat shock protein (HSP70), tyrosine-protein kinase (SYK) in BTT (p<0, 05). Proteins involved in redox imbalance protection such as glutathione S-transferase kappa1 (GSTκ1), isocitrate dehydrogenase 1/2 (IDH1/2) and glucose-6-phosphate dehydrogenase (G6PD) were higher in BTT than in controls (4.2, 4.1 and 2.1 fold respectively). These results showed changes in the gene expression of some redox regulators together with modifications in the erythrocyte proteome generated by the global redox imbalance underlying in this pathology.

135. (167) **REDOX AND INFLAMMATORY IMBALANCE IN PATIENTS WITH DEBUT OF ACUTE LEUKEMIA**

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Several studies have reported the oxidative stress influence on the pathogenesis and evolution of hematologic neoplasms. The aim of this work was determine the behavior of redox and inflammatory biomarkers at transcriptional and systemic level in acute leukemia (AL) patients at the time of onset. Between 2016–2021, 61 AL patients and 63 controls (C) were evaluated. AL characterization was performed by blood count, cytochemistry and flow cytometry. Antioxidant enzymes *catalase* (CAT), and *superoxide dismutase* (SOD), and the cytokines *tumor necrosis factor-alpha* (TNF-α) and *interleukine-6* (IL-6) gene expression were analyzed with qPCR in peripheral white blood cells. Malondialdehyde (MDA) levels and CAT and SOD enzymatic activity were determined in serum by spectrophotometric methods. Cytokines concentration were measured by Human TNF-α and Human IL-6 ELISA. Statistical analyses were performed by SPSS V.25 statistical software and were considered significant at p<0,05. We detected 41% of acute lymphoid leukemia (ALL), 44% acute myeloid leukemia (AML) and 15% acute promyelocytic leukemia (APL). A significant increased level of lipoperoxidation was found in AML and APL respect to C [MDA μmol/L: AML=1,07 (0,40–6,60); ALP=1,22 (0,54–2,96); C=0,83 (0,33–2,51)]. Also, AML and APL showed higher CAT activity than C [CAT nmol/mg prot: AML=0,47 (0,02–3,62); ALP=0,70 (0,18–0,99); C=0,25 (0,08–2,15)], while SOD activity was a similar behavior between the groups studied. Furthermore, IL-6 concentration was significantly increased in all AL patients respect to C. SOD and IL-6 transcriptional expression were significantly downregulated in AL patients. No statistically significant differences were found in the other genes expression studied. These findings show the imbalance of redox and inflammatory biomarkers in the different AL evaluated and highlight on the differential behavior observed at the transcriptional/systemic level underlying in this neoplasm.

136. (288) **REGULATION OF HEPCIDIN BY ERYTHROPOIETIN IN MACROPHAGES**

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The role of erythropoietin (Epo) as a growth factor in erythropoiesis is well known. Many defects, both congenital and acquired, lead to ineffective erythropoiesis, thus establishing the condition of anemia. The demand for iron (Fe), necessary in biological processes, including erythropoiesis, gives importance to its controlled regulation. Hepcidin (Hep) is a key regulator of systemic iron metabolism and its expression responds to Fe levels. The participation of Epo in the regulation of Hep has been investigated in hepatocytes but not in macrophages, fundamental cells in Fe homeostasis that act as reservoirs of senescent erythrocytes. Therefore, the aim of this work was to investigate the regulation of Hep by Epo in macrophages.
Standardization of the differentiation of monocytic THP-1 cells to macrophages was carried out with different concentrations of phorbol 12-myristate-13-acetate (PMA). According to cell morphology (visible light microscopy), results of viability, proliferation and mRNA levels of the specific differentiation markers CD-14 and CD-68 (RT-PCR), 100 nM was chosen as the adequate PMA concentration to continue with the stated objective. The presence of EpoR was demonstrated by Western blotting (anti-EpoR M20), RT-PCR and flow cytometry, comparing with UT-7 cells as a positive control. Epo treatment of macrophages induced a significant decrease in Hep mRNA levels (RT-PCR, a.u.: C6h 0.39±0.02; *Epo6h 0.11±0.03; *Epo24h 0.09±0.04, *P<0.05 vs. C6h, n=5). To investigate whether this action of Epo takes place via its traditional pathway, evaluation of Hep levels was carried out in the presence of Jak2 (AG490) and PI3K (Ly294002) inhibitors and, under these conditions, Hep expression was found increased. In conclusion, the results show that Hep expression is modulated by Epo in macrophages, an effect that occurs through its receptor and is mediated by the Jak2/PI3K pathway.

137. (306) **ACTIVATION OF TOLL-LIKE RECEPTORS 7 AND 8 ON CD34+ CELLS BY COXSACKIEVIRUS B3 IMPAIRS MEGAKARYOCYTE AND PLATELET PRODUCTION**

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Background: Increasing evidence indicates that hematopoietic progenitor cells (CD34+ cells), megakaryocytes (MKs), and platelets (PLT) express toll-like receptors (TLR) allowing the contribution of these cells to the immune response and inflammation. However,

whether TLRs' activation on CD34+ modulates megakaryo/thrombopoiesis during viral infections is still unclear.
Objectives: We evaluated whether the single-strand RNA Coxsackievirus B3 (CVB3) infection modulates human MK development and PLT production through TLR7/8 activation.
Methods: CD34+ cells from the human umbilical cord were exposed to CVB3 or UV-irradiated CVB3 virus (mock) and then stimulated with thrombopoietin (TPO) in the presence or absence of TLR7/8 antagonists. The total number of cells (counting in a Neubauer chamber), CD34+ differentiation, maturation of MKs, PLT biogenesis (flow cytometry), and expression of Fli-1, RUNX-1, and NF-E2 (qPCR) were determined after 12-17 days post-infection (dpi).
Results: Exposure of CD34+ cells to CVB3 resulted in productive infection as determined by the presence of viral infectious particles in culture supernatants 1 to 11 dpi. Interestingly, CD34+ differentiation towards MKs (CD41+ cells) as well as MK maturation (CD42b+ cells), were markedly impaired (n=6, $p<0.05$) in infected cultures. mRNA expression of Fli-1, RUNX-1, and NF-E2, transcription factors involved in these processes, was also downregulated in infected cells (n=5, $p<0.05$). The reduction in MK growth was associated with an increase in cell apoptosis (n=4, $p<0.05$). CVB3 infection also led to a lower platelet count when compared to mock samples. Decreased cell number, MK maturity, and PLT production were significantly reversed by TLR7/8 antagonists (n=4, $p<0.05$).
Conclusions: These data suggest a new role for TLR7/8 in megakaryo/thrombopoiesis during viral infections that might contribute to a better understanding of the molecular bases underlying thrombocytopenia during infectious processes.

138. (315) **GENE EXPRESSION DYNAMICS OF IMMUNE MEDIATORS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB**

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Imatinib was the first inhibitor approved for chronic myeloid leukemia (CML). Besides its direct oncokinase inhibition, the induction of an immune antitumor mechanism has also been suggested.
The aim was to describe the gene expression dynamics of *IL2*, *CIITA*, *TBET*, *GATA3*, *RORGT*, *FOXP3* and *EOMES* in CML at diagnosis and during the first year of treatment.
Total RNA from peripheral blood samples was collected from 79 CML patients (34 serially followed) at diagnosis (n:23) or under treatment at 3 (n:41), 6 (n42) or 12 (n:26) months classified according to the molecular response at each time. Gene expression was evaluated by quantitative real-time PCR applying the comparative method $2^{-\Delta CT}$ relative to *GAPDH* gene. Statistical analyses were performed using the InfoStat software v2019 and p-values <0.05 were considered statistically significant.
Baseline expression of all immune mediators evaluated was significantly decreased when compared with healthy donors (n:26) (Mann-Whitney test $p<0.0001$). Once on therapy, each gene displayed a particular behavior and similar between responders and non-responders. *TBET* and *EOMES* reached normal levels after 1 year (Kruskal Wallis test-KWt: p=0.2407 and p=0.0904). *CIITA* and *RORGT* continued downregulated throughout the follow-up (KWt: p=0.0136 and $p<0.0001$). *GATA3* and *IL2* showed a normalization on their expression levels at 6 months, followed by a decrease at 12 months (KWt: p=0.0045 and p=0.0083). Finally, *FOXP3* achieved normal levels early at 3 months (KWt: p=0.3859) and was sustained afterwards.
The obtained results agree and complement our previous results describing a suppression of the immune system in CML at diagnosis and a re-activation after imatinib initiation with some mediators requiring a longer exposure. A deeper understanding of the immunological landscape in CML is therefore important to comprehend the role of tyrosine kinase inhibitors in restoring immune surveillance and characterize patients' outcomes.

139. (362) **EXPRESSION OF TRANSFERRIN RECEPTOR 2 IS REGULATED BY ERYTHROPOIETIN IN HEPG2 CELLS**

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The systemic regulation of Fe availability relies on the hepcidin/ferroportin axis, which controls the mobilization of Fe stores for different cellular functions. It is currently recognized that transferrin receptors (TfRs) not only play a role in Fe acquisition but also in modulating its availability. While TfR1 has a high affinity for holo-transferrin (Tf-Fe) —with which it complexes and internalizes thus favoring cellular deposition of Fe—, TfR2 is deemed to regulate hepcidin expression, through mechanisms yet to be clarified.
Erythropoiesis is a major Fe-consuming physiological process, although further research is needed to understand its effect on the different factors involved in Fe homeostasis. In previous studies we found an inhibitory effect of the erythroid survival factor erythropoietin (Epo, 160 ng/mL, 6 h) on hepcidin mRNA levels in HepG2 hepatocarcinoma cells. Here we report that Epo decreased TfR2 mRNA levels in this cell type in the same treatment period (real-time PCR, a.u.: Control=1.0, *Epo 6h=0.4±0.1, Epo 15h=1.3±0.3, *$p<0.05$, n=5). In contrast, TfR1 mRNA expression remained unaffected by Epo (RT-PCR, a.u.: Control=0.5±0.1, Epo 6h=0.5±0.1, n=6). The decrease in hepcidin and TfR2 mRNA levels after Epo treatment was accompanied by a significant reduction of intracellular Fe (potassium ferrocyanide-based colorimetric assay, a.u.: Control=0.13±0.08, *Epo 6h=0.06±0.01, *$p<0.05$, and microscopy, n=4).
In line with the expression patterns previously observed for hepcidin, Epo also showed a tendency to suppress TfR2 mRNA levels after addition of ferric citrate (3 μM), although this effect was not observed in Fe-overloaded cells (100 μM). In contrast, Epo did not alter TfR1 expression at any of the external Fe concentrations studied.
Altogether, our findings highlight the involvement of Epo in the regulation of Fe homeostasis and hint at TfR2 modulation as a possible mechanism for Epo-induced hepcidin suppression in hepatic cells.

140. (580) **EFFECT OF CANNABIDIOL (CBD) ON NORMAL AND HEAT-MODIFIED RED BLOOD CELLS (RBCs)**

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In recent years, cannabidiol (CBD), a cannabinoid compound has emerged as a candidate for the control of different pathologies since it does not have a hallucinogenic effect. Several beneficial effects have been reported in pain, epilepsy, arthritis, inflammation, etc. but the mechanism by which CBD acts remains still in controversia. Also the beneficial effect, several side effects were observed in patients under CBD treatment. Among other side effects, anemia and elevation of transaminases were observed in 20-30% of the patients with refractory epilepsy under CBD treatment. We hypothesized that CBD treatment could promote anemia reducing the RBCs viability. To test this hypothesis, we use human RBCs obtained from samples with normal hematometric parameters. The effect of CBD on osmotic fragility, hemolysis, and morphological characteristics of normal and heat-damaged RBCs was evaluated. In vitro experiments were performed using CBD 10^{-5}M and 1-2% increase in the free hemoglobin concentration was observed while the osmotic fragility remained unchanged. The incubation of normal RBCs with CBD increases a

40% the number of elements, highlighting the formation of microspherocytes. This effect was time dependent and had a maximum at 20 minutes while at 40 minutes it returned to baseline. Heat-damaged RBCs did not show changes in osmotic fragility, hemolysis, and CBD-induced morphology. Together, these results suggest that CBD induces the formation of hemolytic vesicles via protein signaling from the cell membrane, with clinical relevance since the values of released hemoglobin were 10-15 times higher than the cut-off value (4mg%) for free hemoglobin in plasma.

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141. (002) **EARLY ANTIPARASITIC TREATMENT PREVENTS PROGRESSION OF CHAGAS DISEASE: RESULTS OF A LONG-TERM CARDIOLOGICAL FOLLOW-UP STUDY IN A PEDIATRIC POPULATION**

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Objective: To evaluate cardiac involvement in children after pharmacological treatment for Chagas disease (CD).
Methods: A descriptive study of a cohort of pediatric CD patients treated with benznidazole (Bz) or nifurtimox (Nf) was conducted by convenience sampling, 95 children with at least 6 years post-treatment follow-up and who attended a clinical visit between August 2015 and November 2019 were invited to participate in the study. They were evaluated with 24-hour Holter monitoring and speckle-tracking 2D echocardiogram (STE). As a control of the incidence of ECG non pathological findings a group of non-infected people were included.
Results: In enrolled treated patients: 24-hour Holter showed alterations in 3/95 (3%) patients, but only one was considered probably related to CD involvement. This patient presented a complete right bundle branch block (cRBBB). No contractility damage was found in 79/95 (83%) patients evaluated by STE.
In non-infected cardiological control group: 24-hour Holter showed alterations in 3/28 (10%) patients. No contractility damage was found in 25/28 patients evaluated by STE.
Benznidazole was prescribed in 87 patients and nifurtimox in 8 patients. Baseline parasitemia data was available for 65/95 patients. During follow-up, 59/61 (96%) treated patients achieved constant negative parasitemia evaluated by qPCR. A decrease in T.cruzi antibodies titers was observed and seroconversion occurred in 53/95 (56%) treated patients. These results showed a good efficacy of treatment in parasite clearance.
Conclusions: A good treatment response with a low incidence of cardiological lesions related to CD was observed. This suggests a protective effect of parasiticidal treatment on the development of cardiological lesions and highlights the importance of early treatment of infected children.

142. (004) **VALIDATION OF POOLED TESTING FOR SARS-CoV-2 USING DROPLET DIGITAL PCR**

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The outbreak of COVID-19 has become a public health emergency. Viral nucleic acid detection by reverse transcription PCR (RT-PCR) is the gold standard method for diagnosis of COVID-19. Droplet digital PCR (ddPCR) is a highly sensitive PCR technology based on the generation of 20,000 nanodrops per tube. This technology is rarely used in clinical laboratories, due to its higher cost when compared with PCR. Cost could be reduced by using pooled testing. A negative test result indicates that all individuals in the pool are negative while a positive result indicates that at least one individual is positive. Pooled testing may be particularly useful to communities with low prevalence of COVID-19. We proposed to validate the use of ddPCR to detect SARS-CoV-2 by pooling.
Throat swab samples of 1000 patients were collected and soaked in 2mL saline. RNA extraction was done using automatic magnetic extraction, columns extraction kits, and heat. Firstly, positive and negative samples were identified with RT-PCR, pools of different sizes were designed and ddPCR was performed. Data was analyzed with Quanta Soft analysis software v.1.7.4.0917 (Bio-Rad). This study was granted exception from bioethics committee approval as deidentified remnants samples were used.
We determined the specificity (we measured 100 negatives pools), the limit of detection (three independent octuplicates of the greatest dilution that it is positive) and the robustness of the method (the ability to withstand small but deliberate variations in method parameters by performing 20 repetitions changing the order of pooling and purification; and by measuring RNAs obtained using different extraction method). In the present work, we validated the use of pooled testing by combining up to 34 samples per pool.
We hope that such implementation of a pool test for SARS-CoV-19 would allow expanding current screening capacities, thereby enabling the expansion of detection in the community, as well as in close organic groups.

143. (012) **ERYTHROPOIETIN IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME: A RANDOMIZED CLINICAL TRIAL**

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Background: The efficacy of recombinant human erythropoietin (rHuEPO) in sparing red blood cell (RBC) transfusions in children with hemolytic uremic syndrome related to Shiga toxin-producing Escherichia coli (STEC-HUS) is uncertain.
Methods: We conducted a prospective, two-parallel-group randomized open controlled trial conducted at the Hospital General de Niños Pedro de Elizalde from December, 2018 to January, 2021 (ClinicalTrials.gov NCT03776851). We randomly assigned children with STEC-HUS to the rHuEPO group (subcutaneous rHuEPO 150 U/kg/week + RBC transfusion if hemoglobin ≤7 g/dLand/or hemodynamic instability) or to the usual- caregroup (RBC transfusion if hemoglobin ≤7 g/dL and/or hemodynamic instability). Primary outcome was the number of RBC transfusions received during the hospitalization. Secondary outcomes were to explore whether baseline EPO levels were deficient (according to the relation between observed and predicted level), to correlate selected acute phase parameters with the number of RBC transfusions, and to assess possible adverse events.
Results: Twelve patients per arm were included, all completed the trial. They were comparable at recruitment and coursed a similar acute disease. Median number of RBC transfusions was similar between groups (1.5, p=0.76). Most patients had appropriate baseline EPO levels, which did not correlate with the number of RBC transfusions (r 0.19, p=0.44). Conversely, baseline (r 0.73, p=0.032) and maximum lactic dehydrogenase levels (r 0.78, p=0.003), creatinine peak (r 0.71, p=0.03) and dialysis duration (r 0.7, p=0.04) correlated significantly with RBC requirements. No potential side effect was attributed to rHuEPO therapy.
Conclusion: Administration of rHuEPO did not reduce the number of RBC transfusions in children with STEC-HUS.

144. (019) **HYDATID FLUID FROM *ECHINOCOCCUS GRANULOSUS* INDUCES PHAGOPHORE AND AUTOPHAGOSOME FORMATION IN DENDRITIC CELLS THROUGH AN UPREGULATION OF BECLIN-1**

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Background: The cestode *Echinococcus granulosus* (Eg) is the etiological agent of cystic echinococcosis. This parasite develops cysts filled with hydatid fluid (HF) in the viscera of the intermediate host. Autophagy is a cellular catabolic process that plays a key role in the presentation of endogenous and exogenous proteins, promoting the activation of T cells. The aim of this work is to analyze if HF, constituted by a wide range of parasite proteins, could trigger autophagy in dendritic cells. **Methods:** HF was punctured from the hydatid cysts collected of infected cattle slaughtered. Murine BMDCs were cultured in RPMI 1640 medium, supplemented with FLT3-L. First, lysosome activity was evaluated using Acridine Orange, a fluorophore that can be trapped in acidic vesicular organelles. Then, autophagy induction was evaluated by FACS, qPCR, Confocal and Transmission Electron Microscopy. Rapamycin (20 nM) and chloroquine (100µM) were used to modulate autophagic flux. LC3-attachment to the autophagic membrane, were analyzed by stained DCs with anti LC3-β antibody (clone H50). **Results:** HF significantly increased acridine orange cytoplasmic accumulation compared to control cells (***p <0.001) and enhanced the effect of rapamycin (****p<0.0001). The ultrastructural analysis of TEM showed that in the presence of HF, DCs stimulate the formation of phagophores, double lipid membrane autophagosome, MVBs and autolysosomes. Also, HF-stimulated BMDCs significantly enhanced the mean fluorescence intensity of LC3-positive structures in comparison with unstimulated cells (*p<0.05, **p<0.01, ***p<0.001 HF-stimulated cells vs controls). Finally, we have observed that HF induces a significant increase in the transcriptional expression of LC3 and Beclin-1 (n=3, **p <0.01 vs control) and enhances the expression induced by rapamycin. **Conclusions:** These results suggest that HF of *Echinococcus granulosus* regulates gene expression to increase autophagy-related structures in DCs.

145. (129) **SERUM DETERMINATION OF TAU PROTEIN AS A POTENTIAL PREDICTIVE BIOMARKER OF ENCEPHALOPATHY ASSOCIATED WITH HEMOLYTIC UREMIC SYNDROME**

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Hemolytic uremic syndrome (HUS) is a foodborne disease caused by intoxication with Shiga toxin (Stx) produced by enterohemorrhagic E. coli (EHEC). HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure. A variety of neurological alterations are often associated with poor prognosis and mortality risk, becoming the highest incidence of death due to HUS. In addition to Stx, EHEC is a gram-negative bacterium and thus releases LPS known to be involved in proinflammatory-related events which contributes significantly to the development of the disease. Early detection of neural serum biomarkers during the first days of bloody diarrhea manifestation, and prior HUS signs and symptoms, could be determinant to prevent the progress of the disease. We are currently studying the tau protein associated-neuronal microtubules. Its presence in blood as a neuronal damage consequence confirms a wide spectrum of brain insults. The aim of this work was to determine whether the neuronal tau protein can be considered an early serological biomarker of encephalopathy in the context of HUS. For this purpose, NIH-Swiss male mice were intravenously injected with vehicle, LPS (800ng), Stx2 (3.5ng, 1LD100) or a combination of Stx2 and LPS (Stx2+LPS, same previous amounts). After 1-and 2- days blood samples were collected to test by Elisa (Invitrogen, Viena, Austria) the detection of tau protein. One way ANOVA and Tukey post-hoc tests were employed for statistical analysis. A significant two-fold increase was determined after 2 days in the Stx-2+LPS group (p<0.05) with respect to the vehicle. Non-significant tau protein immunodetection was found in all groups after 1 day of treatment. Assuming that the murine death occurs after the fourth day of treatment, and that significant tau serum immunodetection was determined within 2 days, this protein could be used as a potential biomarker to prevent lethal encephalopathy associated to HUS.

146. (139) **BONE-MARROW DERIVED DENDRITIC CELLS FROM *TOXOPLASMA GONDII* CHRONICALLY INFECTED MICE EXHIBIT ALTERATIONS IN MONOCLONAL AND POLYCLONAL T CELL PRIMING**

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Rationale: We previously showed that splenocytes from chronic *T. gondii* infected mice have a diminished capacity to activate and differentiate OVA-specific Th1 and Th2 cells. Moreover, BMDCs from infected animals presented phenotypic alterations as shown by increases in CD80 and CD86 maturation markers and fewer secretion levels of IL-6 and IL-10, with no differences in IL-12. To extend these previous results, herein, we studied the ability of BMDC from chronically infected mice to activate and differentiate effector T cells. **Methods**: Bone-marrow derived dendritic cells (BMDCs) were obtained from naive and chronically infected mice, by culturing bone-marrow precursors for nine days with GM-CSF-conditioned medium. Afterward, BMDC were fed with OVA and matured during 18h with LPS. Subsequently, BMDC were cultured with DO11.10 OVA-specific CD4+T cells. Also, a mixed lymphocyte reaction (MLR) was performed by co-culturing BMDC with naive C57BL/6 mice splenocytes. **Results**: OVA specific CD4+ T cells co-cultured with BMDCs from infected mice showed lower levels of IFN-γ and IL-5 and increased levels of IL-10 (p<0.05). When analyzing polyclonal T cell responses in MLR assays, T lymphocytes incubated with BMDCs from infected mice showed decreased secretion of IFN-γ and IL-10 (p<0.01). No significant differences were observed for Th2 cytokines. **Conclusion**: The data obtained with BMDCs show that mice chronically infected with *T. gondii* present alterations of bone-marrow precursors. Interestingly, the diminished Th1/Th2 profiles observed in antigen specific co-culture assays are in line with results previously observed in splenocytes from *T. gondii* infected mice. These results suggest that infection with *T. gondii* results in long-lasting alterations in hematopoietic cells that could be involved in the lower susceptibility to developing allergic and autoimmune disorders.

147. (165) **THE NEW CAGE-LIKE PARTICLE ADJUVANT ISPA ENHANCE IMMUNITY OF AN EXPERIMENTAL VACCINE AGAINST CHRONIC *TOXOPLASMA GONDII* INFECTION IN MICE**

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Toxoplasmosis is a disease that affects 30% of the world's population. At present, there are no pharmacological treatments that eliminate the parasite or vaccines that confer protection to the host. The aim of the present work was to study the immunogenicity of vaccine formulations containing a new cage-like particle adjuvant (ISPA) in combination with *T. gondii* recombinant proteins.

METHODS: C57BL/6 mice were intradermally immunized 3-times with a 2-week interval with rGRA7, rTgPF, rTgPI-1 or rROP2 in com-

bination with ISPA, and control groups received ISPA or PBS. Fourteen-days later, mice were orally challenged with a non-lethal dose of *T. gondii* cysts and after one month, brain cyst numbers were determined under optical microscope. Antigen-specific responses were characterized two weeks after the last immunization in sera, spleen (SC) and draining lymph node (DLN) cultures.
RESULTS: Significant reduction in brain parasite load was obtained with rGRA7+ISPA (*p<0.05*), rTgPF+ISPA (*p<0.05*) and rROP2+ISPA (*p<0.01*) formulations. All experimental groups elicited strong humoral responses *(IgG:p<0.0001)* with a mixed profile (Th1/Th2). rTgPF+ISPA induced a strong Th1 systemic cellular response characterized by increased levels of CD4+ and CD8+ cells in SC *(p<0.01 and p<0.005, respectively)* and DLN *(p<0.05)*, and IFN-γ *(SC:p<0.0001; DLN:p<0.0001).* On the other hand, rGRA7+ISPA and rRop2+ISPA formulations induced a mixed Th1/Th2 response since significant IFN-γ, IL-4 and IL-5 production was detected after antigen-specific stimulation *(SC and DLN: p<0.05)*. Only rGRA7+ISPA generated high numbers of CD4+ cells in SC *(p<0.001).* Additionally, IL-10 secretion was observed in all vaccinated groups both in SC and DLN *(p <0.01).*
CONCLUSION: The present results indicate that the use of ISPA as an adjuvant of rGRA7, rTgPF and rROP2 antigens was able to enhance and modulate the specific responses generating partial protection against chronic *Toxoplasma* infection.

148. (174) **DEVELOPMENT OF A HIGHLY SENSITIVE NS1 CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF ACUTE ZIKA VIRUS INFECTION**

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Zika virus (ZIKV) is a flavivirus that is primarily transmitted from bites of infected *Aedes aegypti* or *Aedes albopictus* mosquitoes. There are currently no vaccines to prevent ZIKV infection nor commercially available clinical diagnostic tests demonstrated to identify ZIKV without cross-reactive interference of other related flaviviruses. In this context, the development of sensitive and accurate diagnostic methods is urgently needed for the early detection of ZIKV. Nonstructural protein 1 (NS1) is a highly conserved glycoprotein that is secreted as a hexamer and circulates at high levels in the bloodstream of acute patients. These properties turn ZIKV NS1 (ZNS1) into a good diagnostic marker, allowing early detection and diagnosis of ZIKV infection. In order to develop a highly specific and sensitive capture ELISA, we aimed at obtaining monoclonal antibodies (mAbs) against hexameric ZNS1 protein. We selected 6F6 specific hybridoma clone, which binds to a ZNS1 linear epitope. Cross-reaction studies through Western blotting, indirect ELISA and immunofluoresence staining indicated that 6F6 specifically recognizes ZNS1, and does not cross-react with the NS1 protein from other related flaviviruses. The 6F6 mAb enabled the development of a sensitive, reliable and reproducible capture ELISA with a limit of detection (LOD) of 10.8 ng/ml and a limit of quantification (LOQ) of 29.4 ng/ml. The accuracy of the 6F6 sandwich ELISA was assessed by spike-and-recovery tests, obtaining average recoveries between the ideal range from 80 to 120%. In conclusion, we established a valid capture ELISA that allows the detection and quantification of small amounts of ZNS1 in human sera, and constitutes a promising bioanalytical method for control strategies and the prevention of ZIKV propagation.

149. (196) **OUTER MEMBRANE VESICLES SHAPE THE INTERACTION OF *BORDETELLA PERTUSSIS* WITH NEUTROPHILS**

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Outer membrane vesicles (OMVs) secreted by pathogenic bacteria are usually loaded with virulence factors that get to the host cell without close contact with the bacteria. *Bordetella pertussis* (Bp), the etiologic agent of pertussis, releases OMVs during infection. By proteomics analysis we confirmed the presence of most of Bp virulence factors in OMVs, adenylate cyclase toxin (CyaA, a strong immunomodulatory toxin) among them. In this study we investigated whether the presence of OMVs affects the outcome of Bp encounter with neutrophils (PMN), the main defense of the host against invading pathogens. We first studied the effect of the OMVs in the innate encounter of Bp with PMN. The presence of OMVs led to a significant decrease in bacterial uptake, which proved to be dependent on the delivery of CyaA from these vesicles, as determined by studies performed with OMVs isolated from a Bp CyaA deficient mutant (OMVs-CyaA-). Confocal microscopy studies showed a significant decrease in bacterial colocalization with the late endosomal/lysosomal marker LAMP-1 in PMN incubated with OMVs as compared with PMN treated with media or OMVsCyaA-, suggesting a bactericidal modulating effect of CyaA. Our results further showed that OMVs might protect Bp from PMN even in immune hosts. By means of double immune staining and fluorescence microscopy in combination with the use of cytochalasin D, we here observed that in the presence of specific antibodies the OMVs get opsonized and compete with opsonized bacteria for FcgR on PMN, leading to a significant decrease in the number of bacteria taken up by PMN. Confocal microscopy studies also showed that bacterial colocalization with LAMP-1 was significantly lower in PMN incubated with opsonized OMVs as compared with PMN treated with media alone, favoring the odds of bacterial survival also in immunized individuals. Taken together, these results seem to indicate that OMVs delivery should be considered within the immune evasion mechanisms of Bp

150. (200) **FENOFIBRATE MODULATES INFLAMMATORY MEDIATORS AS WELL AS CROSS-TALK BETWEEN TRYPANOSOMA CRUZI- INFECTED CARDIOMYOCYTES AND FIBROBLASTS**

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Chronic chagasic cardiomyopathy is characterized by parasite persistence, chronic inflammation and cardiac cell (Mc) death that may end lead to fibrosis and cardiac insufficiency. Activated fibroblasts (Fb) are involved in this process. Previous results from our group show that fenonofibrate (Fen), a PPARα ligand, modulates the expression of inflammatory mediators, prevents fibrosis and restores cardiac function in *Trypanosoma cruzi* (Tc) -infected mice. To deepen into the knowledge of the role of Mc and Fb in these events, in the present work we used an *in vitro* model of neonatal murine cardiac cells. We studied the modulator role of Fen on the inflammatory response of Mc and Fb infected with Tc. Mc and Fb were characterized by the expression of troponin C and s α-SMA, respectively, using Western blot (Wb). Wb and RT-qPCR analysis showed that Tc stimulated the expression of pro-inflammatory and pro-fibrotic enzymes like NOS2 and MMP-9 in Mc and Fb, whereas treatment with Fen (100μM) inhibited such expression in both cell lineages. In addition, after 48 hs of infection we observed increased expression of CTGF, MMP-2 and TGF-β by RT-qPCR in Mc and Fb, whereas Fen inhibited such increment (p<0,05).
Besides, the rise of TNF-α and IL-6 after 2 to 6h of infection was precluded by Fen. Furthermore, while NFκB was activated at 30 min post-infection in Mc and Fb since cytoplasmic IκBα was significantly reduced as determined by Wb, treatment with Fen precluded such activation. Finally, the ability of Mc- and Fb -conditioned media to promote the expression of NOS2 in Fb and Mc was analyzed. Both media induced the expression of NOS2 in Fb and Mc while Fen inhibited its expression as assessed by Wb. These results emphasize the role of Mc and Fb in the inflammatory and pro-fibrotic response to Tc and the interaction between these cell lineages that Fen is able to modulate.

151. (202) **FENOFIBRATE INCREASES THE POPULATION OF NON-CLASSICAL MONOCYTES IN ASYMPTOMATIC CHAGAS DISEASE PATIENTS AND MODULATES INFLAMMATORY CYTOKINES IN PBMC**

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Chagas heart disease (CHD) is the most important clinical manifestation of *Trypanosoma cruzi* (Tc) infection. Peripheral blood mononuclear cells (PBMCs) infiltrate the tissue and differentiate into inflammatory macrophages. Advances in pathophysiology show that myeloid cell subpopulations contribute to cardiac homeostasis, emerging as possible therapeutic targets. In this work we investigated the spontaneous release of inflammatory cytokines and chemokines, changes in the frequencies of monocyte (Mo) subsets and the effects of fenofibrate (Fen) on PBMC of patients with different clinical forms of Chagas disease. PBMC isolated from CHD by Ficoll® display higher levels of IL-12, TGF-β, IL-6, MCP1 and CCR2 than cells from uninfected individuals (HI) or asymptomatic (Asy), as tested by RT-qPCR, (P<0,05). Fen reduces the levels of pro-inflammatory mediators and CCR2 in both Asy and CHD (P< 0,05). Also, CHD patients display a significantly higher percentage of classical Mo in comparison with Asy and HI (P<0,05). Besides, Asy have a significantly higher percentage of non-classical Mo than CHD or HI (P<0,05). However, no difference in the intermediate Mo subpopulation was found between groups. Moreover, Mo from Asy or CHD patients exhibit different responses upon stimulation *in vitro* with Tc lysates and Fen treatment. Tc stimulation significantly increased the percentage of classical Mo and decreased percentage of intermediate Mo in the Asy group. Also, there were no changes in their frequencies in CHD or HI. Notably, stimulation with Tc did not alter the frequency of non-classical Mo in any of the groups. Moreover, Fen treatment of Tc-stimulated PBMC increased even more the frequency of non-classical Mo in Asy patients. Summing up, our results stress a potential role for Fen as modulator of Mo towards an anti-inflammatory profile in different stages of chronic Chagas disease.

152. (209) **AQUAPORINS CAN BE INVOLVED IN THE SWELLING CAUSED BY SHIGA TOXIN TYPE 2 ON HGEC AND HK-2 CELLS**

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Hemolytic uremic syndrome related to Shiga toxin–producing *Escherichia coli* (STEC-HUS) is the principal etiology of acute kidney injury in children in Argentina.

Previously, we demonstrated that Shiga toxin type 2 (Stx2) damages human glomerular endothelial cells (HGEC) and HK-2 human proximal tubular epithelial cell line by inducing swelling and detachment. In this work, we analyzed cell volume changes of HGEC and HK-2 exposed or not to Stx2 or a hypoosmotic (HYPO) medium, in the presence or not of aquaporins (AQPs) inhibitors (mercuric chloride: $HgCl_2$ and tetraethylammonium: TEA), or an inhibitor of Stx2 receptor (Gb3) synthesis, Eliglustat (EG). For controls, an isosmotic (ISO) medium was used.

Cells were grown on 12 well plates and pretreated for 30 minutes with $HgCl_2$ (10 μM) or TEA (100 μM) or pretreated during 24 h with EG (10 μM). Then, HGEC and HK-2 were incubated with Stx2 (50 μM) for an additional 40 minutes. Cell volume was analyzed by light microscopy and measuring cell area by using Image J software.

After Stx2 and HYPO medium treatments, a significant increase in the cell volume of HGEC (Stx2: 42%; HYPO: 36%, n= 3, p<0.05) and HK-2 (Stx2: 70%; HYPO: 55%, n= 3, p<0.05) was detected respect to ISO medium. However, when HGEC and HK-2 were pretreated with $HgCl_2$ or TEA a significant swelling prevention was obtained for HGEC (Stx2+$HgCl_2$:100%; Stx2+TEA:86%; HYPO+$HgCl_2$: 42.5%; HYPO+TEA: 83%, n=3, p<0.05) and HK-2 (Stx2+$HgCl_2$: 90%; Stx-2+TEA: 85%; HYPO+$HgCl_2$: 55%; HYPO+TEA: 75%, n=3, p<0.05). In addition, EG also was able to prevent HK-2 swelling in 87 % (n=1) with respect to Stx2 treatment.

Results show that AQPs may be involved in the water movement inside HGEC and HK-2 induced by Stx2, since $HgCl_2$ and TEA avoided this effect. Furthermore, binding of Stx2 to Gb3 could be the initial step for the development of cellular mechanisms that possibly trigger the entry of solutes into the cells and the consequent osmotic gradient responsible for the hypotonic effect.

153. (210) **FABF8:STX2 RECOMBINANT MONOCLONAL ANTIBODIES AVOID THE DELETERIOUS EFFECTS OF SHIGA TOXIN TYPE 2 ON HUMAN MICROVASCULAR ENDOTHELIAL CELLS**

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Hemolytic Uremic Syndrome (HUS) associated with Shiga-toxigenic *Escherichia coli* (STEC) infections is the principal cause of acute renal injury in pediatric age groups in Argentina. Neither a licensed vaccine nor effective therapy for HUS is available for humans. Previously, we demonstrated the *in vitro* cytotoxic effects of Shiga toxin type 2 (Stx2) on human glomerular endothelial cells (HGEC). Recently, recombinant antibodies against Stx2, produced in bacteria, were developed, and characterized. In this work, we studied the ability of anti-Stx2 FabF8:Stx2 antibody to neutralize the Stx2 activity on primary cultures of HGEC. Cells were plated in 96-well plates and grown to confluence. Then, cells were treated in growth-arrested conditions for 72 h with different pre-incubations (1 h at 37ºC) or co-incubations of FabF8 with Stx2. Antibodies were used from 10 μg/mL to 0.001 μg/mL and Stx2 at the dilution required to kill 50% of cells (0.5 ng/mL). Finally, cell viability was assessed by neutral red uptake. In addition, cells were seeded on gelatine coated glass coverslips and then treated, as it was previously mentioned, with 1 μg/mL FabF8 and 0.5 ng/mL Stx2, during 72 h. Percentage of necrotic and apoptotic cells were established by fluorescence microscopy after staining with acridine orange/ethidium bromide. Under both conditions evaluated, FabF8:Stx2 significantly neutralized, in a dose-dependent manner, the cytotoxic effects caused by 0.5 ng/mL Stx2 in HGEC (p <0.05, n = 3). HGEC viability was protected by 10 μg/mL FabF8 in about 67.5% at the co-incubation condition, and about 83% at the pre-incubation condition. Additionally, FabF8:Stx2 significantly prevented HGEC necrosis (pre: 60%; co: 92.5%) and apoptosis (pre: 93% and co:75%) (p <0.05, n = 3). The results demonstrate the high efficiency of FabF8:Stx2 to avoid the cytotoxic effects of Stx2 on HGEC, therefore, they could be used as a therapeutic strategy to prevent the renal damage described in patients with HUS.

154. (240) ***B. PERTUSSIS*** **COMPROMISES THE EPITHELIAL BARRIER AND SURVIVES IN NON-DEGRADATIVE INTRACELLULAR COMPARTMENTS**

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Bordetella pertussis (Bp) is the etiological agent of whooping cough, a reemerging respiratory tract disease. Current vaccines do not prevent transmission and epidemiological data indicates that vaccinated asymptomatic carriers are important reservoirs and constitute a source of transmission to vulnerable unvaccinated subjects. Recent studies suggest the existence of an intracellular niche of persistence inside host macrophages. We here investigated the potential role of the respiratory epithelial barrier in the infectious process and the eventual development of persistent infections. In binding assays Bp showed a clear tropism for tight junctions (TJ) of polarized 16HBE14o- cells as determined by fluorescence microscopy. This tropism seemed to be directed by the bacterial preference for basolateral membrane (BLM) mediated by FHA as addressed using a FHA defective strain. Our results further showed that wild type Bp but not an adenylate cyclase toxin (CyaA) deficient Bp strain disrupted TJ integrity as determined by confocal microscopy. This suggests that access of Bp to BLM in intact monolayers might be granted by the action of local high concentrations of CyaA released by the bacteria attached near TJ. The study of bacterial intracellular trafficking revealed that most internalized bacteria did not colocalize with lysosomal marker cathepsin D two days after infection suggesting that Bp avoids phagolysosomal fusion. Furthermore, we observed intracellular bacteria colocalizing with recycling pathway marker transferrin at this time point, indicating that Bp might survive in non-degradative vesicles with access to nutrients. Accordingly, antibiotic protection assays showed high intracellular survival levels over the time post infection. Taken together, these results show that Bp can compromise epithelial barrier, invade cells and persist in intracellular location in the respiratory epithelium, pointing out its potential relevance as another persistence niche.

155. (303) ***OLD DRUGS, NOVEL USES:*** **COMBINATION OF IVERMECTIN AND HEMIN AS A PROMISING TREATMENT AGAINST SARS-COV-2 INFECTION**

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel virus that is the causal agent of the Coronavirus disease 2019 (COVID-19). Given the urgent need for drugs to halt COVID-19 infection, one of the most valuable strategies is drug repurposing. The aim of this study was to analyze the antiviral effect of hemin and ivermectin (IVM), two drugs approved by Food and Drug Administration (FDA). The effect of hemin and IVM was evaluated in human pulmonary epithelial cells (A549 cell line). We evaluated the expression of genes related to SARS-CoV-2 infection, antiviral genes and anti-inflammatory genes by RT-qPCR. First, we established the optimal doses of hemin (80 uM) and IVM (10 uM) in this experimental model. Then, we cultured A549 cells with hemin and IVM, alone or in combination during different timepoints. We found that IVM treatment resulted in an increased expression of MX1 ($p<0.001$), an antiviral response gene against a great diversity of viruses. Moreover, in this *in vitro* model, hemin induced the expression of HMOX1 ($p<0.001$), a gene that encodes for the anti-inflammatory protein HO-1, and this effect was enhanced when hemin was combined with IVM ($p<0.001$). Concerning the proteins associated with virus entry into the host cell, we found that both IVM and hemin decreased the expression of BSG ($p<0.01$), a membrane receptor that facilitates SARS-CoV-2 entry, and the combination of these drugs increased the expression of ADAM17 ($p<0.001$), whose activity is related to viral entry inhibition. Finally, we mimicked viral infection using Poly(I:C), a synthetic analog of viral double-stranded RNA. Poly(I:C) treatment increased NF-κB and IRF3 expressions, validating the Poly(I:C) responsiveness of A549 cells. In this viral simulation context, IVM treatment also boosted MX1 expression ($p<0.05$) and hemin induced HMOX1 ($p<0.001$). Altogether, our results ascertain the potential antiviral action of hemin and IVM combination.

156. (329) **B. PARAPERTUSSIS ADENYLATE CYCLASE TOXIN PLAYS A KEY ROLE ON RESPIRATORY EPITHELIAL CELL INFECTION BY SUBVERTING THE EPITHELIAL BARRIER FUNCTION**

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B. parapertussis (Bpp) is one of the causative agents of whooping cough and a key factor contributing to its reemergence. The development of improved strategies to control it requires identifying the mechanism of persistence within the host to eventually eradicate the carriage. We previously found that an intracellular stage in epithelial cells might be important for its persistence. In that work we found a tropism of Bpp for tight junctions (TJ). Like for other pathogens, this might indicate the existence of a mechanism to subvert epithelial barrier function. We here investigated the role of the barrier function during Bpp infection. To this end we used the 16HBE14o- bronchial cell line that polarize in vitro and form TJ. Two experimental models were used. A fully 7-day polarized confluent monolayer in which apical and basolateral (BL) components are largely separated by TJ and a 1-day confluent monolayer, which lacks of TJ and hence barrier function. Cells were infected with Bpp and the outcome of this interaction was evaluated. Microscopy analysis showed that 6 h after infection the attachment and internalization were higher in the 1-day model than in the 7-day model, indicating that the infection efficiency increased when barrier function are absent and Bpp access BL components. We further evaluated how Bpp accesses the BL components in a monolayer with intact TJ. Microscopy analysis showed that early after infection of the 7-day model Bpp adheres to and progressively disrupts TJ in an adenylate cyclase toxin (CyaA) dependent way, suggesting that Bpp might access the BL components by subverting epithelial barrier. Accordingly, a CyaA defective mutant showed a reduced efficiency to access the intracellular location in a 7-day model with intact TJ. These results suggest that CyaA-mediated epithelial barrier disruption might grant Bpp access to the intracellular location of epithelial cells and/or the possibility to disseminate to underlying tissues.

157. (334) **MOLECULAR CHARACTERIZATION OF CIRCULATING TREPONEMA PALLIDUM CLUSTERS IN PEDIATRIC PATIENT LESIONS**

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Syphilis is caused by the bacterium *Treponema pallidum pallidum* (TPA). The scare about the biology of TPA during the complex clinical stages and the multiple routes of transmission allows syphilis to cause a large proportion of morbidity in children. Thus, there are not data in children about molecular biology techniques (MBT) in the diagnosis of syphilis in Argentina or about the strains of TPA worldwide. We started a multidisciplinary study evaluating the MBT for the diagnosis of syphilis in children. A strict evaluation detected 11 cases of acquired secondary syphilis transmitted through nonsexual contact (AsNs) with lesions of caretakers with untreated syphilis. Our aim was evaluate the use of PCR in swab lesions for the diagnostic of syphilis and examine the clusters of *TPA* by multilocus sequencing in an exploratory study of 11 AsNs cases (mean±SD: 5±2 years old) from October 2018 to August 2021. Lesions swabs were mainly in oral and perianal zones (n=17) and were processed for DNA extraction followed by PCR for Tpp47 gene (conventional

PCR) and dnaA gene (real time PCR by Taqman probes). Frequency of Tpp47-positive samples was 47%, while dnaA-positive was 94%. Also, nested PCR for the TP0136, TP0548, TP0705 and 23s genes were performed. The sequences were sequenced by commercial kit (BigDye) in a genetic analyzer (3500 analyzer).Then, edition and alignment were performed compared to the reference sequences of cluster SS14 (CP004011.1), Nichols (CP004010.2) and Escherichia coli 23s rRNA genes at the positions 2058 and 2059 (V00331) for macrolide resistant mutation. As adults in Argentina, the Nichols clade was greater than 10% and macrolide resistant mutation (A2058G mutation in 1 patient) was nearly 10%, although in our pediatric cases the prevalence of Nichols clade was higher (57%) that the reported. Among patients, positive PCR corroborate active lesions while clade and macrolide resistant evaluation shares similarity with studies in adults in Argentina.

158. (349) **CHARACTERIZATION OF SARS-COV-2 INFECTION USING RT-PCR IN SALIVA SAMPLES AND ACE2 GENOTYPING**

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Introduction: New biological matrices (i.e. self-collected saliva) has been postulated as a strategy to massify testing for SARS-CoV-2. In adittion, it has been shown that SARS-CoV-2 uses the ACE2 protein as a receptor to enter host cells, and although genetic variants are known, their frequency has not been described in the local population.

Methods: Nasopharyngeal swabs (reference control) and saliva samples were processed by RT-PCR for the detection of SARS-CoV-2. An additional blood sample was used to genotype the ACE2 gene variants 2158A> G (N720D, rs41303171) and 631G> A (G211R, rs148771870).

Results: 95 patients were included. The analysis of the characteristics of the studied population showed an average age of 48±22 years. The time between the onset of symptoms and the hospital evaluation was 4±2 days. A high correlation was obtained between nasopharyngeal swab and saliva obtained using the column extraction methodology, with an analytical sensitivity of 92%. The disaggregated analysis based on population characteristics showed greater sensitivity in patients with more severe symptoms (requiring hospitalization and high-flow oxygen) and long lasting symptoms at the time of consultation (> 2 days). Saliva samples showed higher Cycle threshold (Ct) amplification results compared to nasopharyngeal swab samples. By RT-PCR, the amplification cut-off points are between 37-40 cycles. Several Saliva samples, although "detectable" (Ct <37), amplified at higher cycles (around 5 cycles) compared to nasopharyngeal swab samples. Analysis of the variants of the ACE2 gene show the wild-type form for 2158A>G and 631G>A in all the cases analyzed.

Discussion: The detection of SARS-CoV-2 in saliva seems to be an appropriate method for the diagnosis of COVID-19, presenting excellent sensitivity, which increases depending on the severity and duration of the condition. ACE2 genetic variants do not appear to be common in the local population.

159. (392) **IMPLEMENTATION OF AN EPIDEMIOLOGICAL SURVEILLANCE STRATEGY FOR COVID-19 FOCUSED ON GROUPS AT HIGH RISK OF SARS-COV-2 INFECTION**

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The COVID-19 pandemic has been a challenge to implement strategies to mitigate the transmission of the SARS-CoV-2 virus. Asymptomatic transmission among healthcare workers (HCWs) at the front line of care is particularly concerning due to the potential emergence of outbreaks at healthcare centers. With the final goal of early identification and isolation of asymptomatic infected HCWs, we validated a pooled-sample screening and assessed implementation outcomes and results of an epidemiological surveillance strategy carried out during a 12-month period at public health institutions in Bahía Blanca. To develop and validate a coronavirus detection method, RNA was extracted from nasopharyngeal swabs and identification of the viral *E* gene was done by an "in-house" RT-qPCR using Taqman probes and the human gene *RNAseP* as a control. Validation against a commercial kit demonstrated high sensibility and specificity of our test (95%, IC 95%: [85%-100%]). To increase our testing capacity, we validated sample pooling (n= 5) prior to RNA extraction. The results showed a sensibility of 73% (IC 95%: [46%-99%]) and specificity of 100% against individuals. A tailor-made software called "VIGI-COVID" was designed to properly manage data. A prospective cohort study was conducted since 15/09/20 to 15/09/21. 860 HCWs were included in the epidemiological surveillance and 1765 swabs were performed. The annual cumulative incidence was 2,30% IC95% [1,26% - 3,39%] (20/860), and 43% of the 860 HCWs were swabbed more than once. Our study demonstrated the utility of comprehensive screening of asymptomatic HCWs during the COVID19 pandemic. Early identification and isolation of infected HCWs prevent the onward transmission of SARS-CoV-2, reducing the risk of healthcare-associated outbreaks.

160. (413) **INTRACELLULAR HISTONE H2A IS RECOGNIZED BY CE AND OTHER PARASITOSIS IMMUNE SERA**

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Echinococcus granulosus (*Eg*) causes cyst echinococcosis (CE), a worldwide distributed parasitic disease that affects livestock and humans. Our laboratory has developed the EGPE cell line from bovine *Eg* G1 protoscoleces (Echeverria et al, 2010) for studies of antiparasitic drugs and identification of relevant molecules in diagnosis and treatment of CE. This work **aimed** to identify and characterize proteins from *Eg* in the EGPE cells recognized by CE patients' sera. **Materials and methods:** total proteins extract from EGPE cells 20 days grown was passed through a gel filtration column. Protein fractions were concentrated through a 3K cut-off membrane concentrator. Reactive fractions to Western Blot were passed through affinity columns with CE or other parasitosis patient's sera. Then isolated proteins were identified by proteomic in CEQUIBIEM (FCEyN, UBA). Proteins were modeled using Robetta platform (TrRosetta method) and validated by molecular dynamics simulations with software NAMD 2.14. Epitope prediction was performed with IEDB (linear epitope prediction and Discotope 2.0) and ABCpred. **Results:** We identified *Eg*'s Histone H2A (W6U0N3, 195 aa) recognized by sera from CE and others parasitosis patients. W6U0N3 has 81% identity in the first 58 aa with *Fasciola hepatica*'s histone H2A. Generated model was validated showing in 10-50 ns section of the trajectory an averaged potential energy resultant -168208 ± 198 kcal/mol. This model presented two regions with different RMSD values for the trajectory taking the averaged structure as reference: for 1-14 aa region, 3.32 +/- 0.77 Å and for 14-195 aa region, 2.16 +/- 0.61 Å. Linear epitopes were predicted in 123-138, 138-153 and 170-185 aa, meanwhile conformational epitopes were predicted in the 1-14 aa region. **Conclusion:** Intracellular Histone H2A from EGPE

cells is recognized by CE and others parasitosis patient's sera, that could mean a marker for specific stages in the development of some parasitosis.

161. (459) **HIGUCHI ALGORITHM ANALYSIS OF COVID-19 OCCURRENCE FREQUENCY FOR PREDICTIVE PURPOSES IN THE ARGENTINE REPUBLIC, SANTA FE PROVINCE AND ROSARIO CITY**

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COVID-19 temporal occurrence frequency would reproduce a fractal rhythm. This behavior can be analyzed by mathematical algorithms that determine the Fractal Dimension (FD) and the predictive determination coefficient (R^2). FD would express virus temporal repetition and R^2 would express response capacity to environment demands, its value ranges between 0 and 1, greater than 0.5 would indicate persistence of the system. The aim of this work was to analyze temporal distribution of COVID-19 using Higuchi Algorithm (HA) for predictive purposes in the Argentine Republic (AR), Santa Fe Province (SFP) and Rosario City (RC) according to the epidemiological week (EW). Observational, longitudinal and prospective study. COVID-19 positive cases (PC) were considered by testing (nasopharyngeal swab-PCR) according to daily reports from the Ministry of Health, from the first PC in EW 10 and 11 (2020) to EW 32 (2021). HA was applied by EW. Median (M) and standard deviation (±) were obtained from FD and R^2, and Pearson's correlation coefficient (r) between FD and R^2 according to the territory. Results: FD (AR): M=2.47±0.36, R^2 (AR): M=0.96±0.03; FD (SFP): M=1.7±0.44, R^2 (SFP): M=0.74±0.13; FD (RC): M=1.63±0.45, R^2 (RC): M=0.72±0.14. Correlation for AR: r=0.37 ($p<0.17$), SFP: r=0.89 ($p<0.0001$) and RC: r=0.74 ($p<0.0015$). Conclusion: COVID-19 behavior in AR shows a growing and sustained temporal manifestation, and interaction system-environment, while SFP and RC have found limitations of environment interaction. COVID-19 could be sustained for a longer time in the AR regarding SFP and CR if the current conditions are maintained. AR PC decrease would not be accompanied by a fall off in the adaptation capacity of COVID-19 to the environment, that would not occur in SFP and RC. This conduct requires studying the impact of sanitary measures.

162. (508) **EPIDEMIOLOGICAL SURVEILLLANCE OF SARS-COV-2 OCURRENCE IN WASTEWATER FROM GRAN SAN MIGUEL DE TUCUMÁN, ARGENTINA**

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The ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been a public health emergency of international concern. SARS-CoV-2 is a member of the Coronaviridae family, consisting of a group of enveloped viruses with single-stranded RNA genomes which cause diseases ranging from common colds to acute respiratory distress syndrome. Although the main transmission routes for SARS-CoV-2 are the inhalation of aerosols/droplets and person-to-person contact, current evidence available indicates that the viral RNA is present in wastewater, suggesting the need to better understand this route as a potential source of epidemiological data and human health risks. To this aim, 32 sewage samples were collected between May and June 2020 from wastewater in the cities of San Miguel de Tucumán and Yerba Buena, Argentina. Before viral concentration, composite samples were heat inactivated to increase handling safety. Next, wastewater samples (200 ml) were mixed with polyethylene glycol (PEG) and NaCl. Mixtures were left to stand overnight at 4 °C. Subsequently, they were centrifuged, and pellets were resuspended in TRIZOL. The extraction of viral RNA was executed using the PURO VIRUS KIT (Productos BIO-Lógicos). A real-time RT-qPCR assay targeting the N gene, using SARS-CoV-2 specific primer and probes set, was performed. RT-qPCR mix was prepared using qScript XLT 1-Step RT-PCR (Quantabio) in a one-step system. While 6 out of 16 samples collected in May were positive, 8 out of 16 from June turned out positive. Interestingly, the increase in positive samples correlates with the increase in the number of human cases detected, further supporting wastewater-based epidemiology as a sensitive tool to study spatial and temporal trends of virus circulation in the population.

163. (539) **ASSESSMENT OF SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* O157:H7 PATHOGENICITY IN THE PRESENCE OF SHORT CHAIN FATTY ACIDS AND LACTATE**

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen, which can lead to the life-threatening Hemolytic Uremic Syndrome (HUS). There is no treatment available in order to reduce HUS outcome up to date. Short chain fatty acids (SCFAs), including acetate (A) and butyrate (B), are produced by the intestinal microbiota. Lactate (L) is the main metabolite of many fermented products. A, B and L can protect mucosa exposed to inflammatory insults. The aim of this work was to evaluate the effects of A, B and L on modulating STEC pathogenicity. We evaluated the inhibition of STEC growth after 3h culture, EspB production by SDS-PAGE and bacterial adherence to Caco-2 cells. We found that 50 mM of A and B, and 100 mM of the three compounds inhibited bacterial growth significantly compared to control: 0;1;10;50 and 100mM (median CFU(IQR) $x10^9$) A=2.2(1.8-2.7);2.3(2.0-2.6);2.2(1.8-2.4);1.4(1.2-2.0);1.1(0.5-2.0);B=2.1(1.9-2.3);1.9(1.8-2.2);1.6(1.5-2.0);1.3(1.0-1.4);0.5(0.4-1.0);L= 2.1(1.7-2.5);2.3(1.6-2.6);1.7(1.5-2.1);1.6(1.5-1.9);1.1(1.0-1.5); $p<0.001$, K W test. Then we evaluated if those concentrations that did not inhibit STEC growth had an effect on the expression of EspB, a protein from type three secretion system that is involved in bacterial adhesion to intestinal epithelial cells. We did not observe differences on EspB expression by SDS-PAGE. However, we observed a significantly reduced percentage of bacterial adherence to Caco-2 cells in the presence of A and B 1mM compared to control media (median%(IQR): Media=81(69-87); A1=13(12-18);A10=35(17-75);B1=8(6-9);B10=52(29-52);L1=52(35-52);L10=35(35-40);$p<0.05$, K W test.
In conclusion, acetate and butyrate were able to reduce bacterial adherence to Caco-2 cells in concentrations that do not inhibit bacterial growth. Since gut colonization is the first step in STEC pathogenesis, it could be interesting to examine the mechanisms involved in the inhibition of bacterial adherence to epithelial cells.

164. (554) **INCREASED PRESENCE OF PROCALCITONIN DURING PULMONARY TUBERCULOSIS. ITS RELATIONSHIP WITH DISEASE SEVERITY, AND DIABETES COMORBIDITY**

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Tuberculosis (TB) continues to be among the top ten leading causes of mortality worldwide being produced by the intracellular bacteria *Mycobacterium tuberculosis*. Previously, we demonstrated that TB patients showed a neuro-immune-endocrine imbalance consisting of elevated plasma levels of proinflammatory cytokines along with an increased cortisol/dehydroepiandrosterone ratio. Former results also revealed elevated lypopolysaccharide (LPS) plasma levels in severe TB. As procalcitonin (PCT) is considered a diagnosis and prognosis biomarker in bacterial infections, we evaluated PCT plasma concentration and its association with cytokine and LPS levels in TB. PCT plasma levels were assessed by an electrochemiluminescence immunoassay (Elecsys BRAHMS PCT, Roche) in an autoanalyzer. Interleukin 6 (IL-6) and interferon-γ (IFN-γ) were measured by commercial enzimoimmunoassays whereas C reactive protein was assessed by an immunoturbidimetric assay (CRP, Wiener-lab). LPS plasma concentration was studied by using a commercial chromogenic endpoint LPS detection assay. Newly diagnosed TB patients (n=37) exhibited increased PCT and LPS values respect to age- and sex-matched healthy controls (HCo, n=20; p=0.0011 and p=0.0007, respectively), mostly in severe cases who showed the highest levels (p<0.0001), with 2-month of specific treatment leading to a significant reduction (p=0.0001). At diagnosis, PCT levels correlated significantly with IL-6 (r=0.39, p=0.0092), IFN-γ (r=0.47, p=0.0018), and CRP (r=0.63, p<0.0001). Further analysis in the context of TB and type 2 diabetes (DBT) comorbidity, showed that TB-DBT patients had even more increased PCT concentration than TB (p=0.049) or DBT (p=0.0129) patients, in addition to HCo (p<0.0001). DBT patients also displayed raised amounts of PCT respect to HCo (p=0.0062). Increased presence of PCT mirrors the accompanying inflammation seen in active TB, preferably in progressive disease and during the DBT comorbidity.

165. (563) **ENVIRONMENTAL ENRICHMENT IMPROVES NEUROCOGNITION AND BEHAVIOR IN A MURINE MODEL OF CHRONIC TOXOPLASMOSIS**

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Currently, accumulated evidence links *T. gondii* chronic infection with different pathologies, including neurocognitive and behavioral conditions. There are no treatments able to eliminate the parasite at this stage or to reduce the adverse effects associated with the infection. Herein, we study the effect of a non-invasive therapy based on environmental enrichment (EE) on brain parasite load and the damaging effects of infection.

METHODS: The EE therapy involves increasing the available space and the addition of novel elements in the habitat. Chronically infected treated (TE), untreated (T) and naive (N) C57BL/6 mice were used to evaluate different abilities by the Open Field (OF), Hole Board (HB), Forced Swim (FS) and Novel Object Recognition (NOR) tests. Data was analyzed using ANOVA test. Brain cyst burden was evaluated at the end.

RESULTS: OF results evidence that the EE treatment on infected mice improved its exploratory ability measured as crossed lines (TE vs T p=0,0059) and rearings (TE vs T p=0,008), leading to similar levels than the N mice. We confirmed this result with the HB, where TE showed a 2,1-fold increase in nose poke behavior than the T group, reaching similar records to N (TE vs T p=0,007). The positive impact of the therapy was also exposed in the memory-learning abilities, measured by NOR, since TE showed higher identification index compared to T and similar performance to N (TE vs T p=0,0014). Also, the EE improved the TE response to stressful situations previously experienced as measured by the FS (TE vs T p=0,0014), with similar results to N. Nevertheless, brain parasite load was similar in TE and T groups.

CONCLUSIONS: This environmental enrichment therapy showed a positive impact in all the studied skills, showing its potential to deal with the harmful effects of chronic toxoplasmosis, improving well-being of the affected individuals. This type of non-invasive therapy could be easily incorporated into translational medicine approaches.

166. (575) **NEOKIT-COVID19, A COLORIMETRIC REVERSE-TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION TEST TO DETECT SARS-COV-2 AND ITS VARIANTS FROM EXTRACTION-FREE SAMPLES**

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Background: The coronavirus disease 2019 caused by SARS-CoV-2 has killed millions of people worldwide. Presently, RT-qPCR is considered gold standard test, but requires sophisticated equipment, expertise and is also expensive.

Objective: We developed a simple colorimetric molecular test for SARS-CoV-2 based on Reverse Transcription Loop-mediated isothermal Amplification (RT-LAMP). Faster, direct, low-cost and versatile.

Methods: we developed the test optimized on nasopharyngeal swab (NP) and saliva samples without an RNA isolation. We optimized of samples pretreatment with lysis buffer and heat-inactivated, temperature, incubation time, enzymes, dNTPs, primers, other components.

We used 3 primers set targeting 2 regions of ORF1ab gene and one in ORF E gene. The test result was defined as a Hydroxynaphthol blue dye (HNB) change violet to blue (visible to eye) as a result of the amplification.

Results: We present a colorimetric SARS-CoV-2 RNA detection method performed using RT-LAMP to achieve specific, rapid, with a detection limit of 25-100 copies per reaction directly from NP or saliva samples, without RNA isolation. We optimized the final condition of temperature (64°C), time (60m) and sample pretreatment with LB with a heat inactivation of 8 minutes.

The assay present 90,6 % of sensitivity and 100 % of specificity.

The kit, **NEOKIT-PLUS**, was authorized by **ANMAT**, Argentina, after validating it using samples previously analyzed by RT-qPCR.

The primer binding sites are well conserved in all the variants of concern (VOC), notified by World Health Organization (WHO). These lineages include **B.1.1.7, B.1.351, P.1** and **B.1.617.2.**

Conclusions: We presents a rapid and extraction-free detection of SARS-CoV-2 from NP swab and saliva by colorimetric RT-LAMP. Simple, sensitive, and cost-effective approach with broad potential to expand diagnostic testing for the virus causing COVID-19. This development gave birth to a technology-based company (EBT), NEOKIT SAS.

167. (585) **THE POTENTIAL OF SIRTUIN ENZYMES AS DRUG TARGETS IN CESTODE PARASITES**

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The anti-parasitic treatment of neglected tropical diseases (NTDs) caused by cestodes, such as echinococcosis and cysticercosis, relies on a small number of approved anthelmintics that are not well tolerated by some patients and often only partially effective. Thus, the identification of novel drug targets is critical. In this work, we identified Sirtuins (SIRTs), a family of NAD^+-lysine deacetylases, in cestodes and evaluated their potential as drug targets. By bioin-

formatic analysis of genome data, we identified six SIRT-encoding genes -belonging to SIRTs classes I, III, and IV- in species from *Echinococcus*, *Mesocestoides* and *Taenia* genera. RNA-seq data analysis in *Echinococcus* spp. showed transcriptional expression of these genes throughout several developmental stages; being SIRT2 the most expressed SIRT gene in all analyzed stages. Furthermore, we experimentally determined the anthelmintic effect of SIRT inhibitors by a motility assay in the cestode model *Mesocestoides vogae*. The SIRT inhibitor Mz25 showed a strong and irreversible cestocidal activity at various concentrations. This activity was time and dose dependent and with a value of IC_{50} significantly lowers than that of the current anthelmintic albendazole. Ultrastructural features; studied by SEM showed that Mz25 induced extensive damage on the general morphology and highlighting damage at the tegument. Structural analysis by homology modeling showed a high conservation of the canonical SIRT structure for cestode SIRT2s. No mutations were found in the residues implicated in zinc coordination, or in those implicated in the binding to NAD^+ cofactor or Mz25. However, some not conservative mutations were found in the selective pocket; representing a promising lead for developing selective cestode SIRT2s inhibitors. This report provides the basis for further studies to understand the roles of SIRTs in cestode biology and for developing selective inhibitors to treat NTDs caused by these parasites.

168. (592) **EPIDEMIOLOGICAL STUDY OF CANCER AND COVID-19 IN A DENSELY POPULATED SUBURBAN AREA**

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Diverse studies and data reports from 2020 showed that cancer patients were more prone to die by COVID-19, in special those undergoing hematologic malignancies and lung cancer. Information on how different cancer treatments influences the COVID-19 condition still remains unclear presenting some controversies. As pandemic drove the agenda of the Universidad Nacional de Hurlingham (UNAHUR) to set up a diagnostic center for SARS-CoV-2 detection by RT-qPCR that analyzes hundreds of samples from nearby municipalities on a daily basis, we proposed to carry out a retrospective cohort study to elucidate relations and influences between cancer, cancer treatments, and COVID-19. Furthermore, we proposed to standardize the RT-qPCR technique to quantify the viral load of the patients to also relate it with the presence of the oncological disease. Eligible patients are those with a measurable oncologic disease whose samples were positive for SARS-CoV-2 infection at our diagnostic center. Data are systematized to carry out the analysis, anonymizing the patients, and including the severity of COVID-19 disease, type of cancer, and oncologic treatments, among others. All procedures were approved by both the ethical committees of UNAHUR and of Buenos Aires Province.

Absolute viral load was quantified by constructing a plasmid with a sequence from the E viral gene —the viral target for PCR— to obtain a calibration curve from serial dilutions.

Therefore, we are analyzing possible relations between SARS-CoV-2 infection and clinical features of oncologic patients. We will present the preliminary results of the data analysis as well as sharing our research experience within the pandemic context.

169. (012) **ERYTHROPOIETIN IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME: A RANDOMIZED CLINICAL TRIAL**

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Background: The efficacy of recombinant human erythropoietin (rHuEPO) in sparing red blood cell (RBC) transfusions in children with hemolytic uremic syndrome related to Shiga toxin-producing Escherichia coli (STEC-HUS) is uncertain.

Methods: We conducted a prospective, two-parallel-group randomized open controlled trial conducted at the Hospital General de Niños Pedro de Elizalde from December, 2018 to January, 2021 (ClinicalTrials.gov NCT03776851). We randomly assigned children with STEC-HUS to the rHuEPO group (subcutaneous rHuEPO 150 U/kg/week + RBC transfusion if hemoglobin ≤7 g/dLand/or hemodynamic instability) or to the usual- caregroup (RBC transfusion if hemoglobin ≤7 g/dL and/or hemodynamic instability). Primary outcome was the number of RBC transfusions received during the hospitalization. Secondary outcomes were to explore whether baseline EPO levels were deficient (according to the relation between observed and predicted level), to correlate selected acute phase parameters with the number of RBC transfusions, and to assess possible adverse events.

Results: Twelve patients per arm were included, all completed the trial. They were comparable at recruitment and coursed a similar acute disease. Median number of RBC transfusions was similar between groups (1.5, p=0.76). Most patients had appropriate baseline EPO levels, which did not correlate with the number of RBC transfusions (r 0.19, p=0.44). Conversely, baseline (r 0.73, p=0.032) and maximum lactic dehydrogenase levels (r 0.78, p=0.003), creatinine peak (r 0.71, p=0.03) and dialysis duration (r 0.7, p=0.04) correlated significantly with RBC requirements. No potential side effect was attributed to rHuEPO therapy.

Conclusion: Administration of rHuEPO did not reduce the number of RBC transfusions in children with STEC-HUS.

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170. (011) **REDISTRIBUTION OF B-CELL SUBSETS DURING PREGNANCY**

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Previous studies from our group showed a decrease in B-cell lymphopoiesis in bone marrow during pregnancy. This state of generalized lymphopenia prevents an immunogenic reaction against the semi-allogenic fetus, but still allows the immune system to react to the exposition to foreign antigens. B-cells travel from the bone marrow to the spleen to differentiate. They arrive as Transitional 1 (T1) B-cells, and if maturation pathways are activated, they differentiate into T2 B-cells which in turn will originate Marginal Zone (MZ) or Follicular (FO) subsets. Lastly, T2 cells can be selected away from the B cell developmental pathway into T3 anergic B-cells. During pregnancy, our group described an expansion of mature and pre-activated MZ subset of B-cells in the spleen that suggest a compensatory mechanism of the immune system to guarantee the availability of effector B-cells for defense. In this work, our aim was to confirm these results using a genome-wide transcriptome profiling on isolated splenic B cells from pregnant (P) and non-pregnant (NP) mice. Using the gene expression profiles (GEP) from the mixed population of B-cells subsets, we estimated cell fractions of T1, T2 & T3 based on a matrix of 950 genes using CIBERSORTx deconvolution tool (https://cibersortx.stanford.edu/). We observed an abundance of GEP of T2-subset in P compared to NP mice ($p<0.001$, F-test, N=4). The relative abundance of T1 GEP was similar in both groups, however, the proportion of B-cells in NP mice that does not correspond to T2 subset is in turn represented by T3, which suggests a selection against differentiation in NP mice during physiological conditions. These results hints that the differential expression of genes in P mice will activate the pathways needed for further differentiation into the MZ subset of B-cells. Further analysis of GEP may allow us to predict with more accuracy B-cell redistribution in the spleen

during pregnancy.

171. (018) ***BRUCELLA SUIS ΔMAPB*** **OUTER MEMBRANE VESICLES POTENTIAL AS VACCINE CANDIDATES AGAINST BRUCELOSIS**

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Outer membrane vesicles (OMVs) have been used in development of vaccines against Gram negative bacteria. Here we evaluated *B. suis* M1330 (wt) and *B. suis* Δ*mapB* OMVs immunogenicity and protective capacity against both systemic and mucosal *B. suis* challenge.

Female Balb/c mice were immunized intramuscularly (i.m.) with OMVwt (20 µg), OMVΔ*mapB* (20 µg) or saline at 0 and 30 days. One week after last immunization serum, bronchoalveolar lavage, feces and saliva samples were obtained to measure OMV-specific antibodies. One-week later mice were challenged with virulent *B. suis* through the intraperitoneal (i.p.) or intratracheal (i.t.) routes. CFU counts were determined in lungs and/or spleens 20 days after challenge. Antibodies capacity to neutralize *Brucella* infection was determined in lung epithelial cells culture (A549 cell line).

Vaccination with both OMVs induced serum specific IgG ($p<0.0001$), sera from OMVΔ*mapB* animals reached higher IgG titers than OMVwt group (1600 OMVΔ*mapB* vs 400 OMVwt). In addition, OMVΔ*mapB* mice showed high levels of serum specific IgG1 (51200), IgG2a (3200) and IgA (400), while in OMVwt animals only low levels of specific IgA were detected (100). A slight increase in specific IgA levels at the respiratory and oral mucosa was detected in mice vaccinated with OMVΔ*mapB* ($p<0,01$). Serum specific antibodies from OMVwt and OMVΔ*mapB* mice reduced *B. suis* adherence to and invasion of A549 cells ($p<0.01$).

OMVwt and OMVΔm*apB* immunization achieved the same reduction of lung (0.7 log;$p<0.05$) and spleen (1.2 log; $p<0.05$) burden after i.t. infection; and a 1.67 log and 2.11 log reduction, respectively, of spleen burden after i.p. infection ($p<0.0001$).

Vaccination with *B. suis* wt and Δ*mapB* OMVs induced systemic and mucosal specific humoral immune response, which may contribute to prevent *Brucella* mucosal entry and its dissemination.

172. (085) **TONSILLAR GERMINAL CENTER REACTIVITY REGULATION AND AGING**

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While tonsillar hyperplasia is the most frequent cause of tonsillectomy in children younger than 10, abscesses and acute infections are responsible for most tonsillectomies in teenagers. Wherever extracellular ATP (a DAMP) becomes abundant in the body, ectonucleotidases CD39 and CD73 are considered vital in the generation of an immunosuppressive microenvironment through adenosine production. Our goal was to investigate a potential metabolic adaptation of tonsillar B cells, promoting a suppressive behavior upon years of hyperplasia due to local chronic inflammation. By analyzing tonsillar mononuclear cells (TMC) using FACS, we compared co-expression of the ectonucleotidases CD73 and CD39 on CD20+ cells at different donor ages. We found that samples from older patients presented a statistically significant higher double positive CD73+ CD39+CD20+ cell population than those from younger children (37.7% ± SD 10% vs 25.8% ± SD 8.8% respectively, n=40, $p<0.01$). By culturing TMC with IL2/IL4/CpG/CD40L, we also show that activated B cells reliably downregulated CD73, presumably to prevent autocrine adenosine signaling. Thus, we found that neither IL10+CD20+ cells nor IL17+CD20+, generated upon stimulation, expressed CD73. In contrast, changes in CD39 expression with B cell activation resulted more variable between patients. Finally, we used the percentage of germinal center B cells (GC) as a read out of the effector immunological activity of the organs. We found that the proportion of GC within CD20+ cell population steadily declined with increasing age. GC B cells represented approximately one third of all the B cells from tonsils within the (2-9) year old range (29% ± SD 14%). That value declined to 15.7% ± SD 9.7% in tonsils from 10 to 18. Differences between the means were statistically significant (n=50, $p<0.01$). We concluded that the progression on the cause of tonsillar disease with age might illustrate the adaptation of the tonsillar tissue to constant inflammation.

173. (101) **B1 B CELLS ACQUIRE A PROLIFERATIVE AND ANTI-INFLAMMATORY PROFILE DURING PREGNANCY IN MICE**

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B1 B cells are a distinct subpopulation of B cells characterized by their unique capacity of self-renewal and the ability to secrete IgM without foreign antigen exposure (natural antibodies). In addition, upon activation, B1 B cells produce large quantities of the potent anti-inflammatory cytokine IL-10. Though the mechanisms that control natural antibodies production are not fully elucidated, it was recently associated with a down-regulation of CD1d expression in B1 B cells. Taking into account that both, IL-10 and natural antibodies are known to be fundamental components in pregnancy wellbeing, the aim of this study was to evaluate proliferation status as well as CD1d expression and IL-10 production by B1 B cells during pregnancy. Flow cytometry analysis, on splenic B1 B cells from pregnant (P) and non-pregnant (NP) mice was performed to evaluate ki-67 (proliferation marker) and CD1d expression as well as IL-10 production upon LPS stimulation.

We observed significantly higher expression levels of Ki-67 in splenic B1 B cells from P compared to NP (Unpaired t-test $p<0,0001$; n=3) mice which was mirrored by higher percentages of B1 B cells in the spleen of P mice (Unpaired t-test $p=0,0095$; n=11). In addition, B1 B cells from P mice expressed lower levels of CD1d as compared to NP mice (Unpaired t-test $p<0,0001$; n=3). Furthermore, LPS-stimulated B1 B cells from P mice produced significantly higher levels of IL-10 compared to NP mice *in vitro* (Unpaired t-test $p=0,015$; n=5).

Overall, our results demonstrate that not only B1 B cells are expanded in the spleen during pregnancy but they also seem to acquire the capacity to produce higher levels of natural antibodies and IL-10 during this period, suggesting their critical role in the intricate process of pregnancy tolerance.

174. (205) **EXTRACELLULAR ATP DRIVES T CELL IMBALANCE IN PEDIATRIC COVID-19**

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Background: Profound Tregs perturbations correlate with COVID-19 severity in adults. Extracellular ATP increases in inflammatory milieus with its concentration being regulated by CD39, mainly ex-

pressed on Tregs. There is no data regarding Tregs and Th17 cell balance in children with COVID-19.
Aims: 1) to analyze the frequency and phenotype of Tregs children with COVID-19 and controls; 2) to quantified the levels of ATP release by stimulated PBMCs and in serum; 3) to study the effect of extracellular ATP on CD4+ T cell balance.
Methods: We used sera, PBMCs and/or purified T cells from children with COVID-19 (n=54) and controls (n=24) to evaluate frequency and phenotype of cells subset and proliferative response by flow cytometry; ATP levels by luminometry; cytokines levels by multiplex assays.
Results: We observed a decreased frequency of Tregs in children with COVID-19, mainly in those with severe disease ($p<0.01$). These Tregs showed an activated phenotype with a strong suppressive profile including a great expression of CD25, CTLA-4 and CD39. Severe patients expressed increased levels of the ectonucleotidase cd39 in CD4+ T cells in comparison with non-severe ($p<0.05$) and controls ($p<0.0001$). Additionally, we found that stimulated PBMCs from severe children released the highest levels of ATP as well. We also detected that ATP promoted a fall in the proliferative response of purified T cells ($p<0.0001$) as well as in the Th1 and Th2 cytokine patterns. Interestingly, IL-17A and IL-17F levels did not decrease. As expected, ATP reduced the percentage of FOXP3+ and increased the expression of RORC in T cells, that were abrogated with the P2X7R antagonist, showing the involvement of this receptor. Finally, the levels of ATP in plasma correlated inversely with the frequency of Tregs.
Conclusions: We demonstrate that signaling through purinergic receptors drives Th17 but impairs Tregs immune response which have implications in the pathogenesis of pediatric COVID-19.

175. (229) **NOVEL HETEROZYGOUS MUTATION IN STX11 IN A PEDIATRIC PATIENT WITH EVANS SYNDROME**

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Natural killer (NK) and CD8+ T cells play an important role in the immune response. STX11 encodes a t-SNARE protein necessary for the final fusion of lytic granules with the plasma membrane of these cells. Biallelic mutations in STX11 results to a "particular" Familial Hemophagocytic Lymphohistiocytosis type 4. Heterozygous mutations have been identified in several patients, although the clinical/functional relevance of these mutations remains poorly understood. Our aim was to determine the functional relevance of an heterozygous STX11 variant (R129P) identified in a pediatric patient diagnosed with Evans syndrome. Targeted sequencing showed that the patient's mother was heterozygous for the mutation.
PBMC from healthy donors (HD), patient, mother and a patient with Chediak Higashi syndrome (negative control, NC) were used.
We analysed degranulation capacity of CD8+T cells and degranulation and cytotoxicity ability of NK cells, using flow cytometry assays. We observed a reduction of all these functions in the R129P-STX11 patient and mother in comparison to HD. Nevertheless, these reductions were less defective than observed in NC cells. IL-2 in vitro treatment restored these functions.
The RNA levels (qPCR) of patient and mother were similar to HD but the protein expression (WB) was reduced.
Finally, we performed in-silico structural analysis of R129P substitution using available STX11:Munc18-2 complex structure. R129 is part of a helix in the NHabc domain displaying a rich hydrogen bond network with Munc18-2. In this context, this variant is expected not only to impact helix stability, but also protein-protein interaction.
Altogether, we demonstrated that the novel R129P-STX11 mutation can play a pathogenic role by impairing degranulatory activity of NK and CD8+T cells and cytotoxic activity of NK cells. The aberrant functionality of NK cells have been reported in several autoimmune disorders. This novel mutation may explain the clinical patient Evans Syndrome phenotype.

176. (232) **BACTERIUM-LIKE PARTICLES DERIVED FROM THE RESPIRATORY COMMENSAL BACTERIA *CORYNEBACTERIUM PSEUDODIPHTHERITICUM* 090104 AS A PROMISING MUCOSAL ADJUVANT**

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Corynebacterium pseudodiphtheriticum is a Gram-positive *bacterium* that is part of the nasopharyngeal microbiota. This bacterium was shown to prevent the colonization of the respiratory mucosa by pathogenic bacteria including *Streptococcus pneumoniae* (Sp). We recently demonstrated that *C. pseudodiphtheriticum* 090104 (Cp), when nasally administered to mice; differentially modulated the respiratory immune responses triggered by TLR2 and improved the resistance to pneumococcal pneumonia. We also reported that bacterium-like particles derived from Cp (BPCp) had the ability to modulate the innate respiratory immunity. These results allowed us to hypothesize that Cp or BPCp could be used as mucosal adjuvants to enhance the respiratory adaptive immunity. In this work, infant Swiss-albino mice were nasally immunized with 6.25 pg of Pneumovax23® vaccine (PV), PV plus Cp (10^8 CFU) or PV plus BPCp at days 0, 14 and 28. Seven days after the last immunization samples of bronco-alveolar lavages (BAL) and serum were collected for specific antibodies determinations. In addition, immunized mice were challenged with Sp serotypes 6B or 19F (10^6 CFU) and the resistance to the infection was evaluated 2 days after the challenge. Mice in the PV+Cp and PV+BPCp groups had significantly higher levels of BAL anti-Sp IgG and IgA ($p<0.01$) as well as serum IgG and IgM ($p<0.01$) in comparison with mice immunized only with PV. Of note, the PV+Cp immunization was more efficient than PV+BPCp to improve respiratory and serum antibodies levels. PV+Cp and PV+BPCp mice had lower Sp counts in lungs than controls, as well as negative hemocultures. In addition, PV+Cp and PV+BPCp groups had higher levels of BAL and serum TNF-α, IFN-γ and IL-4 ($p<0.05$) than controls after Sp infection. Again, Cp was more efficient than BPCp to induce protection against Sp 6B and 19F. The results show that both Cp and BPCp are promising mucosal adjuvants for the development of nasal vaccines to combat respiratory infections.

177. (265) **CD4+ T CELL SPECIFICITY AND TRPV1-INITIATED NEUROINFLAMMATION IN DRY EYE DISEASE**

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In dry eye disease (DED), it is likely that CD4+ T cells specific for an unknown ocular antigen cause corneal epitheliopathy and that corneal nerve damage contributes to ocular surface inflammation.

We tested both hypotheses in murine models of surgical DED by restricting the T cell repertoire [Balb/c (wild-type) vs DO11.10 mice (partial ovalbumin-restriction)] and exploring the role of the corneal nerve polymodal nociceptor TRPV1, known to trigger neuroinflammation. We assessed corneal epitheliopathy (CE), corneal mechanical sensitivity (MS), capsaicin sensitivity (CS, TRPV1 agonist), eye-closing ratio (ECR, pain surrogate) and corneal intraepithelial nerve density (IEND).
Remarkably, DO11.10 mice were capable of developing DED, showing increased CE, decreased MS, ECR and IEND (n=10-16, $p<0.05$). However, Balb/c mice developed worse DED, with similar MS and ECR but worse CE (n=16, $p<0.05$) and lower IEND (n=12, $p<0.05$). Prior immunization of DO11.10 mice with their cognate antigen did not affect DED severity (n=16, $p<0.05$). Furthermore, the opposite eyes of Balb/c mice with unilateral DED showed lower MS (n=62, day>4, $p<0.05$), ECR (n=9, day>7, $p<0.05$) and CS (n=9, day>4, $p<0.05$) and higher CE (n=9, day>4, $p<0.05$) compared with control eyes, and lost mucosal tolerance later on (day 8). Also, TRPV1 antagonist-treated mice developed milder disease with respect to CE (n=20, $p<0.05$) and ECR (n=9, $p>0.05$), and also had higher IEND(n=11, $p<0.05$).
Thus, T cell repertoire restriction leading to a milder DED phenotype suggests that antigenic specificity is relevant in DED, while immunization of DO11.10 mice not worsening disease suggests that bystander activation of CD4+ T cells is not. Both findings point towards an still unidentified corneal autoantigen. In addition, both the improvement in DED phenotype by TRPV1 blockade and the disease-like findings in the opposite eyes of unilateral DED mice suggest that TRPV1-initiated neuroinflammation contributes to DED pathophysiology.

178. (300) **CHARACTERIZATION OF SPECIFIC CAMELID SINGLE DOMAIN ANTIBODIES, VHH OR NANOBODIES, AGAINST HUMAN IgG4 FOR DIAGNOSTIC APPLICATION IN IgG4 RELATED DISEASE**

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The adaptive immune systems of camelids comprise classical antibodies and heavy-chain only antibodies (HCAb), where antigen-binding is mediated by one variable domain, called VHH or nanobody (Nb). Nbs present unique characteristics conferring great potential in the development of more sensitive diagnostic methods, including high solubility, physicochemical stability and low production cost. Moreover, Nbs are able to recognize cryptic epitopes, not targeted by conventional antibodies. On the other hand, IgG4 is increased in the serum of patients with IgG4 related disease (IgG4-RD). Along with other characteristic symptoms, serum IgG4 levels allow the diagnosis of IgG4-RD. For this reason, we aimed to characterize specific VHH against human IgG4 (hIgG4) for the development of new tools in IgG4-RD diagnosis. To achieve our goal, two immune VHH libraries were previously constructed starting from llama blood. Specific Nbs against hIgG4 were selected by Phage Display methodology. The reactivity of 90 selected Nbs was studied against the four human IgGs by ELISA. As a result, 61 out of 90 VHH were specifically reactive against hIgG4. The diversity of these Nbs was analyzed by fingerprinting, showing 20 different digestion patterns. From these VHH, the 10 most reactive Nbs were confirmed as unique by sequencing. Six of these Nbs were expressed as soluble protein in *E. coli* WK6 strain. Then, reactivity of soluble-expressed Nbs against sera of different species was assessed by Dot Blot. Furthermore, the reactivity against all human IgGs was studied by ELISA, confirming IgG4 specificity. In conclusion, 61 specific Nbs against hIgG4 were successfully selected by Phage Display. Moreover, the 10 most reactive out of these 61 VHH showed to be unique. Six out of these Nbs were expressed as soluble protein and showed high reactivity against only hIgG4 in Dot Blot and ELISA. Further characterization of these Nbs will allow us to develop new tools for diagnostic innovation in IgG4-RD.

179. (301) **DISCOVERY OF NEW ADJUVANTS FOR ORAL VACCINE FORMULATIONS**

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In our laboratory, we have shown that U-Omp19 would be an ideal mucosal adjuvant because it can inhibit gastrointestinal proteases while it induces adaptive immune responses. To our knowledge there are no other published reports describing the use of protease inhibitors from bacteria as immune vaccine adjuvants.
Based on these previous results, we studied if other microbial protease inhibitors also have adjuvant activity. After an *in-silico* screening using MEROPS database plus literature, we have selected 11 protease inhibitors present in human pathogenic microorganisms representing different families of protease inhibitors.
We made a screening based on 3 different selection criteria: i) purification yield, ii) protease inhibitor activity against gastrointestinal proteases and iii) immunostimulatory properties. Our results indicate that 5 putative protease inhibitors can inhibit the protease activity of gastrointestinal proteases and pancreatin extract ($p<0.001$ ANOVA + Bonferroni). We also shown that these molecules have the capacity to activate immune cells. We have found a significant increase in the production of IL-6 in BMDCs from C57BL/6 and C3H/HeJ mice when the cells were stimulated with the protease inhibitors, compared with medium ($p<0.001$ ANOVA + Bonferroni) in 3 of them. Finally, we have found a significant increase in the proliferation of OT-I CD8+ T cells after oral administration of OVA plus each of the 3 bacterial protease inhibitors, compared with OVA alone (*$P<0.05$, **$P<0.01$ ANOVA + Bonferroni)
Together our results show that the new identified bacterial protease inhibitors could be mucosal adjuvants, since they are able to increase adaptive immune responses to orally co-delivered model Ag.

180. (320) **DIFFERENTIAL EXPRESSION OF MIRNAS IN IBD MUCOSA AND INTESTINAL FIBROBLASTS: POTENTIAL BIOMARKERS FOR ASSOCIATED COLORECTAL CANCER**

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MicroRNAs (miRNAs) are small and noncoding RNAs which have recently gained relevance for their role in IBD pathogenesis as epigenetic modifiers of gene expression. MiRNAs are believed to be involved in the gut inflammation in IBD and to be implicated in the transformation process from chronic inflammation to colorectal cancer (CRC). Besides, mucosal myofibroblasts are key stromal cells involved in IBD pathogenesis and in the CRC tumor micro-environment. miR-21-5p, miR-155-5p, and miR-31 have repeatedly been identified and seem to be the most studied miRNAs related to IBD. In this work, we aimed to study whether there is differential expression of miR-21-5p and miR-155-5p in IBD mucosa, and in intestinal fibroblasts from IBD patients, compared to CRC patients and healthy control intestinal mucosa, as potential early biomarkers to predict CRC outcome in patients with chronic intestinal inflammatory disorders.
Total RNA was obtained from mucosal explants from IBD patients, healthy control patients, polyps biopsies and colon tumor biopsies. Intestinal fibroblasts were isolated from colon surgical pieces and primary cultures were established. cDNA from biopsies and/or fibroblasts was obtained and miR-21-5p and miR-155-5p expression was quantified by real time qPCR with specific primers.
We detected higher expression levels of miR-21-5p in inflamed tissue compared to non-inflamed mucosa, as well as in tumor biopsies from CRC patients, whereas expression of miR-155-5p was lower in inflamed mucosa compared to non-inflamed mucosa, as well as in tumor biopsies from CRC patients compared with healthy patient biopsies.
Further studies including more samples with different pathological features are underway, in order to confirm that the differential ex-

pression of these microRNAs could be a useful tool to predict IBD outcome to early prevent CRC onset.

181. (340) **NANOVACCINE PLATFORM CONTAINING TLR-9 AGONIST REQUIRES IFN-γ FOR INDUCE ANTIGEN-SPECIFIC IgG2a ANTIBODY RESPONSE**

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The subunit vaccines have many advantages; however, they need appropriate adjuvants to enhance immune response. Our group developed an adjuvant strategy in which OVA and CpG-ODN are formulated with a nanostructure (Coa-ASC16) formed by self-assembly of 6-O-ascorbyl palmitate (ASC16). We have previously shown that this nanoformulation (OCC) elicited stronger OVA-specific antibodies, and Th1 and $CD8^+$ T-cell responses compared with a solution of OVA and CpG-ODN (OC). OCC elicited early IFN-γ secretion in lymph nodes (LN) draining the injection site peaking at 24 h after injection, and this secretion was higher than the one produced by the OC group. Conversely, the IFN-γ concentration in serum was higher in OC mice than in OCC mice. Here, we study the source of innate IFN-γ production and its requirement to induce an adaptive immune response. Wilde-type and IFN-γ-deficient mice were subcutaneously immunized with a single-dose of OC or OCC. LN and spleen from wilde-type mice were collected 24 h after injection and IFN-γ producing cells were assessed by flow cytometry after incubation with RPMI in the presence of Brefeldin A and Monensin at 37 °C 5% $C0_2$. The frequency of IFN-γ^+ cells found in LN was higher in OCC mice than in OC mice ($p<0.001$), but no differences were observed in spleen. Furthermore, the relative proportion of IFN-γ^+ cells was different between both groups, the main difference being the increased size of IFN-γ^+ NK1.1 cells population in LN of OCC mice compared to OC mice ($p<0.001$). In IFN-γ-deficient mice, OCC elicited lower OVA-specific IgG2a titers than in wild-type mice; however, OVA-specific CD4+ T cells proliferation and frequency of SIINFEKL-K^btetramer$^+$ CD8+ T cells were not affected. Understanding the mechanism by which adjuvants engage the immune responses is critically important for the development of vaccines. Our results support the hypothesis that early IFN-γ production is important for obtaining productive responses to adjuvanted vaccines.

182. (351) **SEX DIFFERENCES IN THE INCREASED ASTHMA RISK IN ADULTS CAUSED BY MATERNAL STRESS**

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BACKGROUND: Allergies are increasing worldwide. The presence of atopic diseases in the mother propagates the onset of allergic diseases in the offspring with a considerably stronger penetrance than atopic diseases of the father. Such observation challenges genetic predispositions as the sole cause for allergic diseases. Epidemiological studies suggest that caregiver stress in the perinatal period may predispose offspring to asthma. We have shown that maternal stress during pregnancy results in an increase of litter susceptibility to develop allergic lung inflammation OBJECTIVES: We aimed to study if there are sex differences in susceptibility in adult mice. METHODS: Pregnant BALB/c mice were subjected to a single restraint stress exposure at day 15 of gestation. Pups were separated by gender and after puberty and treated with two i.p.injection of ovalbumin (OVA)/alum(day 4 and 47), challenged with antigen aerosol(days 50-52) and euthanized(day 54). Negative controls included pups of non-stressed dams subjected to the same protocol or i.p. sensitized and aerosol challenged with PBS. RESULTS: Female and male adult mice born to stressed dams were more susceptible to developing pulmonary allergic inflammation, since an increase in the number of eosinophils in bronchoalveolar lavage (BAL), a greater peribronchial and perivascular infiltrate, higher proportion of mucus-producing cells, and increased IL-4 and IL-5 levels in BAL were detected compared to control mice. These parameters were more pronounced in females than males. Moreover, only females from stressed dams showed an increase in IgE levels. CONCLUSIONS: Increased litter susceptibility to develop allergic lung inflammation induced by maternal stress is stronger in females than in male mice.

183. (384) **NANOVACCINE PLATFORM CONTAINING TLR9 AGONIST IMPROVES GERMINAL CENTER RESPONSE**

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We have previously reported that the nanoformulation of OVA and CpG-ODN with a nanostructure (Coa-ASC16) formed by self-assembly of 6-O-ascorbyl palmitate (ASC16) elicited an OVA-specific antibody response sustained for over 160 days and cellular immune response superior in magnitude and quality to those induced by vaccine components in solution. Here, we study the effect of various vaccine components formulations in the antigen-specific $CD8^+$ T cells, germinal center B cells, and Tfh cells. We also evaluate the effect of ASC16 sterilization (gamma irradiation) on the immune response. Mice were subcutaneously immunized with a single dose of OVA and CpG-ODN nanoformulated with Coa-ASC16 (OCC), an OVA and CpG-ODN solution heated and then cooled down to RT (OCø), an OVA solution heated and then cooled down to RT plus a CpG-ODN solution at RT (**øO**/C), or with an OVA solution at RT plus a CpG-ODN solution heated and then cooled down to RT (O/**øC**). Heating and cooling processes recreated the conditions of the nanoformulation preparation. ELISA and flow cytometry techniques were used. In the group of mice immunized with OCC we found a response higher than the response elicited in the other groups of antigen-specific $CD8^+$ T cells ($CD3^+$ $CD8^+$ SIINFEKL-K^b tetramer$^+$) in blood 7-days post-immunization ($p<0.0001$), of Tfh cells ($B220^-$ $CD3^+$ $CD4^+$ $CXCR5^+$) in the lymph node 10-days post-immunization ($p<0.05$), and of OVA-specific germinal center B cells ($CD3^-$ $F480^-$ $CD19^+$ IgM^- IgD^- IgG^+ $GL7^+$ $CD38^-$ OVA^+) in the lymph node 14-days post-immunization (6-days post-intraperitoneal challenge with OVA/CpG-ODN) ($p<0.05$). No changes were observed in the OVA-specific immune humoral and cellular response elicited by sterile ASC16 vs non-sterile ASC16. These data showed that the nanoformulation of vaccine components enhanced the germinal center response, which led to robust antibody responses. In addition, it was shown that the ASC-16 sterilization process does not affect performance.

184. (408) **DYSBIOSIS ASSOCIATED TO AZITRHOMYCIN ADMINISTRATION**

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The gut microbiota is a complex community of microorganisms that plays an important role in the development and maintenance of the immune system and gastrointestinal functionality. Alterations in the microbiota composition (dysbiosis) could initiate or enhance autoimmune or inflammatory diseases. Among the factors that cause dysbiosis, the administration of antibiotics is one of the most frequent. In this work we evaluated the impact of acute administration of azithromycin (AZM) on the intestinal microbiota. Adult male C57BL / 6 mice received AZM (50 mg /kg/day) in drinking water for 5 days and stool samples were collected to infers a microbial community structure based on analyses of terminal-restriction fragment length polymorphisms (T-RFLP) of 16S rRNA gen and density for flow cytometry (FC), samples of small and large intestine (SI and LI) to evaluate mucus by colorimetric method, luminal content to determine metabolic profiles by GC-MS and proximal and distal mesenteric lymph nodes (MLN) to evaluate CD3, CD4, CD8 and CD19 lymphoid subsets by FC. After the administration of AZM, the relative abundances of the main phyla showed modifications with an increase in Firmicutes ($p < 0.001$) and a decrease in Bacteroidetes ($p < 0.001$) compared with control group. Analysis of control samples from two different years showed a similar composition in terms of phyla, evidencing the stability of the microbiota in our colony. The antibiotic produced a decrease in mucus levels (30 to 50% in SI and 30 to 75% in LI); analysis of the colon content of AZM and control groups showed similar profiles. For the lymphoid subsets, main differences in percentage of CD19+ cells ($p = 0.07$) and CD19+ and CD8+ cells ($p=0.07$) were found in proximal and distal MLN respectively. Our results provide microbiological, biochemical and molecular findings that contribute to characterize the intestinal dysbiosis associated with the administration of AZM in adult mice.

185. (438) **IDENTIFICATION OF ANTIGEN CROSS-PRESENTATION POTENTIATING DRUGS FOR VACCINE DEVELOPMENT**

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The cytotoxic CD8 T cell (CTL) mediated-immune response is crucial for tumor immunotherapy and for protective immunity against intracellular pathogens. Dendritic cells (DC) have the ability to internalize and present exogenous antigens (Ag) bound to MHC I to activate *naïve* CD8 T cells through a process known as cross-presentation. Subunit vaccines are often poorly immunogenic and adjuvants are required to boost immunity. The complexity of antigen cross-presentation pathways makes difficult to identify therapeutic targets that can act as adjuvants able to generate protective CTL responses. We performed a high throughput screening of libraries of drugs approved by international agencies to identify compounds and molecular pathways capable of enhancing Ag cross-presentation in DCs. For it, we developed a high-performance screening method by adapting the colorimetric B3Z presentation assay using JAWSII DC cell line and Ovalbumin (OVA) as Ag. After assayed 1760 drugs, we found 1.1% of them increased OVA cross-presentation. We validated these hits and functional analogs by performing dose-response assays with both JAWSII and GMCSF BMDCs. Almost all of hits are lysosomotropic drugs that could promote biomacromolecules accumulation. As lipid bodies (LB)-formation has been associated to DCs cross-presentation ability, five hits were evaluated for LBs formation in JAWSII cells by fluorescence microscopy. We found that Tolonium Chloride, Amodiaquine Dihydrochloride and Perhexiline Maleate increased the amount of LBs per cell. None of these five drugs produced an important increase neither in the MHCI-surface expression nor in the soluble OVA-endocytosis by JAWSII cells. In conclusion, we established a sensitive, fast and robust screening platform for compounds-search capable of stimulating Ag cross-presentation in DCs. Although the mechanism of action of these drugs is still under investigation, our preliminary results indicate that it is likely related to lipid metabolism.

186. (473) **SPUTNIK V SECOND DOSE MAKE THE DIFFERENCE IN NON-COVID-19 PATIENTS**

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OBJECTIVES: to analyze antibodies levels against SARS-CoV-2 along time in Sputnik V vaccinated healthcare workers. Two cohorts of patients samples (n=280) were used in this work. First, serum samples from patients vaccinated with Sputnik V that previously had a positive SARS-CoV-2 qPCR (A); and second, serum samples from patients vaccinated with Sputnik V that previously had a negative SARS-CoV-2 qPCR (B). Patients had an age range between 25 and 80 years. To perform the analisys of the antibody levels we used proteins Spike and RBD in a single enzyme-linked immunosorbent assay (ELISA) plate (COVIDAR IgG). Most patients that had COVID-19 presented mild to moderate symptoms. Results are expressed as median (min-max). ANOVA was performed in order to statistical evaluation. RESULTS: antibodies levels in group A were significantly higher than in group B, both after the first doses 13.4 (0.2-16.9) vs 1.4 (0.1-13.5); $p<0.001$ and the second doses 13.5 (2.2-17.4) vs 6.8 (0.1-17.9), $P<0.01$. In the A group there was no significant differences between the antibodies levels comparing from 20 days after the first dose vs 20, 90 and 180 days after the second dose. Noteworthy, in group B seroconversión did not occur in most patients over 45 years old after 20 days of the first dose, unlike what was observed in group A ($P<0.001$). Antibodies levels in group B from 20 days after the first dose of vaccine vs 20, 90 and 180 days after the second dose, were significantly higher in groups over 35 years old after the second dose ($P<0.05$). Concluding remarks, antibodies levels raised much more after vaccinating patients who previously had positive qPCR than patients who did not have COVID-19. Second doses would be mandatory to all patients who did not have COVID-19.

187. (598) **ACTIVATION – INDUCED MARKER ASSAY, AN ALTERNATIVE STRATEGY TO STUDY *TRYPANOSOMA CRUZI* SPECIFIC CD8⁺ T CELLS**

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In the investigation of the immune response induced by vaccines or generated against infectious diseases, the determination of the specific CD8 response is of particular importance. Although there are various techniques for this purpose, such as the use of specific tetramers or ELISPOT, the activation – induced marker (AIM) technique emerges as a simpler experimental approach. In this work, we tested and optimized AIM to analyze the response of CD8⁺ T cells specific for *Trypanosoma cruzi* antigens, generated after infection of C57BL/6 mice with the Tulahuén strain of *T. cruzi*. Animals were infected intraperitoneally with 5000 *T. cruzi* trypomastigotes. At 12 days post infection, at the peak of the CD8⁺ T response, the animals were sacrificed and spleens and lymph nodes were harvested. Splenocytes and lymph node cells were incubated for 15 h with different concentrations of a particular peptide (TsKb20, derived from the Transialidase protein or PAR4, derived from the PAR4 protein of the flagellum, from *T. cruzi*). Cells incubated with Concanavalin A were used as a positive control, and non-restimulated cells were used as a negative control. The specific CD8 response was determined by flow cytometry evaluating the activation markers CD25⁺ and CD69⁺ in the population of CD8⁺ T cells. Through a two-way ANOVA anal-

ysis, it was found that the specific CD8+ T cell response for TsKb20 in infected animals, measured after restimulation with 50 ug/mL of the TsKb20 peptide, was significantly higher than the response measured in uninfected animals. No difference was evidenced in the specific CD8 response for PAR4. These results suggest that the AIM technique could be used to determine anti-*T. cruzi* specific CD8 response.

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188. (023) **MYCOBACTERIUM TUBERCULOSIS TRIGGERS THE GLYCOLYTIC PATHWAY IN DENDRITIC CELLS THROUGH TLR2 LIGATION**

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Tuberculosis remains a major global health problem. Its causative agent, Mycobacterium tuberculosis (Mtb), is a highly successful pathogen that interferes with dendritic cells (DC) functions impairing the onset and development of adaptive immunity. Since several studies are now revealing the importance of metabolic pathways involved in DC functions, we wondered whether Mtb could regulate the glycolytic activity of DC.

For this purpose, we generated monocyte-derived DC from healthy donors and stimulated them with equivalent doses of either irradiated Mtb (iMtb) or viable Mtb. Thereafter, we evaluated lactate release and glucose consumption by enzymatic assays, the expression of HIF1a, a transcription factor that promotes the glycolytic pathway, and lactate dehydrogenase A (LDHA), enzyme which catalyzes the conversion of lactate to pyruvate, by FACS and/or qPCR.

We found that DC stimulated with iMtb or infected with Mtb released lactate and consumed glucose at higher rates than untreated-DC ($P<0.05$). In line with it, either iMtb- or Mtb-treatment resulted in the upregulation of HIF1α expression at protein and mRNA levels ($P<0.05$), as well as the glycolytic enzyme LDHA ($P<0.05$). Additionally, we wondered whether the blockade of the main surface TLRs involved in Mtb recognition, TLR2 and TLR4, may result in an attenuation of the glycolytic pathway in iMtb-stimulated DC. We found that, unlike TLR4 ligation, TLR2 ligation was required to trigger the glycolytic pathway in iMtb-stimulated DC ($P<0.05$). In line with it, the treatment of DC with either synthetic (Pam3Cys) or mycobacterial (peptidoglycans) TLR-2 agonists induced both lactate release and glucose consumption without affecting cell viability ($P<0.05$). In sum, we demonstrated that both viable and irradiated-Mtb induce glycolysis in monocyte-derived DC through TLR2 ligation at least at early time of infection. We are currently addressing the functional impact of the glycolytic pathway on DC maturation.

189. (059) **EARLY TREG CELL DEPLETION DURING *TRYPANOSOMA CRUZI* INFECTION PROMOTES TCONV AND CD8+ T CELL IMMUNITY IN THE ACUTE PHASE**

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We reported that after *Trypanosoma cruzi* (Tc) infection, Tregs undergo a marked and sustained reduction in frequency. This natural contraction of the Treg response was critical to allow the emergence of protective anti-parasite CD8+T cell immunity in the acute phase. In line with this, we previously demonstrated that Treg depletion at day (d) 5 post-infection (pi) but not at d11pi impacted on the magnitude of anti-parasite CD8+T cell response and the ability to control parasite replication in the acute phase. Thus, we hypothesized that Tregs may exert a role during early events of T cell priming. In order to assess this, DEREG mice were infected with Tc and injected with diphtheria toxin (DT) or PBS at d5 and 6pi. However, DT treatment only induced modest effects on APCs shortly after the injection. Specially, CD86 expression was upregulated on splenic DCs, macrophages and NKT cells of DT-injected mice in contrast to controls ($p<0.05$), but no differences were observed in the expression of a range of innate immunity activation markers. In turn, we observed a significant increase in the numbers of Tconv cells of blood and liver at d11pi in DT-treated animals compared to control mice ($p<0.05$). This boost on Tconv cells in Treg-depleted animals was previous to the expansion of anti-parasite CD8+T cells observed at d20pi, suggesting a correlation between these two populations. Furthermore, Tconv cells from d11pi of DT-treated mice display an activated/effector phenotype, shown by the upregulation of CD44, PD-1 and CD25. At this time point after Treg cell depletion, CD8+T cells upregulate markers of early activation, however they show no changes in the expression of the proliferation marker Ki-67 nor in effector cell differentiation markers such us BATF, IRF4 and T-bet. Altogether, our results suggest that during Tc infection Tregs suppress CD8+T cell immunity at the acute phase through indirect mechanisms that involve the previous modulation of the Tconv cell response.

190. (089) **HISTONE H2A (W6UJM4) IS RECOGNIZED BY SERA FROM PATIENTS WITH CYSTIC ECHINOCOCCOSIS IN EGPE CELLS COLONIES SUPERNATANT**

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Cystic echinococcosis (CE) is a zoonotic disease produced by *Echinococcus granulosus* worldwide distributed. The discovery of new antigens would improve the CE diagnosis. EGPE is a cell line obtained from bovine *E granulosus* protoscoleces G1 in our laboratory (Echeverria et al, 2010). We have previously demonstrated that EGPE cells are a good source of antigens for CE diagnosis (Maglioco et al, 2019). The aim of this work was to identify the EGPE proteins recognized by CE patients' sera. Materials and Methods: EGPE cells were grown in agarose 2% (20000 cells / well) for 5 days. The supernatant of cell colonies was passed through G-protein affinity columns performed with sera from patients with 1) CE or 2) other parasitoses. Eluted proteins were concentrated (3K cut-off membrane concentrator), run in 15% SDS-PAGE, stained, and isolated for protein identification in CEQUIBIEM (FCEyN, UBA). Molecular modeling of the protein was performed using TrRosetta method from Robetta platform. Validation of the model was performed by molecular dynamics using NAMD 2.14. Epitope prediction was performed using IEDB (linear epitope prediction and Discotope 2.0) and ABCpred. All protocols were approved by the Ethics Committee of the "UAI". Results: The Histone H2A (W6UJM4), a protein of 189 amino acids (aa), was identified among other proteins only in eluates from CE column. The model was validated. In the 10-50 ns section of the trajectory, the averaged potential energy was -165510 +/- 190 kcal/mol. The protein has two regions with different dynamic behavior: the RMSD for the trajectory taking the averaged structure as reference was 2.76 +/- 0.65 Å for 1-160 aa region and 4.59 +/- 1.04 Å for 160-189 aa region. The linear and conformational epitopes were predicted in 27-42, 55-70, 92-107, 142-157 aa and 54-75, 100-104, 169-189 aa, respectively. Conclusions: The histone H2A is a candidate for serological detection of CE. Further studies are required to prove its diagnostic accuracy.

191. (098) **STUDY OF THE NUCLEAR RECEPTORS NR4A PARTICIPATION IN THE IMMUNE-ENDOCRINE RESPONSE DURING TUBERCULOSIS**

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Tuberculosis (TB) is a major infectious disease caused by *Mycobacterium tuberculosis* that infects alveolar macrophages and hence promotes a cellular immune response, which becomes harmful when prolonged over time. Nuclear receptors (NRs) are factors that may modulate the immune response (IR) and inflammation, with a role in the regulation of homeostasis and bacterial pathogenesis. Within the NRs, NR4As orphan receptors have emerged as important regulators of immune cell polarization and NF-κB signaling, which can lead to a switch from acute to chronic inflammatory responses. NR4A receptors modulate NF-κB activity in a dynamic manner, either repressing or enhancing target gene expression. In this sense, this work aimed to evaluate the RNAm expression of NR4A1 and 2, NFKB1 (Nuclear Factor Kappa B Subunit 1) and its inhibitors, NFKBIA and B, in Peripheral Blood Mononuclear Cells from TB patients, who were classified according to the severity of the disease into mild, moderate and severe. Besides, possible associations between them and other plasma mediators of the immune-endocrine response were also analyzed (IL-6, IL-10, IFNϒ, and DHEA). With regards to NR41 RNAm levels, they were diminished in TB patients with respect to Healthy Controls (HCo) ($p=0.03$), meanwhile, NR4A2 transcript levels in severe TB patients were higher than in HCo ($p=0.01$). On the other hand, NFKBIA and B transcripts were also augmented in TB patients ($p<0.05$, both of them). When analyzing correlations between them, NR4A1 was positively associated with NR4A2, NFKB1, and NFKBIB in HCo, and in moderate TB patients ($p<0.05$, all of them). NR4A2 was positively correlated with NF-κB ($p<0.01$) and NFKBIB ($p<0.01$). In turn, NR4A2 also showed a negative and a positive association with IFN-ϒ ($p<0.05$) and DHEA ($p<0.05$) plasma levels, respectively. These results suggest that NR4As would participate during the IR in TB to dampen the chronic inflammatory process.

192. (099) **DURING ACUTE PHASE OF *Trypanosoma cruzi* INFECTION, DISTURBED MITOPHAGY CONTRIBUTES TO DAMAGED MITOCHONDRIA ACCUMULATION IN EFFECTOR CD4 T CELLS LEADING TO APOPTOSIS**

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Chagas disease is characterized by inefficient host immune response during acute phase of infection, enabling the establishment of chronic disease. We have recently demonstrated that acute infection triggers mitochondrial ROS (mROS) production and mitochondrial alterations in effector CD4 T cells leading to functional alterations and apoptosis. The aim of our work was to evaluate the mechanism involved in the accumulation of damaged mitochondria, and if this could be prevented by the antioxidant N-acetyl cysteine (NAC) or the mitophagy inducer Nicotinamide Riboside (NR). To achieve this, CD4 T cells were isolated from spleen of non-infected (NI), acute (AP) and chronic phase (CP) infected BALB/c mice, with 500 trypomastigotes. Mitophagy was evaluated using mitochondrial potential independent probe (MTgreen) and antibodies for LC3 and LAMP1. Cells were cultured with or without chloroquine and colocalization was evaluated by confocal microscopy. CD4 T cells from AP cultured with chloroquine did not show significant increase in MTgreen and LAMP1 colocalization compared to CCCP-treated NI CD4 T cells used as positive control (*p<0,05) suggesting a defect in mitophagy. Then, we aimed to evaluate by flow cytometry, mROS production, frequency of cells with damaged mitochondria and apoptosis in effector CD4 T cells from AP infected mice treated with NAC, NR and vehicle as control. We did not found differences between NAC and control group. In contrast, NR treatment reduced the percentage of CD4 T cells with damaged mitochondria (*$p<0.05$), although we did not observed difference in mROS production. Moreover, apoptosis frequency was also diminished (**$p<0.01$). Depolarized mitochondria accumulation, probably due to a defect in mitophagy, could be restored by NR, and thus prevent apoptosis. Taken together, this evidence stablishes association between accumulated damaged mitochondria, and impaired mitophagy leading to apoptosis in CD4 T cells during acute *T. cruzi* infection.

193. (208) **ROLE OF B LYMPHOCYTES IN THE IMMUNE RESPONSE TO SHIGA TOXIN- PRODUCING ESCHERICHIA COLI INFECTION**

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We previously demonstrated a better outcome in weaned BALB/c mice (BALB) compared to C57BL/6 (C57) after Shiga toxin (Stx)-producing *E. coli* (STEC) infection. The main difference found was the early appearance of specific anti-STEC (aSTEC) and anti-Stx antibodies (aStx) in BALB. The aStx developed by infected BALB not only protected them against an intravenous (i.v.) Stx challenge, but also passive immunization with their sera protected C57 after STEC infection. The aim of this work is to determine if B cell-dependent response triggered after infection is necessary to guarantee the survival of infected BALB.
We administered a single i.v. dose of anti-B220 antibody (aB220; 4 mg/mouse) to BALB to deplete B cells. We analyzed the percentage of CD19 positive cells (%$CD19^+$) in mesenteric lymph node (MLN) and spleen at different times (4, 24 and 48 h) by flow cytometry. This treatment induced a significant B cell depletion in MLN at 4, 24 and 48 h vs controls (control vs B-depleted: 4, 24 and 48 h, $p<0.001$; ANOVA). %$CD19^+$ cells in spleen at 4, 24 and 48 h were significantly lower than controls; however, at 48 h the %$CD19^+$ cells in B-depleted mice started to increase (control vs B-depleted: 4, 24 and 48 h, $p<0.0001$; B-depleted at 48 h vs B-depleted at 4 and 24 h, $p<0.05$; ANOVA).
To study the role of specific B-dependent response, i.v. aB220 or PBS were administered to BALB 1 h before and 3 h post infection. To guarantee B-cell depletion, aB220 injection was repeated twice a day till the third day of infection intraperitoneally. B-depleted mice showed increased mortality rates ($p<0.05$, Log-Rank test), higher urea levels ($p<0.05$, t test) and a significant weight loss on day 3 p.i. ($p<0.0001$, ANOVA). Also, they didn't develop significant levels of aSTEC IgA at day 4 p.i., assayed as IgA coated bacteria by flow cytometry ($p<0.01$, t test).
We concluded that B cell stimulation and the consequent antibody response play a key role in protection against STEC infections.

194. (217) **CASE REPORT: VIRAL SHEDDING FOR 120 DAYS IN AN ALLERGIC CHILD WITH COVID 19**

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Since it was first detected in Dec 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread through the planet causing the novel coronavirus disease, Covid-19. Here, we report the case of a 9-year-old girl with persistent viral shedding based on RT-PCR detection. Her first positive (Ct=36) dated from March 4 2021, came in a preoperative physical examination (scheduled tonsillectomy), with no apparent symptoms. Her father fell ill and tested positive soon after that casual finding. Surgery was postponed. The patient came positive again on July 1 2021 (Ct=33) and on July 29 2021 (Ct=29). She had a persistent cough, which was also compatible with her allergic condition. Her surgery could not be postponed any longer and was operated on July 30 2021. Excised adenoids and tonsils were extensively rubbed with a swab to test whether the material detected resulted infectious or not, on Vero E6 cell cultures. Based on the absence of any cytopathic effect, we found it was not infective, even upon an intended amplification by a second passage. RT-PCR was negative when performed on the last supernatant. The histological pattern of her tonsillar and adenoid tissue was analyzed through H&E staining and immune cell populations were examined by FACS. Both aspects were compatible with her hyperplastic condition and also with a viral infection. We tested the anti-Spike specific response by ELISA on serum samples taken on Aug 6 2021 (IgM=1.34, cut off=0.584 and IgG=3.27, cut off=0.364). Finally, we determined the neutralizing antibodies titer on the same serum, using the wild type SARS-CoV-2 (titre=32, the mean of infected adults is 64). We concluded that, albeit the long period the genetic material of the virus was detected on her swabs, the patient does not seem to have a major immunological deficiency and could mount an appropriate immune response against the virus. Importantly, we demonstrated she was not able to transmit virus at the time of the surgery.

195. (244) **ADENOSINE REGULATES CYTOTOXIC CD4 T LYMPHOCYTES IN *TRYPANOSOMA CRUZI* INFECTION**

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Growing evidence demonstrates a critical role of purinergic system in the modulation of the immune response and to determine the outcome of infections. Damaged cells release the pro-inflammatory molecule ATP, which is metabolized by the ectonucleotidases CD39 and CD73 to the anti-inflammatory mediator adenosine (ADO).
The mechanisms involved in the induction of CD4 T cell population with cytotoxic effector functions (CD4 CTL) have not been evaluated in the setting of experimental *T. cruzi* infection. Here, we explored the effect of CD73 activity abrogation (CD73KO) on the differentiation and functional capacity of the CD4 CTL population in *T. cruzi* infected mice.
Through flow cytometry, we found that expression of multiple effector molecules (granzyme B, perforin, IFN-γ and TNFα) and the degranulation marker, CD107a, were increased in CD4 T spleen cells of CD73KO mice compared to WT mice at 14 days post-infection (dpi) ($p < 0.05$). Moreover, deficient CD4 cells exhibited higher cytotoxicity capacity evaluated by *in vitro* cytotoxicity model (% of dead cells WT vs KO: $p<0,05$).
Furthermore, the frequency of CD4 CTL and their multifunctional capacity decreased when CD73-deficient cells were cultivated in an ADO-enriched medium (CD73KO vs CD73KO+ADO: $p<0,05$). As expected, when WT cells were cultivated in an ATP-enriched medium, to mimic CD73-deficient environment, an increase in the multifunctional capacity of CD4 CTL was observed (WT vs. WT+ATP: $p<0.05$) reaching similar levels as was observed for these populations in CD73KO cells.
During acute (21dpi) and chronic (258 dpi) phase of the infection, the frequency of infiltrating CD4 CTL was higher in CD73KO cardiac tissue associated with diminished parasite burden (measured by real-time PCR), compared to the WT counterpart ($p<0.05$).
These data suggest that CD73 activity and ADO balance frankly influence the functionality of CD4 CTL during the immune response against *T. cruzi* infection.

196. (251) ***"*EVALUATION OF THE IMMUNE RESPONSE INDUCED BY TWO *STAPHYLOCOCCUS AUREUS STRAINS* WITH DIFFERENT GENOTYPE AND ADAPTABILITY TO THE BOVINE MAMMARY GLAND IN AN EXPERIMENTAL INFECTION*"***

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The aim of this study was to evaluate and compare the ability of two *S. aureus* strains with different adaptation genotypes (low and high) to the bovine mammary gland (MG) to induce immune response after an experimental intramammary infection (IMI). Three animals were challenged in two mammary quarters with *S. aureus* 806 strain (non-persistent NP) or 5011 strain (persistent P), and three animals were inoculated in two mammary quarters with saline solution (control group). Milk samples were collected at 0, 0.5, 1, 2, 3, 4, 7, 14 and 21 days post inoculation(pi) and bacteriological and quality milk examination, somatic cell count (SCC), nitrite, lactoferrin (Lf) and cytokines levels (IL-1β, IL-6 and IL-4) were evaluated. The NP strain (806) was able to induce mild clinical mastitis, triggering a rapid and effective inflammatory response to control IMI. The P strain (5011) was able to induce a subclinical IMI, triggering a less and later inflammatory response than the NP strain. A significant effect of infection was observed in SCC and nitrites concentration over time ($p<0.001$, $p=0.041$ respectively), finding differences between groups. Only in mammary quarters inoculated with P strain, nitrite levels increased at 21 days pi. For Lf levels, a significant effect of infection was observed over time ($p=0.004$), finding differences between the groups. Maximum Lf concentrations were detected in quarters inoculated with NP strain, where an increase was observed since day 3 pi. A significant effect of infection was observed in IL-1β, IL-6 and IL-4 levels over time ($p=0.001$, $p<0.001$ and $p<0.001$ respectively), finding differences between groups. The levels of the three cytokines were higher in quarters inoculated with NP strain compared to P strain. Results confirm previous *in vitro* studies suggesting that the phenotypic and genotypic characteristics of the different *S. aureus* strains and their ability to adapt to the MG determine the type of immune response induced in the host.

197. (255) **HIGH SALT CONCENTRATIONS POTENTIATES REVERSAL OF HIV-1 LATENCY THROUGH AN NF-κB-DEPENDENT MECHANISM**

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BACKGROUND. The HIV-1 reservoir comprises a pool of infected $CD4^+$ T cells in which the expression of the viral genome remains latent, constituting the main barrier to a sterilising cure. The 'shock & kill' strategy involves reactivating HIV-1 transcription in these cells to facilitate their elimination. Candidate Latency Reversal Agents (LRAs), however, have failed in clinical trials. There is, therefore,

an unmet need to develop new approaches aimed to reactivate viral transcription. Considering that high salt concentrations are a common finding in secondary lymphatic organs, here we study its effect on HIV-1 latency.
METHODS. In this study, we exploit a Jurkat cell line latently infected with an HIV-1 clone containing GFP in place of the viral gene *nef* (J-Lat). We expose J-Lat cells to control or hypernatremic media (+50 mM NaCl) for 4 h before treatment with LRAs. HIV-1 reactivation, monitored by GFP, is measured by flow cytometry after 24 h. To evaluate the role of p38 and NFAT5 we silence the expression of the proteins with shRNAs. To measure the activity of NF-κB we use a reporter cell line together with the degradation of IKBα by western blot.
RESULTS. First, we find that transient exposure to hypernatremic media sensitises J-Lat cells to HIV-1 latency reversal by two LRAs (PMA: 5,2 ± 2,4% vs. 17,7 ± 4,7% and TNF-α: 10,2 ± 2,6% vs. 30,7 ± 10,0%; control vs. hypernatremic media, n = 4, p < 0,001). Then, we confirm this effect is independent of the activity of MAPK p38 and the transcription factor NFAT5. Finally, we analyse the transcriptional activity of NF-κB and observe a significant (p<0.01) exacerbation of this pathway in cells exposed to hypernatremic media.
CONCLUSIONS. Here, we have shown that transient exposure to a high NaCl concentration, reflecting those encountered in different tissue microenvironments, sensitised $CD4^+$ T cells to LRA-induced viral reactivation. Understanding the mechanisms involved might lead to new therapies to counteract HIV-1 latency.

198. (262) **EVALUATION OF THE ROLE OF TISSUE REPAIR REGULATORY T CELLS DURING ACUTE *Trypanosoma cruzi* INFECTION**

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Tissue repair regulatory $Foxp3^+$ $CD4^+$ T cells (trTreg) are a specialized subset that exhibit tissue-specific phenotypic, functional and transcriptional profiles. trTreg maintain tissue homeostasis and also display conventional immunoregulatory properties. *T. cruzi* (Tc) triggers a strong effector response that controls parasite spreading but promotes pathological tissue damage. We previously showed that during the acute phase of Tc infection, there is a reduction in trTreg frequency and numbers in Spleen (Sp), Skeletal Muscle (SM) and other tissues that correlates with decreased systemic levels of their growth factor IL-33 and increased markers of tissue damage. We also found that trTreg, obtained from infected spleen, can be expanded *in-vitro* by IL-33.
In the current work we aimed to increase trTreg numbers in Sp and SM of acutely infected (INF) mice to evaluate their impact on disease progression. To this end, Foxp3-GFP C57BL/6 mice infected with 5000 Tc parasites (Tulahuen) were treated on days 12, 15 and 18 post infection (pi) with intraperitoneal or intramuscular injection of IL-33 or PBS. Sp and SM infiltrate was evaluated by flow cytometry at day 20 pi. Systemic IL33 treatment (Tx) induced a mild expansion of ($ST2^+KLRG\text{-}1^+$) trTreg in spleen but not in SM, producing no changes on parasite-specific CD8+ T cells or pro/anti-inflammatory macrophage numbers, total body or SM weights, % of survival, biochemical markers of tissue damage levels and parasitemia. Local IL33 Tx could not increase SM trTreg numbers and had no effects on any of the parameters mentioned above. Both Tx could, however, expand ILC2 in INF mice and trTreg in non-INF animals, indicating functional response to IL-33 in these settings.
Considering these data, we speculate that acute Tc infection may induce signals that could counteract IL33 effect on trTreg. Future studies will be aimed at identifying these signals in order to be able to modulate trTreg and, likely, tissue damage during infection.

199. (279) **THE DOWN-MODULATION OF IFN-GAMMA-INDUCED MHC-I EXPRESSION BY *BRUCELLA ABORTUS* CAN BE EXTRAPOLATED TO CELLS OTHER THAN MONOCYTES**

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Brucella abortus (*Ba*) is an intracellular pathogen capable of surviving inside macrophages. Since the disease is presented in multiple forms, many different cells are susceptible to be infected by *Ba*. This bug is able to evade the host immune system. We have previously demonstrated that infection of human macrophages with *Ba* diminishes the IFN-ϒ-induced MHC-I surface expression. One of the PAMPs that triggers this down-modulation is *Ba* RNA. MHC-I total expression is not altered, instead these proteins are retained within the Golgi Apparatus (GA). However, we acknowledged whether this event could be triggered in other cells able to be infected with *Ba*. So, we started by stimulating the lung epithelium cell line (Calu-6) and the endothelial microvasculature cell line (HMEC) with different doses of *Ba* RNA in the presence of IFN-ϒ. MHC-I expression was assessed by flow cytometry. *Ba* RNA (10 ug/ml) diminished the IFN-ϒ-induced MHC-I surface expression (p<0.05) in both cell lines. To start evaluating whether MHC-I molecules were retained in GA, we performed confocal microscopies of *Ba* RNA-treated Calu-6 cells in the presence of IFN-ϒ (for 48 h). We observed that there is colocalization of MHC-I and GA marker GM130, although to a lesser extent than human macrophages (p<0.05). Conversely to what we expected, supernatants from Calu-6-*Ba* RNA-treated cells had higher IL-8 production compared to those from untreated cells (p<0.05). IL-10 and TNF-α were not detected in supernatants from Calu-6-*Ba* RNA-treated cells when compared to supernatants from macrophages stimulated with *Ba* RNA (p<0.05). Our preliminary results also show that *Ba* infection of Calu-6 diminished the IFN-ϒ-induced MHC-I surface expression. Despite the need for more studies, together these results show that *Ba* could persist successfully within the host, remaining unnoticed and evading $CD8^+$ T cell surveillance.

200. (280) ***B. ABORTUS* RNA ACTIVATES MACROPHAGES TOWARDS A PRO-INFLAMMATORY PROFILE EARLY ON DURING INFECTION**

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Brucellosis is a zoonotic disease caused by *Brucella* spp bacteria. These pathogens can survive inside macrophages, persisting inside the host. We previously demonstrated that *Brucella abortus* (*Ba*) RNA is a PAMP involved in the immune evasion mediated by *Ba*. One of the mechanisms displayed by this bacterium is the down-modulation of MHC molecules when Th1 response is being held, *i.e.*, in the presence of IFN-ϒ. Nevertheless, we acknowledged whether *Ba* RNA could activate macrophages early on during the infection, before Th1 response is settled. To evaluate this, M0 (undifferentiated) macrophages were stimulated with *Ba* RNA (10 µg/ml) and at 24 and 48 h M1 (classical macrophages) or M2 (alternative macrophages) markers were assessed by flow cytometry. Regarding M1 markers, CD86 and MHC-II expressions did not change neither at 24 nor 48 h. Surprisingly, CD64 expression was reduced in *Ba* RNA treated macrophages (p<0.05). *Ba* RNA stimulates the secretion of pro-inflammatory cytokines (IL-8, TNF-α and IL-1β) only at 24 h (p<0.05). With respect to M2 markers, CD206 expression was reduced at 48 h in *Ba* RNA-treated macrophages (p<0.05) but DC-SIGN and CD163 expressions did not change compared to untreated cells. *Ba* RNA induced IL-10 secretion, mostly at 24 h (p<0.05). We also performed functionality assays of M1 macrophages. Nitrite Oxide production (assessed by Griess reaction) was stimulated in *Ba* RNA treated macrophages at 24 h (p<0.05). Moreover, glucose consumption and lactate production were also augmented by *Ba* RNA (p<0.05), all hallmarks of M1 profile. These results show that *Ba* RNA can activate macrophages into a pro-inflammatory profile -at least for a short time- early on during infection. These results also lay the ground for studying more deeply the modulatory properties of bacterial RNA in the context of brucellosis, other intracellular infections and tumors.

201. (298) **KLEBSIELLA PNEUMONIAE ST258 DIFFERENTIAL-**

LY MODULATES NEUTROPHIL FUNCTIONS

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Klebsiella pneumoniae carbapenemase (Kp)-producing bacteria are associated with significant mortality in immunocompromised patients. We have previously reported that neutrophils (PMN) failed to release neutrophil-extracellular-traps (NET) when challenged with Kp. Our aim was to deepen the study of evasive mechanisms of PMN responses, mediated by Kp. For this purpose, we determined NETosis on purified PMN in response to a Kp MOI 10 and/or PMA 50nM, a potent NET inducer. NET release was quantified after 3 h using confocal microscopy after DNA and Elastase staining. Also, NET-associated DNA released by nuclease treatment was measured in the supernatants.
We found that Kp was able to decrease NETosis induced by PMA (NETs area, µm2: Ctrl=944±326, Kp=1250±605, PMA=78781±21065*; PMA+Kp= 34513±13420# ; Released DNA, ng/mL:Ctrl=137,9±19,4, Kp=155,7±12,7, PMA=448,9±85,2*; PMA+Kp=299,8±54,6# ;
p<0.05 vs. Ctrl and Kp, # p<0.05 vs. PMA; n=6). Since NETosis is associated with an increase in lactate production, we determined lactate release in response to Kp and PMA after 4,5 h, using a commercial kit. PMA induced lactate release while Kp did not (Lactate release, mg/dl: Ctrl=6,1±1,1, Kp alone=not detectable, PMN+Kp=7,5±1,3 ; PMA=17,9±4,5;
*p<0.05 vs. Ctrl and Kp; n=6).
Moreover, we study the release of IL-1β and IL-8, two key inflammatory cytokines induced by different activation pathways, using commercial ELISA kits. PMN were incubated 4 h with MOI 10 of Kp or E.coli (Eco), as positive control, and supernatants were collected for IL-1β and IL-8 measurement. Kp was able to induce IL-8 release, but did not produce IL-1β release. (IL-1β, pg/ml: Ctrl=32,4±5,4, Kp=80,9±48,3, Eco=1270,2±106,7*; IL-8, pg/ml: Ctrl=110,9±86,1, Kp=5278,1±218,8*, Eco=6278±389*, *p<0.05 vs. Ctrl; n=3).
In summary, Kp inhibits NETosis induced by PMA, which could be related to a poor lactate production. Our results indicate that Kp is able to subvert some important PMN responses, but not all.

202. (302) **ROLE OF cGAS-STING AXIS IN IMMUNE RESPONSE AGAINST RESPIRATORY *BRUCELLA ABORTUS* INFECTION**

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The cGAS-STING axis is activated upon microbial DNA recognition in the cytosol to initiate an innate immune response, including proinflammatory cytokines and type I interferons. We evaluated the role of these receptors in the response to respiratory infection by *Brucella abortus*.
In vitro infections on samples from knock-out (KO) and wild type (WT) mice revealed that STING, but not cGAS, restricts intracellular growth of *B. abortus* in murine lung explants (LE) and alveolar macrophages (AM) at 24h (p<0.05) and 48h (p<0.005) post-infection (p.i.). Moreover, STING KO AM secreted lower levels of TNF-α (p<0.05), IL-1β (p<0.005) and IL-6 (p<0.0005) at 48h p.i. These three cytokines were also reduced in STING KO LE (p<0.0005), as was also IP-10 (p<0.05).
Brucella usually disseminates systemically after airborne infection. After intratracheal infection with *B. abortus,* STING KO mice displayed a significantly higher bacterial burden in lungs (p<0.0005), liver (p<0.05), and spleen (p<0.005) at 7 p.i. compared with WT animals (similar differences for 14 d p.i.). STING KO AM obtained *ex vivo* after infection evidenced less protein expression of p-NF-κB and caspase-1 by Western Blot. In concordance with these results, we detected lower levels of TNF-α (p<0.05), IL-1β (p<0.05), IL-6 (p<0.005) and IP-10 (p<0.05) in bronchioalveolar lavage fluid of STING KO mice at 7 d p.i. In STING KO lung homogenates, we found lower levels of TNF-α (p<0.0005), IL-1β (p<0.005), IL-6 (p<0.005), IP-10 (p<0.0005), and lower IFN-β expression (p<0.05). Furthermore, we detected lower concentrations of IL-1β (p<0.0005), IL-6 (p<0.05), and IP-10 (p<0.005), and lower IFN-β expression (p<0.05) in spleen homogenates of STING KO mice.
In conclusion, our results demonstrate that STING plays an essential role in the local and systemic immune response to respiratory *B. abortus* infection, both by reducing the intracellular growth of the pathogen and enhancing the cytokine response.

203. (327) **SEXUAL DIMORPHISM OF CELLULAR AND HUMORAL IMMUNITY IN EXPERIMENTAL VACCINES AGAINST TRYPANOSOMA CRUZI**

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There is evidence that vaccine efficacy is affected by diverse parameters like sex between other. Thus, the inclusion of both sexes in studies of immune function becomes increasingly imperative. Usually, vaccine research against *T. cruzi* (Tc) has been focused on antibody response evaluation. Thus, we aim to evaluate possible variations in humoral and cellular response induced after experimental vaccine administration against Tc between BALB/c males (M) and females (F). Thus, mice (n=5/group) were immunized intranasally [3 doses, one every 2 weeks with 10μg of TS emulsified in ISPA (I) or c-di-AMP (A) adjuvants]. Controls were treated with saline (S) or TS alone. Fifteen days after the last immunization, in vivo cell-mediated (delayed hypersensitivity test -DHT-) and specific humoral (ELISA) responses were assayed. Later, mice were orally challenged with 3000 Tc (sub-lethal challenge). Parasitemia and clinical score were evaluated until day 100 post-infection (pi). In both M and F, the DHT was higher in TS+I and TS+A groups compared to their respective controls (p<0.05 in all cases). Nevertheless, DHT was always enhanced in F than M (p<0.05 in all cases). TS-specific IgG_{2a} and IgG_1 levels observed in TS+I or TS+A immunized F were increased compared to S or treated with TS alone (p<0.05 in both cases). In comparison, M showed no differences in both subclasses of antibody levels between all groups, and in addition, their levels were minor than F (i.e., IgG_{2a} p<0.05). After oral infection, M showed more enhanced parasitemias than F, being significantly different between TS+I and TS+A (i.e., 17 days pi, F vs M p<0.05 in both cases). Moreover, circulating parasites disappear one week later in M than F. Clinical affectation was also less evident in F than M (clinical score, p<0.05). We can conclude that there are substantial differences in the immunogenic and protective effects of TS-based vaccine formulations between M and F.

204. (357) **MUCOSAL VACCINE BASED IN A FRAGMENT OF RECOMBINANT TRANS-SILIDASE PROTECTS AGAINST EXPERIMENTAL ORAL CHAGAS DISEASE**

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Oral Chagas disease is a frequent form of infection in some countries of Latin America. Although there are drugs for its treatment,

currently there are no prophylactic vaccines to combat the disease. Here, we evaluated the immunogenicity and prophylactic efficacy against oral infection generated by a N-terminal recombinant fragment of Trans-sialidase (TS_{Nt}), containing different B and T epitopes confirmed by bioinformatics. For mucosal immunization, 10μg of TS_{Nt} was combined with different adjuvants: c-di-AMP(A) or ISPA(I). Thus, female BALB/c mice (n=6/group) were immunized intranasally (3 doses, one every 2 weeks). As control groups we used mice not immunized (NI) or only treated with TS_{Nt}. To evaluate the immunogenicity, 15 days after the last immunization, we performed an *in vivo* cell-mediated test (delayed hypersensitivity test, DHT), splenic multifunctional T cell (IFNγ+/RORγt+) detection by flow cytometry after *in vitro* stimulation with TS_{Nt}, and specific humoral response by ELISA. Next, we evaluated prophylactic efficacy during acute phase. Thus, animals were orally challenged with 3000 Tulahuen strain/mice (sub-lethal challenge). Parasitemia, clinical affectation (score), muscle and liver damage (plasma CK, GOT, GPT) was also assessed. In terms of immunogenicity, TS_{Nt}+A and TS_{Nt}+I vaccines developed an enhanced DHT until 72 h, compared to control groups (in all cases, $p<0.05$). Moreover, the same groups showed enhanced levels of IgG_{2a} and IgG_1 (in all cases, $p<0.05$). Multifunctional CD4+T cells able to secrete both IFNγ and IL-17 were enhanced in TS_{Nt}+A splenocytes ($p<0.05$ vs. NI). Clinical affectation was less evident in TS_{Nt}+A and TS_{Nt}+I groups, while parasitemia and muscle and hepatic damage were fewer in TS_{Nt}+A animals (in all cases, $p<0.05$). Taken together, these results suggest that TS_{Nt}+A formulation may be a good vaccine candidate for the development of a prophylactic mucosal vaccine against oral *T. cruzi* infection.

205. (371) **METFORMIN TREATMENT MODULATES MACROPHAGE RESPONSE AGAINST *T. CRUZI* INFECTION**

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In acute phase of *T. cruzi* infection, both innate and adaptive immunity are necessary to control parasite replication. Macrophage (Mf) and T cells orchestrate the inflammatory response that controls parasite burden. However, an exacerbated immune response results in tissue damage, mainly by ROS and RNS release. Metformin (Mf), a type 2 diabetes drug, reduces inflammation in models of aging and pollution. In our *in vivo* model of *T. cruzi* infection in Balb/c mice, we observed that peritoneal and spleen Mf increase iNOS expression during acute phase. Pretreatment of BMDM with Mf prevents intracellular parasite replication and promotes proinflammatory cytokine production. We also infected RAW cells and then were treated with PBS or Mf 1mM. This treatment decreased ROS production ($p<0.05$). Peritoneal cells (PC) of infected mice treated 48 h with Mf reduce ROS production and iNOS expression assesed by flow citometry ($p<0.05$). To determine the effect of Mf in *T. cruzi* infection, we infected i.p. mice with 500 trypomastigotes (tp) and then were treated with PBS or 100 mg/kg of Mf daily by gavage. At 18 d.p.i. we obtained blood samples, spleen, inguinal lymph nodes (LN) and PC, including control mice groups. Parasitaemia were assessed in both groups of infected mice showing less tp/mL in Mf treated mice ($p<0.05$). We found that both peritoneal infected Mf subsets, LPM and SPM increase mROS production ($p<0.001$) but Mf has no effect neither cROS/mROS production nor iNOS expression. Spleen Mf showed more iNOS+ cells in response to infection and Mf exhibited a slight revert. In LN, CD169+ Mf capture and prevent pathogens spread and initiate immune response driving B cell activation. We found a decrease in CD169+ Mf in infected mice that Mf could not restore. Surprisingly, these remaining cells showed more percentage of iNOS+ Mf ($p<0.05$). These results suggest that Mf could be a promising anti-inflammatory molecule to control tissue damage and modulate immune response to *T. cruzi*.

206. (376) **STUDY OF THE PHENOTYPE OF PERIPHERAL T, B AND NK CELLS AND SYSTEMIC PROFILE OF SOLUBLE IMMUNE MEDIATORS IN HIV$^+$ PEOPLE WHO HAVE UNDERGONE SARS COV-2 INFECTION**

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The immune response to SARS CoV-2 in people living with HIV (PLWH) has not yet been fully elucidated. Our aim was to investigate the impact of HIV infection on cell populations and cytokines/chemokines involved in the SARS CoV-2 immune response. We investigated by flow cytometry the phenotype of circulating T, B, NK cells and monocytes, as well as the plasma concentration of cytokines and chemokines in both 29 PLWH on HAART and 33 HIV-negative (HIV^{neg}) persons. Subjects were studied during convalescence of SARS-CoV-2 infection. Data were analyzed using non-parametric tests and considered statistically significant when the p values were <0.05.

We observed a decrease in antibody-secreting cells ($p>0.05$) among PLWH compared to HIV^{neg}, despite similar proportions (%) of B cells between the groups. Both groups presented similar % of Th1, Th17, Th1/Th17 and Tregs. Likewise, PLWH showed an increased % of total ($p<0.05$) and CXCR3+ Tfh cells ($p<0.05$) with respect to HIV^{neg}. Furthermore, we found a negative correlation between anti-SARS-CoV-2 IgG titers and % Tfh in the PLWH group (R=-0,654; p=0,001; Spearman r), which suggests a Tfh cell dysfunction during the immune response against SARS CoV-2 in PLWH. We detected an increment in the % of HLA-DR$^+$CD8$^+$ T cells in PLWH ($p<0.05$), with no differences in the memory/effector or exhaustion profile between groups. We observed similar proportions of NK cells in both groups, with an expanded % of CD95, HLA-DR and HLA-DR/CD38 cells in PLWH ($p<0.05$), indicating a higher activation of NK cells. We did not find significant differences in monocyte subsets between groups. PLWH depicted decreased levels of IL-8 and increased levels of IP-10, with no differences in MCP-1, CCL2, MIG or RANTES between groups. Furthermore, we noted statistically significant decreased levels of plasma IFN-γ, TNF-α, IL-17A, IL-6, and IL-10 in PLWH compared to HIV^{neg} ($p<0.01$). These data contribute to the understanding of the impact of HIV on SARS-CoV-2 infection.

207. (386) **STAPHYLOCOCCUS AUREUS PROTEIN A AS A PROPHYLACTIC TARGET DURING CHRONIC OSTEOMYELITIS**

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Osteomyelitis caused by *Staphylococcus aureus* is an important and

current healthcare problem worldwide. The treatment of this disease fails due to the high incidence of antimicrobial resistance
and the ability of the bacteria to form biofilms and to persist in the bone for decades. We have demonstrated that staphylococcal protein A (SpA) induces inflammation, exacerbated osteoclastogenesis and increased bone matrix degradation during osteomyelitis. The aim of this study was to evaluate the potential of anti-SpA antibodies as an adjunctive strategy to control bone damage.
Groups of BALB/c mice were immunized via intraperitoneal route with anti-protein A antibody (75 mg/kg) or rabbit IgG as placebo one day before the challenge with *S. aureus* FPR3757 (1-2x10^6 CFU) and at days 6 and 10 thereafter. Data were analyzed using Student's *t* Test or one-way ANOVA and Bonferroni's multiple comparison test. *Ex vivo* assays using bone marrow cells obtained 48 hours post-challenge showed that immunization against SpA reduced priming of osteoclast precursors (p<0.01). Fourteen days post-infection TNF-α and IL-6 levels were significantly decreased in bone of immunized mice compared with the placebo group (p<0.01). Bone histomorphometric analysis and qRT-PCR determinations revealed that the number of osteoclasts per bone perimeter (p<0.05), the osteoclast surface per bone surface (p<0.05) and cathepsin K expression (p<0.05) were significantly reduced in the immunized group. Moreover, the amount of trabecular bone in mice immunized was comparable to the control group inoculated with PBS (non-significant) whereas a significant loss in trabecular bone was observed during infection in the non-immunized group (p<0.01). These results demonstrate the feasibility of using anti-SpA antibodies *in vivo* to block the inflammatory response and bone damage induced by *S. aureus* during osteomyelitis.

208. (387) **EVALUATION OF DIFFERENT IMMUNO-ENDOCRINE FACTORS INFLUENCING MIGRATORY ACTIVITY OF T CELLS IN INDIVIDUALS WITH CHRONIC CHAGASIC MYOCARDITIS**

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T cell-inflammatory infiltrates are evident in the heart of individuals with chronic chagasic myocarditis (CCM). T cell trafficking into the hearts of CCM patients may be modulated by systemic inflammation and by *in situ* expression of chemotactic or haptotactic factors. In addition, systemic glucocorticoids (GC) might modulate T cell activation and migratory response. Our objective was to evaluate immuno-endocrine factors that can differentially influence the migratory capacity of T cells in individuals with CCM. Thus, we evaluated in the heart tissue from CCM and seronegative (Co) individuals subject to cardiac transplant (n=3/group), inflammatory infiltrates and fibrosis (by H&E and trichome stain) and the expression of chemotactic/haptotactic factors as fibronectin (FN), CXCL12 and TNFα (by immunofluorescence). In addition, we determined in PBMCs from CCM and Co (n=20/group) the expression of HLADR as an activation marker and VLA4 (FN receptor) by cytometry and TNFR1/2 by RTqPCR. Moreover, GC sensibility was estimated by GC receptors (GRα as functional receptor and GRβ as inhibitor receptor) and 11β-HSD1 (catalyzes the conversion of GC from inactive to active form) expression by RTqPCR. CCM tissue sections exhibited obvious infiltrates and fibrosis and an increased immunoreactivity for FN, CXCL12 and TNFα (p<0,05 vs Co). HLADR and VLA4 expression was enhanced on T lymphocytes from CCM patients (p<0,05 vs Co). In CCM PBMCs, the expression of RGα do not differ from the Co group. In our samples, RGβ was not detectable, while the mRNA of the enzyme 11β-HSD was found to be increased in the CCM group (p<0.05 vs Co). Conclusion: The increased expression of chemotactic and haptotactic factors in the hearth of CCM patients could promote the recruitment and accumulation of activated T cells, driven in part, by enhanced expression of the FN receptor VLA4. The proinflammatory phenotype seen in these patients not seems to be linked to GC sensibility in immunocompetent cells.

209. (407) **COVID-19 PANDEMIC. LABORATORIO DE MEDICINA GENÓMICA DE LA FACULTAD DE MEDICINA DE LA UNNE EXPERIENCE IN THE DETECTION OF INFECTED AND/OR VACCINATED PATIENTS**

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INTRODUCTION: In December 2019, the World Health Organization was informed of an ongoing global pandemic coronavirus disease 2019 (COVID-19) due to a novel coronavirus, SARS-COV-2. COVID-19 represents an enormous society burden, affecting the economy and sanitary systems all over the world. A timely and accurate diagnosis are essential for disease propagation control.
OBJECTIVE: The main object of the present work is to compare the immunological response of oncologic patients who has overcome the disease or who has been vaccinated, independently of dose or type of vaccine received.
MATERIALS AND METHODS: 211 patients were study. Comorbidity, type and doses of vaccines, previous COVID-19 infections, symptoms and other concerns where assess in order to compare results. Of total patients' samples, 23 oncological ones were selected. Immune response and correlation to type and dose vaccine were analyzed considering oncological treatments. COVIDAR-IgG y COVID-19 Spike 1 & 2 IgG where used for immunoglobin assays.
RESULTS: 23 (10,9%) oncological patient samples were analyzed. One (4,34%) patient has no detectable results. 2 patients (8,69%) had low antibodies values. All the rest, had normal detectable values. No significant differences were observed according to either type or dose vaccination and medication.
DISCUSSION AND CONCLUSION: One of the most important control of COVID-19 disease is the detection of antibodies in the population. It is possible that idiosyncratic reactions occur during vaccination, specially among risk populations. This is important in order to enhance public health system in every community. It is important to continue with epidemiological studies in order to obtain more accurate and significant results of these reactions variations.

210. (417) **DEVELOPMENT AND CHARACTERIZATION OF NEW VACCINES EXPRESSING THE RV2626C PROTEIN TO PREVENT LATENT *MYCOBACTERIUM TUBERCULOSIS* INFECTION.**

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Tuberculosis (TB) has not been eliminated from any country until now. Moreover, almost 2 billion persons are latently infected with *Mycobacterium tuberculosis* (*Mtb*) and at risk of disease reactivation. Thus, new effective vaccines that might prevent TB infection are required. Hence, multi-state vaccines including early secretory antigens plus latency associated antigens from *Mtb* were proposed. Here, Rv2626c latency antigen was evaluated as a new candidate vaccine by combining a recombinant Modified Vaccinia virus Ankara (MVA) expressing the Rv2626c (MVA2626c) with Rv2626c DNA (DNA vaccine -pCI). Accordingly, Rv2626c DNA was amplified by PCR and ligated into a pCR-TOPO vector. Then, Rv2626c gene was sub-cloned into pCI vector for DNA vaccine (pCI-Rv2626c) or into a transference vector VT (VT-Rv2626c). VT also contains the expression cassette for the β-glucuronidasa enzyme and the flanking sequences of the MVA086R gene (which codifies for the thymidine kinase enzyme). Moreover, VT-Rv2626c was then transfected into CEFs previously infected with *wt*MVA and recombinant viral clones were subsequently isolated. Then, to evaluate the immunogenicity of these preparations, Balb/c mice were immunized with pCI-Rv2626c/pCI-IL12 (two doses every 14 days). After two weeks, a boost of

MVA2626c was administered. Eight days later, splenocytes were obtained and *in vitro* stimulated with Rv2626c protein. After three days, IFN-γ production and plasma IgG levels were determined by ELISA. Our findings showed that pCI-Rv2626c /MVA2626c induced specific IgG and IFN-γ responses against Rv2626c as compared to non-immunized animals. Furthermore, we observed a significantly increase in IFN-γ ($p<0.05$) and IgG levels against Rv2626c ($p<0.001$) when pCI-IL12 was used as an adjuvant. Therefore, in the present work, we successfully developed a new tool that might be employed in the control of latent *Mtb* infection.

(420) **DEVELOPMENT OF AN ALTERNATIVE METHOD TO DETERMINE THE PRESENCE OF ANTIBODIES AGAINST SARS-COV-2 ANTIGENS IN PATIENT SERA AND TO ANALYZE THE CHARACTERISTICS OF THE HUMORAL IMMUNE RESPONSE**

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Coronavirus disease (COVID-19) caused by SARS-CoV-2 has emerged as a global pandemic. The immunological response has shown to be extremely complex and the severity of the cases requires a quick diagnostic. Humoral parameters are especially important to analyze the harshness of the disease and to distinguish between infected and vaccinated patients. However, the human seroprevalence of antibodies against SARS-CoV-2 has shown a variable response among the population.

Thus, this work aims to design surface plasmon resonance (SPR) assays to determine the presence of antibodies anti-SARS-CoV-2 and their kinetic characteristics in patient sera. In addition, we evaluated the presence of pro-inflammatory cytokines in early infection. Sera were obtained from infected patients from Hospital Álvarez confirmed by specific PCR and evaluated by commercial tests. For the antibody analysis, assays using full-length r-Spike protein (20/20), r-RBD domain (19/20), and r-Nucleoprotein (15/20) immobilized into a CM5 chip were performed. ELISAs and WBs were used to corroborate the results. SPR assays with r-RBD showed that 10% of the sera from infected patients display a distinct behavior, expressing kinetic association rates two times higher than control samples (10^4 vs $10^5 M^{-1}.s^{-1}$, $p<0.05$). Also, when analyzing in vitro its ability to neutralize the infection capability with a pseudovirus particle, we found it was eight times higher than control sera ($p<0.05$). Also, TNF-α in positive sera showed higher levels than sera from not infected in half of the patients ($p<0.5$) analyzed by SPR using a TNF receptor capture on a CM5 chip.

In conclusion, we successfully developed an alternative method to determine the seroprevalence of antibodies against three antigens of SARS-COV-2 in real-time. In addition, it allows us to analyze the kinetic parameters of the antibodies and the humoral response, which would correlate with the neutralizing capacity of the sera.

211. (451) **THERAPEUTIC POTENTIAL OF 16α-BROMOEPIANDROSTERONE AS ADJUVANT FOR THE TREATMENT OF TUBERCULOSIS INFECTION**

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Purpose: Despite being preventable and curable, tuberculosis (TB) is one of the principal causes of death worldwide. Macrophages play a key role in controlling *Mycobacterium tuberculosis* (*Mtb*) infection. In search of an adjuvant for TB treatment, we aimed to study a synthetic derivate from DHEA, 16α-Br-epiandrosterone (HE2000) as a modulator of bacterial growth and *Mtb*-induced immune response in human macrophages.

Methods: Minimal inhibitory concentration (MIC) test was performed by growing *Mtb* H37Rv in the presence of HE2000. Bacterial intracellular growth was evaluated by incubating *Mtb* infected THP-1 macrophages at different time points in the presence of HE2000. After 21 days of culture, colony-forming units (CFU) were quantified. Phagocytic activity was assessed by treating infected macrophages with HE2000 and stained by the Ziehl Neelsen technique. Nonparametric tests were used and a $p<0.05$ was considered significant.

Results: MIC assay showed different concentrations at which HE2000 inhibited bacterial proliferation, finding significant differences with control at 0,5 µg/ml ($p<0.001$), 2 µg/ml ($p<0.05$) and 16 µg/ml ($p<0.01$). Also, the concentrations under study were effective in enhancing the phagocytic capacity of infected macrophages, which was evident within 1 hour post-infection ($p<0.001$). These observations were confirmed by Ziehl Neelsen staining, showing a significantly higher number of bacteria per cell in HE2000-treated macrophages ($p<0,01$). Finally, cells exposed to HE2000 showed significantly enhanced bacterial killing after 4 days of culture compared to untreated cells ($p<0.01$).

Conclusion: This study suggests that HE2000 enhances the phagocytic and microbicidal activities of *Mtb* infected macrophages. Therefore, this compound may be considered as adjuvant therapy for tuberculosis infection.

212. (474) **NATURAL ANTIBODY RESPONSE AGAINST SARS-COV-2 INFECTION IS A MATTER OF TIME AND AGE**

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OBJECTIVES: To analyze antibodies levels against SARS-CoV-2 along time in natural infection in healthcare workers. Patients' serum samples (n=174) were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 months after positive qPCR results. Most patients presented mild to moderate symptoms and had an age range between 25 and 80 years. To perform the analysis of the antibody levels we used proteins Spike and RBD in a single enzyme-linked immunosorbent assay (ELISA) plate (COVIDAR IgG). Results are expressed as median (min-max). ANOVA was performed in order to statistical evaluation. RESULTS: Antibodies levels increased over time, being that the highest levels were obtained at 10 months. In patients over 55 years old, the antibodies levels were higher than in younger groups (<45 yo) if we consider the total of the samples throughout all the months 8.0 (0.9-14.3) vs 4.3 (0.1-15.8); $p<0.01$. However, if we compare the different age groups at 3 and 6 months after positive qPCR results, no significant differences were detected. Furthermore, no significant differences concerning gender were observed. Concluding remarks, natural infection antibodies levels longlast at least over ten month after positive qPCR and differences between distribution of age were observed. These results contribute to the knowledge of the humoral specific antibody response in unvaccinated patients who had a positive result for SARS-CoV-2 qPCR.

213. (475) **CHRONIC ADMINISTRATION OF THE ANTIDEPRESSANT FLUOXETINE IMPACT ON *YERSINIA. ENTEROCOLITICA* ORAL INFECTION AND REACTIVE ARTHRITIS DEVELOPMENT IN TNFR1 DEFICIENT MICE**

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Fluoxetine (FLX) is a selective serotonin reuptake inhibitor (SSRIs) with antidepressant and immunomodulatory effects. Whether FLX treatment impacts gastrointestinal bacterial infections and their sequelae, such as Reactive arthritis (ReA), remains unknown. We investigated the FLX effect on *Yersinia enterocolitica* (Ye) O:3 infection and ReA in a TNFR1 knockout mouse model. Differences in male and female mice were also evaluated. Male and female TNFR1 KO mice were orally infected with Ye O:3 (1-5x10^8 colony-forming units). From infection day, FLX (20 mg/kg/day) or water (control) was administrated in drinking water. On day 5, the number CFU was determined in stool, spleen, and mesenteric lymphoid nodes. The weight, mobility, mortality, and arthritis score of the mice were recorded. On day 21, splenic dendritic cells (DCs) infiltration and their maturation markers were evaluated by flow cytometry in surviving mice. We found that male TNFR1 KO mice have lower survival and higher clinical score after Ye infection. On day 5, FLX treatment increased bacterial dissemination in males. Surviving mice developed ReA but females treated with FLX showed greater severity than controls. Furthermore, FLX mice showed a lower proportion of splenic DCs without changing in CD86 expression. We conclude that TNFR1 KO male mice are more susceptible than females to Ye infection. The modulatory effect of FLX hinders more the immune response of males increasing systemic bacterial spread. Finally, the chronic administration of FLX did not reduce the severity of ReA and, in contrast, increased it in females. Although DCs infiltration in the spleen was reduced, the expression CD86 marker did not change, so we infer that the increased arthritis severity could be related to defective DCs migration. The results contribute to understanding how antidepressant chronic treatment influences the immune responses against pathogens and the maintenance of immune homeostasis.

214. (481) **STUDY OF GENES REGULATED BY THE GLUCOCORTICOID RECEPTOR IN PERIPHERAL BLOOD AND PLEURAL FLUID MONONUCLEAR CELLS FROM PATIENTS WITH PLEURAL TUBERCULOSIS**

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One of the commonest extrapulmonary manifestations of tuberculosis (TB) is pleural TB (PLTB). Since the cellular immune response (IR) is essential for the containment and resolution of the infectious process, PLTB constitutes a model to study the protective IR at the site of infection, and the ensuing endocrine response. In previous studies, we have shown that patients with PLTB have a greater inflammatory and cellular response at the pleural compartment (increase in IL-1β, IL-6 and IFN-γ concentrations) respect to the systemic level. Furthermore, mononuclear cells (MC) from pleural exudates (PEMC) had an *in vitro* high specific proliferative capacity compared to peripheral counterparts (PBMC), with cortisol levels being only increased at the peripheral compartment. To expand this issue, PBMC and PEMC from PLTB patients (n=12) were analyzed for the expression levels of genes (RT-qPCR) that are positively (ANXA1, GILZ, FKBP5, NFKBIA and NFKBIB) or negatively (IL-1β, IL-6, IFN-γ) regulated by glucocorticoid receptor (GR), in addition to the NFkB subunit 1 gene (NFkB1) and the eventual relationship with the circulating immuno-endocrine profile. While showing an increase in the expression levels of ANXA1, GILZ, NFKBIA in PBMC with respect to PEMC ($p<0.02$), there were no between-group statistical differences in mRNA amounts for FKBP5, NFkB1 and NFKBIB. In parallel, an increase in IL-6, IFN-γ mRNA levels together with decreased IL1β transcripts were found in PEMC respect to PBMC ($p<0.05$). It follows that PBMC from patients with PLTB present a marked expression profile of GR-regulated anti-inflammatory genes in relation to cells located in the pleural compartment, although transcripts for some proinflammatory cytokines continue to be increased. Present results are in line with our former findings about the immuno-endocrine profile seen in the pleural and peripheral compartments as well as the antigen-induced lymphoproliferation.

215. (490) **DYNAMIC OF FOLLICULAR CYTOTOXIC CD8+T CELL RESPONSE IN TRYPANOSOMA CRUZI INFECTION**

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Germinal Centers (GCs) are specialized structures generated within B cell follicles (FO) in response to T cell-dependent antigens. Follicular helper T cells (Tfh) are crucial for GC formation and antibody-affinity maturation. Other GCs-protagonists are follicular cytotoxic CD8+T cells (Tfc), who share gene signatures with Tfh cells and, in some models, contribute to eliminate infected cells inside the FO.

Our aim was to study protagonists of GC response in *T. cruzi* infection and to characterize Tfc cells. C57BL/6 mice were intraperitoneally injected with 5.000 trypomastigotes of *T. cruzi* (Tulahuén strain) or PBS (control group). Tfc, GC B cells, and plasmablasts (PB) were evaluated by FACS and immunofluorescence (IF) staining at different days post infection (dpi) in the spleen.

The peaks of the Tfc (CD8+CXCR5+PD-1+) and PB ($B220^{low}$-CD138+) response were at 18dpi and decreased at 23 dpi. These populations preceded GC-B cell(B220+FAS+GL-7+) response which peaked at 28 dpi. About 80% of Tfc were CD44+$CD62\text{-}L^{low}$ and had a higher expression of Bcl-6, TCF-1, Lag-3, CD40-L and CXCR3 than non-Tfc CD8+T cells ($p<0,05$). Near 15% of Tfc and 6% of non-Tfc, were specific for the immunodominant *T. cruzi* TSKB20 peptide. After *in vitro* stimulation with PMA/Io or TSKB20, Tfc expressed high levels of CD107a+,IFN-y, TNF-a, Granzyme B and Perforin. By IF we detected CD8+T cells inside of FO at 18 dpi; while at 23dpi and 28dpi all CD8+ T cells were outside. Additionally, we observed that IL-15 and IL-21 increased Tfc survival *in vitro*.

To sum up, we observed an activated, effector and temporal CD8+T cell subset whose response was prior to GC, expressed Tfh-related molecules and were in contact with B cells. Co-culture experiments are needed to dilucidate the relationship between Tfc and B cell in *T. cruzi* infection. Tfc could influence antibody response by interacting with PB or GC; or could control the infection since parasite-infected cells were observed inside B cell FO.

216. (499) **KUNITZ-TYPE MOLECULE IN A LIQUID CRYSTAL NANOSTRUCTURE AS VACCINE PLATFORM AGAINST *FASCIOLA HEPATICA* INFECTION IN SHEEP**

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Fasciolosis is a parasitic disease that affects livestock causing huge economic losses. It is also considered by the WHO as an emerging disease. Triclabendazole is the antiparasitic of choice, however the resistance to this drug has been reported. The development of vaccines against *F. hepatica* is an alternative of choice for the control of this disease. In this context, our laboratory has developed a vaccine based on *F. hepatica* Kunitz-type molecule (FhKTM) as an antigen formulated with a nanostructure formed by self-assembly of 6-O-ascorbyl palmitate ester (Coa-ASC16) and oligodeoxynucleotide containing unmethylated cytosine-guanine motifs (CpG-ODN)

which induces protection against *F. hepatica* challenge in mice. Based on these results we immunized lambs, one of the natural host of this disease, and test the ability of the vaccine to induce protection and humoral response.
Two immunizations were performed with an interval of 21 days with FhKTM/CpG-ODN/ Coa-ASC16 in six month old Creole biotype lambs. A control group was immunized with CpG-ODN/Coa-ASC16. Thirty days later, the experimental infection was performed with 100 metacercariae per animal in all groups (vaccinated, infected and control). After eight weeks egg count, number of adults in the liver, total eggs/ adult and IgG antibody titers were evaluated. The data were statistically analyzed using the T test for difference of means and ANOVA for evaluation of variances. The results show statistically significant differences ($p < 0.0001$) between the total egg count values between groups. The egg count test in the feces showed an 87.9% reduction in vaccinated *vs* infected group and a 83% reduction in fertility determined as the ratio between eggs/worms in the vaccinated vs infected group. The reduction in the eggs number correlated with higher titles of IgG antibodies in plasma of vaccinated vs infected lambs showing the ability of the vaccine to reduce the transmission of infection by this parasite.

217. (517) **DEVELOPMENT OF AN IFN-GAMMA RELEASE ASSAY WITH THE CAPACITY TO DIAGNOSE LATENT TUBERCULOSIS INFECTION**

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Current IFN-γ release assays (IGRAs) cannot discriminate active from latent tuberculosis infection (LTBI). Previously, we demonstrated that IFNγ secretion against Rv2626c, a *Mycobacterium tuberculosis* (*Mtb*) dormancy antigen, allows to discriminate LTBI subjects from healthy donors (HD) and tuberculosis (TB) patients. In this work we developed Diagnos-TB, a new IGRA that differentiates TB and LTBI by using a fusion protein (Fp) containing CFP10 and ESAT6 Mtb antigens plus Rv2626c protein. CFP10 and ESAT6 proteins were cloned, expressed and purified in our laboratory. Diagnos-TB comprises four sterile tubes containing i) negative and ii) positive controls, iii) Fp and iv) Rv2626c proteins. Heparanized whole blood is cultured inside each tube during 16-24h and plasma IFNγ is then assayed by ELISA. According to the IFNγ levels measured, an individual can be diagnosed as a TB patient, a LTBI subject or a non-infected person. We first established the cut-off for the Fp tube that differentiated infected subjects (TB patients and QuantiFERON-QFT+PPD+ people without symptoms) from not infected individuals (QFT-PPD- subjects)(ROC analysis: Cut-off = 0.36 IU/mL, sensitivity 85.4%, specificity = 84.4%, Area = 0.9010, $p<0.0001$). In contrast, QFT distinguished Mtb infected individuals (TB patients and PPD+ subjects without symptoms) from PPD- individuals with 73.3% of sensitivity and 60.7% of specificity. Furthermore, by employing only Fp, we obtained higher concordant results compared to QFT (73.3%) and to PPD (59.6%). We next included the Rv2626c tube and compared our LTBI diagnosis results with those obtained with QTF for QFT+ healthcare workers (healthy subjects who had worked at least two years at Hospital areas where tuberculosis patients were confined), detecting 96.6% of LTBI individuals. Together, our findings indicate that Diagnos-TB, an IGRA composed by two antigens tubes containing Fp and Rv2626c, allows to diagnose LTBI with high specificity and sensitivity.

218. (519) **CIRCULATING IgG ANTIBODIES AGAINST IMMUNODOMINANT REGIONS OF THE DORMANCY ANTIGEN Rv2626c DISCRIMINATE LATENT TUBERCULOSIS INFECTION**

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) together with SARS-CoV-2 in 2020-2021, was the leading cause of death from a single infectious agent. Furthermore, 1.9 billion people worldwide are latently infected (LTBI) with *Mtb.* Identifying and treating LTBI constitutes one of the most important impediments to control TB. Moreover, accurate tests to diagnosis LTBI are currently unavailable.We have previously demonstrated that circulating IgG against Rv2626c, an *Mtb* dormancy antigen, are found in plasma of LTBI. Then, here we evaluated particular regions of Rv2626c to be used to identify LTBI with higher sensitivity and specificity. For this, we used peptides sequences derived from Rv2626c to sensitize ELISA plates and then we analyzed the levels of IgG antibodies against those peptides in LTBI, TB patients and healthy donors (HD). The studied regions are overlapping synthetic peptides (13–15-mers, overlapped by 11amino acids; 36 in total) spanning the sequence of Rv2626c. We first examined the levels of circulating IgG against each Rv2626c peptide in LTBI. By doing that, we identified 13 peptides that allow to detect elevated levels of antibodies in the highest percentages of responders. Then, by investigating the specificity of the selected peptides, we could observe that some of them presented a marked higher specificity as compared to HD and TB patients. For example A) with peptide 18 we observed $D.O._{\cdot LTBI} = 0.45 \pm 0.15$, $D.O._{\cdot HD} = 0.09 \pm 0.02$ and $D.O._{\cdot TB} = 0.03 \pm 0.01$ and a percentage of responder individuals of 80% in LTBI, 0% in HD, 1% in TB (**$p<0.01$). B) with peptide 19 we observed $D.O._{\cdot LTBI} = 0.36 \pm 0.18$, $D.O._{\cdot HD} = 0.004 \pm 0.001$ and $D.O._{\cdot TB} = 0.002 \pm 0.002$ and a percentage of responder individuals of 60% in LTBI, 0% in HD, 1% in TB (*$p<0.05$). Together, our findings demonstrate that an ELISA measuring specific circulating IgG against Rv2626c peptides would allow to diagnose LTBI with high specificity and sensitivity.

219. (548) **EVALUATION OF LAST GENERATION ADJUVANTS ON IMMUNOGENICITY AND EFFICACY OF A PROTEIN SUBUNIT VACCINE CANDIDATE AGAINST TRYPANOSOMA CRUZI**

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Background and Aims: Chagas Disease is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*. Currently there is no effective vaccine to prevent or treat the infection. Previously, we have developed Traspain, a chimeric trivalent immunogen. To assess new adjuvants that might improve protection induced by vaccination, we evaluated the TLR3, TLR9 and STING ligands Poly (I:C), CpG oligodeoxynucleotide and Cyclic-di-AMP (CDA) respectively by the subcutaneous route.
Methods: Groups of 6-8 weeks old, female C3H mice (n=6/group) were vaccinated with 3 doses of 10 μg of Traspain plus 25 μg of adjuvant or PBS as placebo. Exploratory bleeding was performed and 20-30 days after the last dose, antigen specific immune responses

were evaluated by ELISA, proliferation, and flow cytometry. Additionally, for efficacy assessment, immunized mice were challenged with the RA strain of *T. cruzi* (DTU VI). Parasitemia and weight loss were employed as readouts.
Results: Significant Traspain-specific IgG levels were detected in sera from CDA and Poly(I:C) groups (IgG titres: 167117*, 60970* respectively, *p<0.01). Interestingly, CDA vaccinated sera exhibited 3- and 25-times higher titres than Poly(I:C) or CpG (p<0.01).
In terms of cell-mediated immunity, proliferation assays showed a significant difference between Poly(I:C) and CDA vs placebo (p<0.05). Production of IL-2, TNF and IFNγ was detected on the CD4+ T cells subset upon protein recall. More than 60% of these cells corresponded to polyfunctional subsets in all groups, having CDA the highest level. All formulations were able to reduce weight loss and parasitemia, resulting significant only in parasitemia for CDA and CpG vaccinated mice (p<0.05).
Conclusions: Subcutaneous administration of Traspain formulated with Poly(I:C), CpG or CDA is immunogenic and confers partial protection against *T. cruzi*. Traspain/CDA represents the best combination in terms of both immunogenicity and anti-*T. cruzi* efficacy.

220. (589) **EXTRAFOLLICULAR PLASMABLASTS EXHIBIT DIFFERENT PHENOTYPE IN DIFFERENT SECONDARY LYMPHOID ORGANS IN TRYPANOSOMA CRUZI INFECTION**

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B cells are the unique population able to differentiate into antibody-secreting-cells (ASC). The Germinal Center-GC-reaction and the extrafollicular-EF-response generate different types of ASC which provide long and short-term humoral immunity against infections, respectively. In our lab, we identified a population of EF-ASC ($B220^{int}$CD138+Blimp-1+Ki-67+, Plasmablasts/PB) with high expression of PD-L1 and CD39, present in murine infection models but not in autoimmune or immunized mice. The aim of our work is to characterize the phenotype and functionality of PB in *T cruzi* infection. Therefore, B6 mice were infected with *T. cruzi* and splenic, lymph node (LN) and bone marrow (BM) lymphocytes were evaluated at different days post infection (dpi). To assess the antibody response, sorted splenic PB were incubated for 6h and the levels of Igs were evaluated in the culture-supernatant by LEGENDplex. IgM, IgG1, IgG2, IgG3 and IgA were detected in the PB supernatant and by ELISA we determined the presence of IgM, IgG and IgA specific for *T. cruzi* antigens. Moreover, by FACS, we found that the 99% of splenic PB expressed high levels of PD-L1 and CD39 until day 23pi and this frequency was significantly reduced from day 28pi (p<0.005). Only the 15% of BM-ASC from mice at chronic phase of infection (130dpi) expressed high levels of PD-L1 and CD39. Interestingly, we observed that splenic PB expressed higher levels of CD80, CD86, MHCII, MHCI, CXCR3, CXCR4, PD-L1 than PB present in LN (p<0.005). Otherwise, PB from spleen have lower levels of transcription factor IRF-4, T-bet and Bcl-2, than PB from LN. Likewise, we found higher percentage of Ki-67 negative ASC in LN than in spleen at 26 Dpi, suggesting than these ASC could be plasma cells. By LEGENDplex, we detected that supernatant of splenic cells had higher levels of IL-6 and lower levels of IL-2 than supernatants from LN cells. This difference in each tissue-microenvironment could be conditioning the different phenotype of PB.

221. (606) **IMMUNOINFORMATIC ANALYSIS OF A VACCINE CANDIDATE AGAINST *T. CRUZI***

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Chagas disease, caused by the infection of the protozoan parasite *Trypanosoma cruzi*, is a tropical neglected disease. To date, there is still no vaccine available against it. In this context, we have developed Traspain, a chimeric antigen containing domains of two *T. cruzi* relevant antigens: Cruzipain (Cz) and Amastigote Surface Protein-2 (ASP-2). Traspain as a vaccine showed promising results in preclinical models. To broaden the understanding of Traspain as an immunological candidate, we have conducted *in silico* studies of its structure and immunological features. Traspain domains and epitopes conservation was evaluated by sequence alignment using Blast. Traspain structure was predicted with the novel algorithm RoseTTAFold. Most prevalent HLA alleles in Latin America and rest of the world were selected based on bibliography and IEDB. Subsequently, human T cell epitopes were predicted using artificial neural networks-based algorithms (NetMHCpan and NetMHCIIpan). Linear and discontinuous B cell epitopes were predicted using the servers BepiPred and DiscoTope respectively. To confirm antibody recognition, Western blot and ELISA employing a pool of human chagasic sera were assayed. Traspain domains showed more than 80% of identity compared to representatives discrete typing units (DTUs) of *T. cruzi*. 119 nonapeptides were predicted as strong binders (SB) for HLA-I molecules covering the 62 most frequent alleles of world population. 69% of these potential epitopes are located within the ASP-2 domain. Regarding HLA-II molecules, 99 15-mer peptides were predicted as SB covering 95.8% of most frequent alleles. 15 continuous B-cell epitopes were predicted. Conformational B-cell epitopes were also successfully predicted. Antibody recognition was achieved. Immuno-informatic analysis showed that Traspain sequence contains several potential human CTL, Th and B cell epitopes. Overall, these results support Traspain as a vaccine candidate to be tested in a first in human trial.

INMUNIDAD ANTITUMORAL

222. (040) **ABSENCE OF CD39 MAY INDUCE A PRE-EXHAUSTED PHENOTYPE ON TUMOR-INFILTRATING CD8+ T LYMPHOCYTES**

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We previously demonstrated that CD39KO tumor-bearing mice exhibit a higher % of tumor-infiltrating (TI) CD8+ T cells with effector phenotype as well as a higher % of specific TI-CD8+ T cells than Wild type (WT). CD39 participates in the generation of adenosine, an immunosuppressive molecule that interferes in the anti-tumor response. In this work, we evaluated the role of CD39 on TI-CD8+ T cells. C57BL/6 WT and CD39KO mice were injected with $1x10^6$ B16F10-OVA tumor cells, 17 days after injection, we evaluated by flow cytometry the expression of inhibitor receptors (IRc) (PD-1 and TIM-3), transcription factors (TF) related to exhaustion (TOX, TCF-1, EOMES, T-bet and IRF-4) and cytotoxic related molecules (granzime B (GzB), perforin, and CD107) on TI-CD8+ T cells. CD39 KO and WT tumor bearing mice exhibited two PD-1 expressing populations: a $PD\text{-}1_{high}$ and $PD\text{-}1_{low}$; however, CD39KO mice exhibited a higher % of $PD\text{-}1_{low}$ TI-CD8+ T cells than WT(p<0.005). CD39KO and WT mice showed a high % of $PD\text{-}1_{high}$ cells co-expressed TIM-3 (**82,4±6,1** and **80,3±17,9 respectively**), an IRc related to terminal

exhaustion, while a low % of PD-1_{low} cells were TIM-3+ (**17,5±11,2** and **34,0±19,9**). PD-1_{low} cells from CD39 and WT mice also exhibited lower expression of TBET, EOMES, and IRF4 than PD-1_{high} cells ($p<0.05$). Whereas most PD-1_{low} TI-CD8+ T cells from CD39 KO and WT were TCF1+ (**65.4±14.4** and **71.4±11.5**), a TF related to pre-exhaustion, PD-1_{high} CD8+ cells were TOX+ and TCF-1- (**92.8±4.2 and 86.4±7.5**). TI-CD8+ T cells cytotoxicity increase as they become more exhausted, accordingly, a higher % of CD8+ GzB+, perforin+ and CD107+ cells were found within PD-1_{high} cells compared to PD-1_{low} ($p<0.05$) TI-CD8+ T cells. Our results suggest that the absence of CD39 may favor a pre-exhausted phenotype on TI-CD8+ T cells, which are known to respond better than terminal exhausted T cells to anti-checkpoints, reinforcing the idea that CD39 could be consider as a promising target in anti-tumor immunotherapy.

CD39, CD8+ T Lymphocyte, Tumor, Exhaustion.

223. (111) **A TH2 SCORE IN THE TUMOR MICROENVIRONMENT AS A PREDICTIVE BIOMARKER OF RESPONSE TO TREATMENT WITH BACILLUS CALMETTE-GUERIN (BCG) IN PATIENTS WITH SUPERFICIAL BLADDER CARCINOMA. A RETROSPECTIVE STUDY**

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Intravesical administration of live attenuated Bacillus Calmette-Guérin (BCG) is the main therapy for intermediate/high grade non-muscle invasive bladder cancer (NMIBC). However, the response rate is ~60%. BCG is a local immunomodulatory that induces a strong inflammatory response that ultimately eliminates the tumor.We hypothesized that pts with pre-existing Th2 tumor infiltrating lymphocytes (TIL) would be more susceptible to BCG-induced Th1 polarization and therefore respond better to therapy.

Searching for a predictive biomarker of BCG response, pre-treatment NMIBC biopsies (n=32) from pts treated with a 6-week induction plus at least one 3-week maintenance instillations were evaluated retrospectively by immunohistochemistry. TIL polarization was assessed quantifying T-bet+(Th1) and GATA-3+(Th2) lymphocytes ratio (G/T) and the density and activation of EPX+ eosinophils. A Th2score was calculated and correlated to BCG response. In non-responders, Th2score was compared in pre and post-BCG biopsies. Also, PD-1/PD-L1 expression was analysed.

Response rate was 65.6% in the study population. In pre-BCG biopsies a higher Th2score was significantly associated with BCG response (p=0.027, Mann Whitney test) and to prolonged recurrence-free survival (p=0.0138, Wilcoxon rank test). A ROC curve allowed discrimination of responders with 91% sensibility and 57.1% specificity (Th2score cut-off >48.1; AUC 0.74 (95% CI 0.56-0.91; p=0.028). Interestingly, in post-BCG biopsies TIL increased their Th2-polarization, probably reflecting BCG failure to induce a pro-inflammatory status and thus lack of antitumor response. PD-L1 expression (tumor cells/TIL) and PD-1 expression (TIL) was not associated to BCG response.

The results support our hypothesis that pre-existing Th2-polarized TIL can predict a better response to BCG immunotherapy. Instead, pts that already have Th1-polarized TIL would not respond to BCG, presumably because the tumor has already developed immune escape mechanisms.

224. (118) **ADAPTIVE NATURAL KILLER (NKA) CELLS FACILITATE EFFECTOR FUNCTIONS OF TRASTUZUMAB IN BREAST CANCER (BC)**

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NK cells have been regarded as primary effectors of innate immunity, but emerging evidence suggests that a subset of these cells have adaptive immune features, including memory-like properties, such as long-term persistence, robust preferential expansion in response to viral infection, and enhanced antibody-dependent effector functions. Adaptive NK cell subsets are characterized by a lack of FcℰRIγ expression and/or high expression of NKG2C.

Objective: To identify adaptive NK cell subpopulations in BC patients and characterize subsets that support robust *ex vivo* anti-BC activity via trastuzumab (TRZ)- mediated ADCC.

With this aim, we analyzed the immunophenotype of NK cells in peripheral blood samples from our cohort of 60 BC patients and 32 healthy donors (HD), both 85% seropositive for human cytomegalovirus. Defining adaptive (NKa) and conventional NK (NKc) cells as FcRγ- and FcRγ+, respectively, NKa subpopulation was detected in 66% of BC patients and 75% of HD. We characterized differential expression of NK-cell inhibitory and activating receptors. NKa cells expressed significantly higher levels of NKG2C (<0,0001) and CD85j (<0,0001), and lower levels of NKp30 (<0,0001), CD161 (<0,0001) and CD16 ($p<001$) compared to NKc cells (paired t-tests), which is consistent with previous observations in HD.

We then, compared the production of IFNγ by NKc and NKa cells. When PBMC were cultured with SKBR3 HER-2+ cells treated with TRZ, NKa cells from BC patients and HD exhibited increased IFNγ production compared to NKc cells ($p<0.01$, paired t-test).

Conclusions: NKa cells have different immunophenotypic characteristics than NKc cells in BC. Ex vivo treatment of PBMC with TRZ reveals superior effector functions of NKa cells. Our results encourage studying NKa cells as a potential candidate for predicting Ab-based therapy outcomes in HER2+ BC.

225. (236) **CROSSTALK BETWEEN ANGIOGENESIS AND IMMUNE MODULATION: HYPOXIA AS A DRIVER OF THE DIFFERENTIATION OF EXHAUSTED CD8 T CELLS.**

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Hypoxia, angiogenesis, and immunosuppression are interrelated events that fuel tumor progression and blunt clinical effectiveness of therapies. During my scientific career, I focused on glycans and glycan-binding proteins as key players in neovascularization and immune responses in cancer, inflammation, and immunity.

Since 2016, I became the head of the "Glycobiology and Vascular Biology" laboratory at the IHEM-CONICET, were we explore several physio-pathological settings converging on the cellular and molecular mechanisms that link hypoxia, neovascularization, and immune responses. In this sense, we are particularly interested in generating relevant systems to study *in vitro* hypoxic-mediated neovascularization and to generate novel molecular diagnostic tools for clinical applications.

Our main areas of research include: 1) The glycome remodeling in virally mediated tumorigenesis in the context of KSHV and HIV co-infection in Kaposi Sarcoma. 2) The effect of hypoxia in inflammation-induced angiogenesis, and the dynamic regulation of intestinal glycome in inflammatory diseases. In this scenario, we also study miRNA expression as mediators of hypoxia-driven epithelial cell glycosylation.

Finally, in an attempt to reconcile seemingly opposite evidence concerning the impact of hypoxia on functional features of exhausted CD8 T cells, we investigate the fine-tuning of CD8 T cell exhaustion by hypoxia and its association with angiogenesis in the tumor microenvironment. In this sense, we found that both hypoxia and VEGF promote the differentiation of PD-1+TIM-3+CXCR5+ terminally exhausted CD8 T cells at the expense of PD-1+TIM-3- progenitor subsets. Moreover, hypoxia accentuated a proangiogenic profile

in exhausted CD8 T cells, MDSCs, and hMSCs cells. Altogether, our findings highlight the reciprocal regulation between hypoxia, angiogenesis, and immunosuppression, providing a rational basis to optimize synergistic combinations of antiangiogenic and immunotherapeutic

226. (324) **CPG-ODN SYSTEMIC ADMINISTRATION BEFORE B16 MELANOMA CELLS INOCULATION INCREASES TUMOR GROWTH AND DECREASES MICE SURVIVAL: ANALYSIS OF THE IMMUNE RESPONSE ELICITED**

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CpG-ODN immunostimulation has been extensively studied in animal models for the therapeutic treatment of solid tumors and as adjuvant for the development of cancer vaccines. However, the efficacy of CpG-ODN as a prophylactic agent in reducing metastatic dissemination has been less frequently studied. Recently, it was demonstrated that a single systemic prophylactic injection of class-C CpG-ODN, administered 24 h before tumor cell injection into the cerebral circulation, was sufficient to reduce brain tumor growth (Benbenishty, A. et al., 2019).
In this work, we aimed to study the effect of prophylactic class-B CpG-ODN administration in B16 melanoma tumor growth. CpG-ODN (50ug/ml) was s.c. administered on days -8, -6,-4 and -2 before s.c inoculation of 5 x 10^5 B16 melanoma cells and then, tumor growth was measured every other day. Pre-treatment with CpG-ODN enhanced tumor growth and reduced mice survival in two separate experiments (n=6, per group; p≤0.05**).** As a control, therapeutic intratumoral inoculation of CpG-ODN reduced tumor size and prolonged survival, as expected.
Flow cytometry analysis of immune populations in spleen and lymph nodes performed after 4 injections of CpG-ODN and 24 h before tumor cell inoculation showed in spleen a significant decrease in the percentage of T cells (CD45+ CD3+, p=0.0016), whereas dendritic cells (CD45+ CD3- CD19- Ly6G- Ly6C- CD11c+) show non-significant changes. In contrast, the frequency of B cells (CD45+ CD19+, p=0,0035) and neutrophils (CD45+ CD3- CD19- Ly6G+ CD11b+, p=0.0019) increases suggesting a possible tumor promoting role for these populations. When tumor infiltrating lymphocyte where studied at day 15 post inoculation, an important reshaping of the tumor infiltrate was observed. Further studies should be done to decipher the immune mechanisms underlying the tumor promoting role of CpG-ODN in this model.

227. (377) **PHENOTYPE AND FUNCTIONAL ALTERATIONS OF HUMAN NK CELLS BY ORGANOPHOSPHATE PESTICIDES**

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Chlorpyrifos (CPF) and Glyphosate (GLY) are organophosphate pesticides widely used in agriculture. Several evidences suggest that CPF- and GLY-based pesticides are genotoxic and their use has been linked to the increased frequency of malignancies observed in highly fumigated rural areas. However, their effect on the immune system, including cells involved in immunosurveillance of tumor cells, has been poorly explored. Here, we investigated the effects of two commercial formulations containing CPF (Clorpi48) and GLY (Roundup Plus) as well as their isolated active principles on the phenotype and function of human NK cells. First, we identified sub-apoptotic doses of these substances by flow cytometry performing a dose-response curve with human peripheral blood mononuclear cells (PMBC). Next, using such sub-apoptotic doses, we analyzed the phenotype of pesticide-treated NK cells. We observed a pesticide dose-dependent reduction in the expression of CD16 and CD62L on NK cells after a 24 h of culture with CPF, GLY, Roundup and Clorpi48. Moreover, a diminished frequency of IFN-γ-producing NK cells was observed upon exposure of cytokine-stimulated NK cells to 5 mM GLY, 0.05 mM Roundup, 0.01 mM Clorpi48 but not to 0.02 mM CPF (p<0.05). Moreover, NK cell-mediated cytotoxicity against K562 cells was also affected by pesticide treatment. After 0.05 mM Roundup and 0.01 mM Clorpi48 treatment, cytotoxicity was reduced by 27% (p<0.01) and 29% (p<0.001), respectively. In accordance with previous scientific evidence, final formulations of pesticides (which include additional compounds such as polyethoxylated alkylamines surfactants that facilitate their absorption by cells and tissues) showed a more potent effect on phenotype and function of NK cells than the isolated compounds. Therefore, we conclude that GLY- and CPF-based pesticides affect NK phenotype and function, which might impact on their ability to detect and eliminate nascent tumor cells.

228. (405) **MUC4 ENABLES IMMUNE TUMOR EVASION IN HER2+ BREAST CANCER**

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HER2+ is a breast cancer (BC) subtype characterized by the overexpression/amplification of HER2. Patients receive trastuzumab (Tz), but 27-42% of them do not respond. We demonstrated that the overexpression of TNFα induces Tz resistance in cells and tumors by upregulating the membrane glycoprotein MUC4, which hides Tz epitope on HER2 impairing its binding and pharmacological effects. Blocking the soluble TNFα isoform with INB03 (DN) reduces MUC4 expression, overcomes Tz resistance and unleashes an antitumor innate immune response (IIR) with a decrease in myeloid-derived suppressor cells, an increase in NK cell-activation and degranulation and a macrophage (Mφ) polarization to the M1 subtype.
We studied Mφ and NK cells contribution to the Tz-mediated antitumor IIR and MUC4 impact in human T-lymphocyte recruitment and differentiation. We genetically modified the Tz-resistant HER2+ BC cell lines JIMT-1 and KPL-4 to express a doxycycline-inducible (Dox) MUC4 shRNA (shMUC4) or a control one (shControl) and injected them into female nude mice, which were treated with IgG or Tz (5 mg/kg), DN (10 mg/kg) or the combination of Tz+DN, with (+Dox) or without (-Dox) shRNA induction.
After Mφ depletion with chlodronate or NK cell depletion with anti-asialo GM1, in -Dox tumors we found that both populations are needed to achieve Tz+DN antitumor effect (p<0.01 and p<0.05, respectively). However, upon MUC4 silencing, only Mφ are required to mediate Tz antitumor effect (p<0.01). Secretome from JIMT-1 and KPL-4 cells with MUC4 silencing promoted differentiation of activated T-cells to effector cells. Finally, in our HER2+ BC patient cohort, we found a negative correlation between tumor infiltrating lymphocytes presence and MUC4 expression (p=0,005).
We conclude that Mφ are key players in the Tz-mediated antitumor IIR and that MUC4 promotes cold HER2+ tumors with poor therapy response. Women with HER2+MUC4+ BC could benefit from the combined treatment of Tz+DN.

229. (418) **TUMORAL PD-L1 ORCHESTRATES DIFFERENT TUMOR-INDUCED IMMUNOSUPPRESSION MECHANISMS DURING BREAST CANCER PROGRESSION**

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One of the main immunosuppressive mechanisms by which cancer avoids eradication by the immune system is the expression of PD-L1, the ligand for T-cell inhibitory receptor PD-1. PD-1 activation by PD-L1 leads to CD4+/CD8+ lymphocyte exhaustion, which is at the focal point of today's cancer immune therapies. However, little is known about which other immunosuppression mechanisms are triggered by tumor-intrinsic PD-L1 expression.
To genetically address tumor-immune system interactions in a triple negative breast cancer (TNBC) model, we developed a CRISPR/Cas9 expressing TNBC-like EO771 cell line platform. Using flow cytometry, we characterized the immune response associated with the progression of EO771 tumors, which resembled immunosuppression signatures associated with poor prognosis in TNBC patients: an increase in pro-tumoral M2 macrophage polarization, a decrease in MHCII+ Antigen Presenting Cells (APCs) and a marked increase of T-cell exhaustion.
To test the role of tumoral PD-L1 in tumor-mediated immune escape, we generated PD-L1 KO EO771 cell lines. Using CRISPR/Cas9 edited EO771 lines KO for PD-L1, we found that tumor intrinsic PD-L1 expression is required for tumor growth. Interestingly, we also found that PD-L1 expressed by the tumor cell exerts a general impact over the tumoral immune infiltrate composition: a) it is required for the differentiation of M2 macrophages and for the enrichment of myeloid derived suppressor cells and b) in the T-cell compartment, unexpectedly, tumoral PD-L1 is needed to exhaustion of effector CD4+ but not cytotoxic CD8+ cells.
All together, these data suggests that tumor-intrinsic PD-L1 plays a key role on TNBC tumor growth by triggering different immunosuppressive mechanisms in the tumor immune landscape. Using this editable EO771 model platform, we will be able to massively test tumoral PD-L1 synthetic interactions to identify candidate genetic targets to overcome PD-1/PD-L1 resistance in TNBC.

230. (427) **B16F10-OVA TUMOR-BEARING MICE INJECTED VIA INTRAPERITONEAL REPRESENT AN EXCELLENT MODEL TO STUDY TUMOR-INFILTRATING B CELLS**

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Previous works demonstrate that CD8+ tumor-infiltrating T cells play a pivotal role in the anti-tumor response. However, the role of B cells is not completely elucidated. In this work, we evaluate tumor-infiltrating (TI) lymphocytes in two different murine melanoma models focalizing the study in the B cell population. C57BL/6 mice were injected subcutaneously (sc) or intraperitoneally (ip) with $4x10^5$ B16F10-OVA tumor cells and on day 14, tumors and spleen were collected. By Flow cytometry, we observed, that compared to mice injected sc, ip-injected mice show higher frequency of TI-CD45+ cells (p=0.03) but no differences in the % of TI-CD8+ T cells or CD8+ exhausted T cells (TIM-3+PD-1+). While there are no differences in Tregs frequency, TI-CD4+ conventional T cells are more frequent in ip-injected mice (p=0,01), moreover the % of TI-CD19+ cells is significantly increased in these mice compared to sc-injected mice (p≤0.0001). Surprisingly, in tumor microenvironment of the ip-injected mice, we detected a high % of B cells (43.4±6.0) exhibiting naïve phenotype and a significant frequency of plasmablast (19.8±8.8). A high frequency of plasmablast express the activation marker CD69 (89.2 ±3.5) and the ecto-enzyme CD39 (60.2 ±23.1). The B cell compartment is different in the spleen from ip-injected mice. Indeed, while the % of naïve B cells is similar (42.4±4.9), the frequency of the plasmablast is significantly lower (4.2±1.0). Additionally, we observed that the frequency of CD69-expressing plasmablast reach a value of 19.6±9.8 while the % of $CD39^{high}$-expressing plasmablast is only 8.4±2.0.
Taken together these results demonstrated that ip- tumor injected mice represent an excellent model for the study of TI- B cell compartment. Future studies will be perform to understand the role of the TI-plasmablast expressing an activation marker and CD39 in the immune response against tumors.

231. (447) **CHARACTERIZATION OF TUMOR INFILTRATING NK CELLS (TINK) AND TYPE 1 INNATE LYMPHOID CELLS (ILC1) IN BREAST CANCER**

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ILC1 and NK cells share several phenotypic and functional characteristics and display plasticity because they can interconvert one into another in a context-dependent manner. Indeed, TGF-β-driven conversion of TINK into intermediate populations of ILC1 (intILC1) and ILC1 has been proposed as a tumor immune escape mechanism. However, the role of ILC1 in antitumor immunity remains ill-explored. Thus, the aim of this work was to investigate TINK and ILC1 in the tumor microenvironment (TME) using the 4T1 triple-negative breast cancer mouse model. BALB/c mice were injected with $3x10^4$ 4T1 cells and after 19 days, mice were euthanized and cell suspensions of tumor, draining lymph nodes, spleen, lungs, and liver were obtained to study NK cells, intILC1 and ILC1 by flow cytometry. Tissues from healthy mice were also obtained. ILC1 and intILC1 were present in tumor and lung, but were absent in spleen and lymph nodes, while only ILC1 were found in liver from both groups of mice. A higher frequency of intILC1 than of ILC1 ($p<0.01$) was observed in the TME. In lung, where 4T1 metastasizes, higher frequencies of ILC1 were observed in 4T1 tumor-bearing mice than in healthy animals ($p<0.05$). Like TINK, both cell types expressed the activating receptor NKG2D in the TME, while 4T1 cells expresses NKG2D ligands. NKG2D expression was higher in ILC1 and intILC1 present in the TME than in liver ($p<0.05$) and lungs ($p<0.05$) either from tumor-bearing or healthy mice. Also, intILC1 and ILC1 from tumor-bearing mice or from healthy mice expressed CD69, supporting their sentinel-like tissue resident characteristics. Moreover, within the TME, ILC1 exhibited higher expression of Ly6C (associated with ILC1 activation) than in the liver of tumor-bearing mice ($p<0.01$) and such expression was higher in ILC1 than in intILC1 ($p<0.05$) and TINK ($p<0.01$). We conclude that the TME contains ILC1 that display an activated phenotype, which suggests that they might be involved in tumor immunoediting.

232. (449) **ANTI-CTLA-4 TREATMENT PROMOTE THE EXPANSION OF CD39+ CONVENTIONAL CD4+ T CELLS**

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Previously we demonstrated that tumors from different experimental mice models are infiltrated with $FOXP3^-$ CD4+ cells (Tconv) expressing CD39. CD39 is an unequivocal marker CD8+ of exhaustion. Tumor infiltrating (TI) CD39+Tconv cells represent an heterogeneous population with features of exhaustion. Transcriptional profiling of

TI-CD39+Tconv cells showed that they exhibit a transcriptional signature associated with cytotoxic CD4+ T cells. In this work, we aim to evaluate the impact of Anti-CTLA-4 treatment on TI-CD39+Tconv cells. C57BL7/6 mice were injected with $0.5x10^6$ MC38 tumor cells. On days, 4, 7 and 10 mice were treated with anti-CTLA-4 (100ug/mice) or IgG (Control). On day 17 we evaluated by flow cytometry the phenotype of the TI-CD39+Tconv cells. As expected, anti–CTLA-4 treated mice exhibited reduced tumor volume and frequency of TI-Tregs respect to controls ($p<0.001$), however they showed higher frequency of TI-CD39+Tconv cells ($p<0.01$). UMAP visualization analysis based on the expression levels of 8 molecules on TI-Tconv cells from treated and control mice shown 10 different clusters. After treatment, clusters corresponding to FOXP3+ (Tregs) cells are almost missing; however, 2 clusters corresponding to CD39+ and inhibitor receptors (PD-1, 2B4, TIGIT, LAG-3) expressing cells are enriched. Interestingly one of these enriched clusters, exhibit higher expression of CD39 and PD-1. The evaluation of transcription factors related to exhaustion (TOX, Eomes, Helios) or pre-exhaustion (TCF-1) as well as CD107a (a marker of cytolytic capability) revealed no differences in the expression of all these markers on TI-CD39+Tconv cells from treated or control mice. All together, we conclude that the anti-CTLA-4 treatment expand the TI-CD39+T-conv cells, and has no significant impact in the phenotype or function of this population. Due to the cytotoxic potential TI-CD39+Tconv cells may contribute to the immune response against tumors.

233. (450) **IMPAIRED ANTITUMOR IMMUNITY IN *LSP1*$^{-/-}$ MICE IS ACCOMPANIED BY AN INTRATUMORAL CYTOKINE DISBALANCE**

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Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes as well as in endothelial cells. This protein in known as an important regulator of actin cytoskeleton remodeling. LSP1 polymorphisms or downregulation are considered risk factors for some types of cancer.

We previously shown that B16-OVA melanoma in *Lsp1*$^{-/-}$ mice grows significantly faster and bigger than in wild type (WT) controls. Also, tumors harvested from *Lsp1*$^{-/-}$ mice show a lower frequency of total infiltrating leukocytes compared to WT mice. Furthermore, there is a reduced extravasation efficiency of *Lsp1*$^{-/-}$ leukocytes into tumor, combined with a defective CD8+ T cell priming in *Lsp1*$^{-/-}$ tumor dLN. We continued this study by comparing the cytokine milieu in melanoma tumors in *Lsp1*$^{-/-}$ vs WT mice. For that, 1.10^5 B16-OVA melanoma cells were implanted in both animal groups and on day 16 tumors were harvested. Aproximately 70 mg tumor/mouse was digested using T-PER™ Tissue Protein Extraction Reagent added with protease inhibitor. Tumor-cytokine content was determined by a multiplex commercial assay using fluorescence–encoded beads that allows simultaneous quantification of 13 mouse cytokines including IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-23, IL-27, MCP-1, IFN-β, IFN-γ, TNF-α and GM-CSF. We found that tumors in *Lsp1*$^{-/-}$ mice have significantly higher levels of IL-1β, IL-10, IL-27, IL-17A and IL-23 ($p<0.01$) as well as higher levels of TNF-α, IL-12p70, IFN-β and GM-CSF ($p<0.001$) than WT mice. However, tumor extracts contained similar levels of IL-1α, INF-γ, MCP-1 and IL-6. Sumarizing, the tumoral milieu in *Lsp1*$^{-/-}$ mice has increased levels of cytokines that can act as angiogenesis promoters, favoring tumoral growth and chronic inflammation, as well as cytokines involved in promoting DC activation.

234. (488) **THYMIC STROMAL LYMPHOPOIETIN (TSLP) IS A TUMOR MICROENVIRONMENT MODULATOR: RELEVANCE IN THE PATHOGENESIS OF GLIOBLASTOMA AS TUMOR IMMUNOMODULATOR**

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Glioblastoma (GBM) is the most devastating brain tumor, with an associated poor prognosis. Despite the advances in surgery and others treatments, the survival of GBM patients has not improved significantly in the past three decades. Thymic stromal lymphopoietin (TSLP) is a cytokine produced primarily by activated epithelial cells and it has been shown to be a key factor in maintaining immune homeostasis and regulating inflammatory responses at mucosal barriers. Furthermore, recent studies have found the participation of TSLP in inflammatory diseases and cancer. This work aimed to elucidate the relevance of TSLP in the interaction between neutrophils and GBM cells. For this purpose, human U251 cell line or tumoral cells obtained from a patient's GBM biopsy (GBM-b) were co-cultured with human neutrophils.

First, we observed a decrease in the neutrophils apoptosis when they were cocultured with the U251 cell line or the GBM-b cells in the presence of exogenous TSLP ($p<0.05$). Importantly, neutrophils from healthy donors and GBM patients expressed TSLP receptor after 24hs of culture ($p<0.05$). Then, we evaluated the expression of endogenous TSLP in the cocultures. By RT-qPCR, we observed that the U251 cell line or the GBM-b cells produced TSLP when incubated with Epidermal Growth Factor (EGF). On the other hand, neutrophils expressed very low to undetectable amounts of TSLP, compared to the tumor cells. Furthermore, neutrophil expression of TSLP was not induced either by coculture with GBM-b cells nor by stimulation with EGF.

Finally, neutrophils from healthy donors and GBM patients increased the IL-8 production in presence of TSLP ($p<0.05$). Notably, neutrophils also produced IL-8 in the presence of U251 cell line or GBM-b cells.

Our findings suggest that TSLP is present in GBM cells and could modulate the inflammatory microenvironment through their crosstalk with neutrophils.

235. (495) **TUMORAL PD-L1 ORCHESTRATES DIFFERENT TUMOR-INDUCED IMMUNOSUPPRESSION MECHANISMS DURING BREAST CANCER PROGRESSION**

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One of the main immunosuppressive mechanisms by which cancer avoids eradication by the immune system is the expression of PD-L1, the ligand for T-cell inhibitory receptor PD-1. PD-1 activation

by PD-L1 leads to CD4+/CD8+ lymphocyte exhaustion, which is at the focal point of today's cancer immune therapies. However, little is known about which other immunosuppression mechanisms are triggered by tumor-intrinsic PD-L1 expression.
To genetically address tumor-immune system interactions in a triple negative breast cancer (TNBC) model, we developed a CRISPR/Cas9 expressing TNBC-like EO771 cell line platform. Using flow cytometry, we characterized the immune response associated with the progression of EO771 tumors, which resembled immunosuppression signatures associated with poor prognosis in TNBC patients: an increase in pro-tumoral M2 macrophage polarization, a decrease in MHCII+ Antigen Presenting Cells (APCs) and a marked increase of T-cell exhaustion.
To test the role of tumoral PD-L1 in tumor-mediated immune escape, we generated PD-L1 KO EO771 cell lines. Using CRISPR/Cas9 edited EO771 lines KO for PD-L1, we found that tumor intrinsic PD-L1 expression is required for tumor growth. Interestingly, we also found that PD-L1 expressed by the tumor cell exerts a general impact over the tumoral immune infiltrate composition: a) it is required for the differentiation of M2 macrophages and for the enrichment of myeloid derived suppressor cells and b) in the T-cell compartment, unexpectedly, tumoral PD-L1 is needed to exhaustion of effector CD4+ but not cytotoxic CD8+ cells.
All together, these data suggests that tumor-intrinsic PD-L1 plays a key role on TNBC tumor growth by triggering different immunosuppressive mechanisms in the tumor immune landscape. Using this editable EO771 model platform, we will be able to massively test tumoral PD-L1 synthetic interactions to identify candidate genetic targets to overcome PD-1/PD-L1 resistance in TNBC.

236. (540) **IN VITRO STIMULATION WITH IL-17 INDUCES DISTINCTIVE RESPONSES IN DIFFERENT TUMOR CELL LINES ACCORDING TO THEIR IL-17R EXPRESSION PROFILE.**

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The role of IL-17 signaling in tumor progression is discussed as it sustains, directly and indirectly, tumor growth and immune-escape but also supports anti-tumoral immunity by boosting CD8+ T and NK cell responses. Our aim is to determine the role of IL-17 signaling in tumor progression dissecting pro- and anti-tumoral effects. For this, we used B16 (melanoma) and EL4 (thymoma) cells that according to previous results show a different IL-17 receptor (IL-17R) expression profile:both expressed IL-17RA and IL-17RD whereas only B16 cells expressed IL-17RC. In addition, B16 and EL4 exhibited divergent tumor growth in hosts deficient in IL-17 signaling. These data led us to hypothesize that IL-17 may trigger different transcriptional programs in both cell lines to promote or restrain tumor progression. To evaluate this, we performed RNA-seq to compare the transcriptomes and differentially expressed genes (DEG) in B16 and EL4 cells after exposure to IL-17 for 24h. We confirmed the different IL-17R expression profiles in both cell lines that were linked to significant differences in the expression of genes of the IL-17 pathway in the basal condition. Exposure to IL-17 led to different outcomes among the cell lines. Comparison of medium vs IL-17 treated cells revealed 374 DEG (179 up and 195 down) in B16 cells, and 535 DEGs (444 up and 96 down) in EL4 cells with only 12 genes in common (6up, 5down and 1 with opposite result) ($p<0.05$, logFC>1.5). Ingenuity Pathway Analysis highlighted within the Top Canonical Pathways enriched in IL-17-treated B16 cells pathways associated to cytokine-mediated responses while within those enriched in IL-17-treated EL4 cells predominated pathways associated to signaling during cellular processes such as cell growth and cell-to-cell interactions. Our results highlight that IL-17-signaling triggered diverse responses in different tumor cells that may modulate tumor growth likely as consequence of particular profiles of IL-17R subunit expression.

237. (551) **ANALYSIS OF THE BINDING CAPACITY OF α2-DOMAIN OF MICA TO NKG2D RECEPTOR**

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To the date, the role of each MICA domains (α1 or α2) in the binding with NKG2D is not well understood and in this work we presented the subcloning, production and purification of α2 domain of MICA, and the behavior with NKG2Dr.
Methodology. The subcloning of a2 MICA domain was done with a pET-JT1 vector, which was introduced into *E.coli* D5Hα by chemical competency. Through a clonal selection, we obtained the DNA and then sequenced the insert with external primers. Second, we introduced the vector with the α2 MICA domain sequence, into *E.coli* BL21 and produced the recombinant protein by fermentation and posterior induction with IPTG. The purification of protein was done separating the medium and cells, and then, lysing the cells, and purifying the proteins through Ni^{+} chromatographic column. Last, we prove the binding functionality of recombinant protein by immuno assay, using a homodimer NKG2D recombinant and we compared its behavior with a construction of recombinant MICA external domain (MICAsp).
Results: After the subcloning, the sequence had a high identity with the original gene. The protein structure model was obtained using the Swiss-Model server. The chromatogram showed a high presence of protein in the supernatant of lysis, but only a low percentage of binding protein. Thanks to design of protein with a polyhistidine (His)-tag, this should be retained by the column, and be into the elution fraction. The α2 domain was bond to the NKG2D recombinant protein, in different concentration, at the same way like MICAsp recombinant protein. MICAsp showed an optical density signal higher than α2 domain, however this signal is half to obtained with MICAsp, indicated that this domain provide, at less, the half of capacity of binding.
Conclusions: This work showed the possibility of production and purification of α2 MICA domain, and its functionality in the binding to NKG2D, allowing to know the importance of the α2 domain in the union with the receptor.

238. (599) **GALECTIN 1 REINFORCES THE IMMUNOSUPPRESSIVE ACTIVITY OF MYELOID-DERIVED SUPPRESSOR CELL EXOSOMES**

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Myeloid-Derived Suppressor Cells (MDSC) represent a major hurdle for cancer immunotherapy by blunting antitumor T-cell responses. Emerging evidence suggests that MDSC may promote immunosuppression via exosomes shedding; however, the molecular mediators that trigger this effect are still elusive. We have recently demonstrated that Galectin 1 (Gal1), a β-galactoside-binding protein, enhances both the immunosuppressive and pro-angiogenic activities of MDSC-derived exosomes. Here, we aimed to described Gal1 role in MDSC exosomes biology. We have differentiated MDSC from mouse bone marrow cells *in vitro*, incubated them with recombinant Gal1 (MDSC-Gal1), and isolated the exosomes shed (control MDSC-exo or MDSC-Gal1-exo). We have characterized MDSC-exo and their parental cell population by flow cytometry. Interestingly, CD63, a well-established marker of tumoral exosomes, was not detected on MDSC-exo surface. In contrast, we have detected high expression of MHC II on MDSC-exo surface. Notably, Gal1 induced

heightened PD-L1 and IDO expression on MDSC-exo ($p < 0.05$ and $p < 0.01$, respectively). However, other immune checkpoints expression as LAG 3 and VISTA was not detected on MDSC-exo surface. By using an in-house-developed array of conjugated plant lectins we have evaluated the glycosylation signature of MDSC-exo by flow cytometry and found higher exposure of asialo-core-1-glycans, a preferred ligand of Gal1, on MDSC-Gal1-exo compared to control MDSC-exo. Accordingly, the binding of both Gal1 and Gal2 to MDSC-Gal1-exo was higher ($p < 0.05$). To further elucidate Gal1 contribution to MDSC-exo immunosuppression capacity, we have co-cultured mouse activated T cells with MDSC-Gal1-exo or control MDSC-exo. Notably, MDSC-Gal1-exo further inhibited T cell proliferation and activation compared to control MDSC-exo in a dose-dependent manner ($p < 0.001$). **In conclusion, we have described for the first time Gal1 effect on the** immunosuppressive capacity of MDSC exosomes.

239. (601) **INFLUENCE OF THE NON-NEURONAL CHOLINERGIC SYSTEM ON THE CROSS TALK BETWEEN THE IMMUNE SYSTEM AND GLIOBLASTOMA MULTIFORME**

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Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. This tumor is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, robust angiogenesis, resistance to apoptosis and genomic instability. Acetylcholine (ACh) is a neurotransmitter, which can also modulates cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells, which has been referred to as a "non-neuronal cholinergic system". The aim of this work was to elucidate the relevance of the non-neuronal cholinergic system in the tumor of glioblastoma and in the interaction between immune and GBM cells.

Human U251 and U373 GBM line cells showed the expression of the coactivation marker ligand OX40 ligand (OX40L) and apoptosis has assessed by flow cytometry, both lines have basal expression of OX40L and it was not modulated by cholinergic system; but the apoptosis was increased ($p<0.05$) when the cells were cultivated with cholinergic agonist. On the other hand, in order to evaluate whether the cholinergic system modulate the cross-talk with immune cells, human dendritic cells (DC) were differentiation monocyte CD14+ isolated by positive selection from peripheral blood of healthy adult volunteer and patients with GBM and then were cultured with GM-CSF and IL-4 during 5 day. It was observed an increase in the % of + cells of OX40L and HLA-DR when the DC of patients with GBM are treated with cholinergic agonist. Conversely, macrophages M2 were co-cultures with GBM line and incubated in the presence of cholinergic agonist and we observed a decreased in the expression HLADR. Conclusions: our findings suggest that the non-neuronal cholinergic system is present in GBM cells and could modulate their cross-talk with the immune system.

INMUNIDAD INNATA

240. (015) **INVOLVEMENT OF TNF-A IN THE ACTIVATION OF GAMMA DELTA T LYMPHOCYTES BY HUMAN GLOMERULAR ENDOTHELIAL CELLS EXPOSED TO SHIGA TYPE 2 TOXIN. ROLE IN THE PATHOGENESIS OF HEMOLYTIC UREMIC SYNDROME**

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The hemolytic uremic syndrome (HUS) associated with diarrhea, a consequence of Shiga toxin (Stx)-producing *Escherichia coli* infection, is a common cause of pediatric acute and chronic renal failure. Stx type 1 and type 2 (Stx1 and Stx2) produced by those bacteria are the main factors related with HUS that trigger kidney damage. Stx2-producing strains are associated with severe cases of HUS in Argentina. γδ T cells are a specialized subset of T lymphocytes, which act as early sensors of cellular stress and infection. They can exert cytotoxicity against infected and transformed cells and produce cytokines and chemokines. In this work, we studied the activation of human peripheral γδ T cells in response to human glomerular endothelial cells (HGEC) exposed to Stx2 (0.01 ng/ml, 24 h) or their conditioned medium. We analyzed CD69, CD107a, and perforin expression by flow cytometry; and cytokine production by ELISA and intracellular staining in γδ T cells after incubation with Stx2-treated HGEC or their conditioned medium. We evaluated by confocal microscopy, the interaction between γδ T cells and HGEC treated or not with Stx2 and perforin distribution. As result, we observed an increase in TNF-α, IFN-γ, and cell interactions ($p<0.05$) in γδ T cells cultured with Stx2-treated HGEC, and perforin polarization; but without changes in CD69. Moreover, γδ T cells incubated with Stx2-treated HGEC conditioned medium showed an increase in TNF-α and IFN-γ production, and CD107a expression and a decrease in intracellular perforin ($p<0.05$). Interestingly, the blockage of TNF-α by Etanercept reverted the increase in TNF-α, IFN-γ, and CD107a, and the decrease in perforin ($p<0.05$) in γδ T cells incubated with Stx2-treated HGEC conditioned medium. Our results indicate that soluble factors released by Stx2-stimulated HGEC modulate the activation of γδ T cells, being TNF-α a key player during this process. This suggest that γδ T cells could be involved in the renal endothelial damage in SUH.

241. (034) **ALVEOLAR MACROPHAGES DEPLETION AFFECTS THE ABILITY OF *DOLOSIGRANULUM PIGRUM* 040417 TO PROTECT INFANT MICE AGAINST PNEUMOCOCCAL INFECTION**

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Previously, we demonstrated that the nasal administration of the respiratory commensal bacterium *Dolosigranulum pigrum* 040417 (Dp04) to infant mice differentially modulates the respiratory immune response triggered by Toll-like receptor (TLR)-2 activation, increasing the resistance to *Streptococcus pneumoniae* (Sp) infection. The nasal priming with Dp04 reduced pneumococcal counts in lung and blood and diminished the levels of lung injury biomarkers. In this work, we aimed to characterize the role of alveolar macrophages (AM) on the immunomodulatory properties of Dp04 in the context of pneumococcal infection. In the first set of experiments, mice were

nasally stimulated with Dp04 (10^8 cells/mouse/day) for 5 consecutive days and then challenged with 10^6 CFU of Sp. Variations in numbers and functionality of resident AM in broncho-alveolar lavage (BAL) samples were evaluated. The number of activated CD11c$^+$SiglecF$^+$MHC-II^{hi} AM was significantly increased after Sp challenge in mice primed with Dp04 than in controls ($p<0.05$). Furthermore, AM obtained from Dp04-treated mice produced *in vitro* higher levels of IFN-β and IFN-γ, as well as IL-10 and IL-27 compared to the control group ($p<0.05$). In a second set of experiments, AM were depleted using liposomes containing clodronate (CLP) before the stimulation of with Dp04. The CLP treatment significantly affected the ability of Dp04 to reduce pneumococcal cell counts, as well as lung injury biomarkers. In addition, AM depletion impaired the capacity of Dp04 to differentially modulate the cytokine profile in the respiratory tract. The ability of Dp04 to increase the levels of BAL IFN-γ, IL-10 and IL-27 in response to Sp infection was abolished when AM were depleted by CLP ($p<0.05$). This results show for the first time that AM have a relevant role in the immunomodulatory effect of Dp04. Our results also mark a significant advance in the positioning of Dp04 as a next-generation probiotic for the respiratory tract.

Key words: Alveolar macrophages, clodronate liposomes, respiratory commensal bacteria, innate respiratory immunity.

242. (092) **MICROVESICLES FROM GLIOBLASTOMA CELL LINE ACTIVATE ΓΔ T LYMPHOCYTES**

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Glioblastoma multiforme (GBM) is the most aggressive malignant type of cerebral tumor in adults and has a median survival of less than a year after diagnosis. GBM is refractory to standard treatments such as gross total resection, followed by radiotherapy and chemotherapy because of its infiltration nature. Therefore, current therapies are only a temporary and limited solution. However, there are new immunotherapy approaches to GBM and one of them involves the adoptive transfer of γδ T cells. This subset of T lymphocytes expresses a restricted repertoire and can recognize stressed and malignant cells, and induce their apoptosis. Within the peripheral blood γδ T cells, those that express TCRVγ9Vδ2 chains constitute the main subsets of γδ T cells in a healthy human. We recently demonstrated that soluble factors released by GBM cells activate γδ T lymphocytes by inducing a Th1-like profile. Moreover, it is well known that tumor cells can secrete extracellular vesicles, among them, microvesicles (MV), which contain molecules that regulate tumor microenvironment to allow their growth and progression. For those reasons, in this work we aimed to evaluate whether MV, as released factors derived from human GBM cells, were involved in the modulation of γδ T lymphocytes' functionality. For this purpose, γδ T cells were purified from human peripheral blood mononuclear cells, by using an anti-TCR γδ MicroBead isolation kit. After purification, γδ T cells were incubated for 24 hours with MV obtained from GBM U373 cell line by differential centrifugation. After incubation, we analyzed the activation state of γδ T cells by measuring the expression of CD69 by flow cytometry, and the production of TNF-α by ELISA. Our results indicated that U373-derived MV induced an increase in the expression of CD69 ($p<0.01$, n= 6) and TNF-α secretion ($p<0.05$, n= 5) in γδ T cells. Our finding suggests that the GBM-derived MV obtained from the cell line U373 can activate γδ T cells.

243. (128) **CERAMIDE 1-PHOSPHATE SKEWS HUMAN MACROPHAGES´ FATE TOWARDS A M2-LIKE PHENOTYPE BY RESTRAINING THE M1**

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Ceramide 1-Phosphate (C1P) is a bioactive sphingolipid released from dying cells after inflammation, increasing locally in the damaged tissue. C1P exerts many biological effects depending on the cell type, including the downregulation of inflammatory mediators and activation markers. Given that macrophages are critical players for both, resolution of inflammation and tissue restoration, here we aimed to decipher the effect of this sphingolipid on human monocytes/macrophages (Mø) under inflamed tissue conditions and predict the Mø fate and behavior. Human CD14+ monocytes (Mo) were isolated from PBMCs of healthy donors, cultured with RPMI+10% FBS + M-CSF (50ng/ml), and stimulated with C1P short chain analog C8-C1P (1 and/or 20μM), together with naturally occurring inflamogens, lipoteichoic acid (LTA) and hyaluronate (HA); the immune-characterization was performed by flow cytometry and qPCR. Principal Component Analysis (PCA) was carried out including all evaluated parameters; for correlation significance, $p\leq0.05$ was considered. Firstly, C1P-primed Mo (1, 20 or 1+20 μM) gave rise to transcriptionally different Mø compared to the untreated cells. The more explicative dimensions (Dim1= 26.2% & Dim2=20.9%) predict that PDGF, MER, FGF2, PPARG, LXRA, TGFB1, MMP9, VEGFA, and GAS6 are the more contributing variables that segregate C1P-treated from non-treated cells. In addition, when Mo were also challenged with LTA and HA and polarization markers were considered, CD206+CD163+, CD64+CD206/CD163- percentages, CD163, CD206 and CD11b MFI and mRNA levels of MER were referred as the main variables explaining 55.6% of total variation (Dim1= 36.5% and Dim2 = 19.1%). These findings highlight that monocytes primed with a high concentration of C1P, and under inflamed tissue conditions would skew macrophages to a pro-resolving program over the inflammatory license. Integrating, C1P is a key messenger also in macrophages to promote pro-inflammatory deactivation and tissue regeneration.

244. (164) **IL-6 SECRETED BY *BRUCELLA ABORTUS*-INFECTED ASTROCYTES INDUCES PHAGOCYTOSIS OF VIABLE NEURONS THROUGH BYSTANDER ACTIVATION OF MICROGLIA**

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Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. We have previously demonstrated that soluble mediators released by *B. abortus*-infected astrocytes induces an inflammatory state in microglia and this bystander activation elicit neuronal death in primary neurons/microglia co-cultures. The aim of this work was to investigate the underlying mechanisms of this phenomenon. Primary cultures of murine astrocytes were infected or not with *B. abortus* for 24 h. After that, cell-free culture supernatants were used to stimulate primary co-cultures of murine neurons/microglia during 48 h. Neuronal density was evaluated by fluorescence microscopy. Blocking the phagocytic receptor vitronectin in microglia using the cRGD peptide (an antagonist specific for this receptor) was sufficient to prevent neuronal loss ($p<0.005$) without accumulation of apoptotic neurons and without inhibiting microglia activation (evaluated by secretion of TNF-α and proliferation; $p>0.05$) in co-cultures treated with supernatants from infected astrocytes. Our previous results demonstrated that IL-6 secreted by *B. abortus*-infected astrocytes is at least one mediator involved in neuronal death through bystander activated-microglia. Neutralization of IL-6 in culture supernatants of infected astrocytes did not change microglial proliferation (assessed by microscopy; $p>0.05$) or microglial secretion of TNF-α (measured by ELISA; $p>0.05$), although did cause a reduced phagocytic activ-

ity of microglia (evaluated by phagocytosis assay with negatively charged fluorescent 5 µm beads; p<0.05). In summary, our results indicate that neuronal death induced by bystander activation of microglial cells occurs by vitronectin receptor-mediated phagocytosis of viable neurons through IL-6. Bystander activation of non-infected microglia could be a mechanism involved in neuronal death during neurobrucellosis.

245. (170) **A PLANT EXTRACT FROM SMILAX CAMPESTRIS (SME) INDUCES MITOPHAGY AND REDUCES ARSENIC-INDUCED PRO-INFLAMMATORY EFFECT IN HUMAN KERATINOCYTES**

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Smilax campestris is an Argentinian plant traditionally used to treat inflammatory skin disorders. Arsenic causes a skin disease known as HACRE and mitochondrial damage with pro-inflammatory effects. Mitophagy (MF) ensures mitochondrial quality. We have previously shown that arsenic trioxide (ATO) increases IL-1 secretion in human keratinocytes (HaCaT) that can be reduced by drug-induced MF. Here we explore if an aqueous extract of Smilax campestris (SME) induces MF and reverts ATO-induced IL-1 production in HaCaT cells. Mitochondrial mass (MM) was evaluated with the probe NAO by flow cytometry. MF was assessed with Hoescht, lysotracker, NAO, expression of Emerald Green-p62, and a mitochondria-targeted tandem EGFP-RFP protein (mtTan), by image cytometry. IL-1 levels were measured by ELISA. We observed that SME (0.1-10µM) decreased MM. ATO-treated cells showed decreased IL-1 production when co-treated with SME (p<0.005). SME (5-10µM) decreased MM in ATO-treated cells (p<0.01). SME increased rounded mitochondria, often associated with MF (p<0.001). SME-treated cells showed increased colocalization of lysosomes and mitochondria (p<0.001); colocalization was decreased by chloroquine (CQ), indicating inhibition of MF (p<0.001). 10µM SME induced bright spots in cells expressing p62EG (p<0.001) and decreased colocalization of red and green fluorescence in cells expressing mtTan indicating induction of MF (p<0.001). ATO-treated cells also showed increased colocalization of lysosomes and mitochondria (p<0.001). CQ caused no change in colocalization rate, which indicated blockage of MF in ATO-treated cells. In contrast, ATO-treated cells exposed to SME showed decreased colocalization with CQ (p<0.001), indicating that SME restored MF. In conclusion, SME induced mitophagy in untreated keratinocytes, and decreased IL-1 levels, decreased MM, and restored mitophagic flux in ATO-treated cells. SME improves mitochondrial quality and restrains the pro-inflammatory effects of ATO.

246. (198) **EFFECTORS MECHANISMS OF INNATE CD8+ T CELLS (T_{IM} CELLS)**

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Innate $CD8^+$ T cells (TIM cells) mature in the thymus as a different lineage from conventional simple positive $CD8^+$ thymocytes and are exported to SLO as a conventional T cell. TIM cells play a protective role during the early phase of infectious processes as reported for certain bacteria, viral and parasite infections. We have previously reported that thymi from *T. cruzi*-infected mice are highly enriched on TIM cells. Functionally, TIM cells act in a TCR-independent way but can exert their cytotoxic capacity through the release of perforin/granzyme. It is also postulated that TIM cells can induce cell death through the killing receptor NKG2D. NKG2D recognizes infected cells expressing different families of ligands, especially RAE-1 receptors. However, this cytolytic mechanism is still poorly described. We evaluated the killing capacity of a bulk population of thymocytes obtained from *T.cruzi*-infected or control mice (efectors) when co-cultured with peritoneal macrophages (PM) infected with *T.cruzi* (targets). As a read-out we evaluated 48h later, the number of parasite either inside macrophages (by IF) or in the culture supernatants 72h later. In both cases, we observed a reduced number of parasite when macrophages were co-cultured with *T.cruzi*-infected thymocytes (<0,05).

Interestingly, PM stimulation with different TLR agonists demonstrate up-regulation of RAE-1γ only after PolyI:C but not after LPS or PGN stimulation (<0,05). Also, PM obtained from *T.cruzi*-infected mice show significantly higher RAE-1γ expression than PM from control mice (<0,05) becoming a possible target of $NKG2D^+ T_{IM}$ cells. Our data intend to contribute in the understanding of the effectors mechanisms of T_{IM} cells.

247. (204) **PURINERGIC SIGNALING MODULATES NEUTROPHILS FUNCTION IN CHILDREN WITH COVID-19**

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BACKGROUND: Evidence implicates neutrophils in the pathophysiology of severe COVID-19. Activated and injured cells release their stores of ATP to the extracellular space, with ATP being generally excitatory to most cells trough purinergic receptors. Here we analyzed plasma levels of ATP in children with COVID-19 and the ability of extracellular purines to modulate the functions of neutrophils from children with COVID-19.

METHODS: Sixty acute COVID-19 infected children (1-16 years-old) and 20 healthy children (HC) were studied. Levels of ATP were measured in plasma by Luminometry. Purified neutrophils were exposed to ATP and/or adenosine agonist. Phenotype and ROS production were analyzed by flow cytometry. NETs release was measured by Fluorometry. Levels of IL-8 were quantified by ELISA.

RESULTS: We found higher levels of ATP in plasma from COVID-19 patients, mainly in those with pneumonia compared with HC (p<0.01) and increased expression of ectoenzyme CD39 in neutrophils (p<0.05). When neutrophils were exposed to Bz-ATP during 3 hs, an increased expression of activation marker CD11b (p<0.05) was observed and the expression of inhibitory receptor LAIR-1 (p<0.01) and calprotectin (p<0.05) were higher in neutrophils from COVID-19 children, but not in neutrophils from HC. Importantly, Bz-ATP exposure promoted PMA-induced NETosis in COVID-19 neutrophils (p<0.005) showing no changes in HC. Also, CGS, an adenosine agonist, reduced significantly NETs release (p<0.007). By contrast, Bz-ATP significantly impaired the spontaneous production of IL-8 in neutrophils from patients enhancing the production in controls (p<0.01). While Bz-ATP exposure did not enhance PMA-induced ROS production, CGS promoted its fall in patients (p<0.01).

CONCLUSION: Our results suggest a relationship between the purinergic signaling and the inflammation caused by neutrophils in children with COVID-19. Further studies are needed to assess how targeting purinergic signaling prevent severe disease.

248. (224) **METABOLIC PATHWAYS IN NEUTROPHILS IN A MODEL OF EARLY MATERNALPLACENTAL INTERACTION**

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Trophoblast cells (Tb) interact with maternal immune cells at placentation favoring an anti-inflammatory microenvironment required for fetal growth. Circulating neutrophils (PMN) appear activated during pregnancy and even more in pregnancies complicated by preeclampsia. Metabolic regulation underlies the functional profiling of immune cells in a number of settings but immunometabolic reprogramming in the context of pregnancy is still enigmatic.
We have shown that conditioned media (CM) from human first trimester Tb (Swan-71 cell line) inhibit PMA-induced neutrophil extracellular trap formation, promote PMN apoptosis and induce an angiogenic profile on PMN.
The aim of this work was to explore the effect of trophoblast cell-derived factors in neutrophils' functional and metabolic profile.
Neutrophils from healthy donors were cultured with Swan-71 conditioned media (CM)-2% FCS. PMN activation profiles were assessed by RT-qPCR and flow cytometry. Treg modulation was explored co-culturing PMN with autologous mononuclear cells and CD4, FOXP3 staining. Glucose uptake and intracellular lipid accumulation were determined by flow cytometry using the glucose fluorescent analog 2-NBDG or BODIPY 493/503, respectively.
PMN pre-incubated with CM increased the frequency of double stained CD4+ FOXP3+ cells in co-cultures, compared to 2% serum media (basal) (% X±SE Basal 3.85±0.64 CM 9.97±3.38, $P<0.05$, n=6). Regarding neutrophils' metabolism, PMN cultured with CM increased glucose uptake (MFI X±SE Basal 897.3±195.4; CM 1112±244.8, $P<0.05$, n=10) although to a lower extent than PMA. A trend increase in the glucose specific transporter GLUT1 was observed. CM also favored lipid droplet formation (MFI X±SE Basal 997.7±93.4 CM 1318.0±115.5, $P<0.05$, n=8).
Our results support an immunometabolic programing of neutrophils upon trophoblast cell interaction in an in vitro model of early maternal-placental interface.

249. (259) **IMMUNOBIOTIC NASAL PRIMING CAN ACELERATE THE RECOVERY OF NASOPHARYNX-ASSOCIATED LYMPHOID TISSUE IN MALNOURISHED MICE**

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Malnutrition induces atrophy of mucosa-associated-lymphoid-tissue. However, little is known about the effect of malnutrition on the nasopharynx-associated-lymphoid-tissue (NALT). In addition, immunobiotic lactic acid bacteria and its postbiotics became an interesting and non-invasive alternative for the recovery of the respiratory immune response in malnourished hosts. We demonstrated that *Lactobacillus rhamnosus* CRL1505 peptidoglycan (PG05) increases the infection resistance against *Streptococcus pneumoniae* (Sp) and improves innate immune response in immunocompromised-malnourished mice. The aim of this work was to study the effect of nasal administration of *L. rhamnosus* CRL1505 (Lr05) and PG05 on the recovery of NALT in malnourished mice. Weaned mice were malnourished with a protein-free diet (PFD) for 21d. Malnourished mice received a balanced conventional diet (BCD) during 7d (BCD group) or BCD with nasal supplementation with *L. rhamnosus* CRL1505 (10^8 cells/mouse/day) or PG05 (8μg/mouse/day) during the last 2 days of treatment (BCD+Lr or BCD+PG groups). Malnourished control mice (MC) received PFD while the well-nourished control group (WC) consumed BCD. Histological studies and flow cytometry studies were carried out on NALT to study the impact on B and T cells, dendritic cells, macrophages and myeloid cells. Protein-malnutrition significantly reduced the number of total cells and the different cell populations. These results were related to a NALT atrophy in the malnourished mice. BCD treatment was not able to normalize these parameters. However, the BCD+Lr and BDC+PG groups showed histological characteristics similar to the WC group, and normal numbers of NALT B, T, dendritic cells and neutrophils. These results highlight the importance of NALT as a target for postbiotics and immunobiotics administration to improve respiratory immunity in immunocompromised malnourished hosts.

250. (260) **LACTOBACILLUS RHAMNOSUS CRL1505 AND ITS POSTBIOTIC IMPROVE EMERGENCY MYELOPOIESIS AGAINST RESPIRATORY INFECTION IN IMMUNOSUPPRESSED HOST**

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Emergency myelopoiesis is critical to control infection with pathogens. Patients undergoing chemotherapy have an impairment in hematopoiesis associated with an ineffective immune response against infections. *Lactobacillus rhamnosus* CRL1505 (Lr05) and its cell wall (CW05) diet supplementation has proved to be an interesting alternative to improve steady-state myelopoiesis. In this work we studied the effect of oral administration of Lr05 and CW05 on the emergency myelopoiesis induced by TLR4 agonist in immunosuppressed mice. Adult Swiss-mice were orally treated with Lr05 or CW05 during 10 consecutive days. On day 6, treated and untreated mice received one intraperitoneal dose of cyclophosphamide (Cy 150mg/kg). On day 3 post-Cy injection, mice were challenged intraperitoneal with LPS (10mg/kg weight). At different post-challenge times, the following studies were carried out: 1) recruitment of phagocytic cells (neutrophils and macrophages) in peritoneal lavage; 2) frequency of hematopoietic stem cells (HSCs) ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$), and myeloid multipotent precursors (MMPs) ($Lin^-Sca\text{-}1^+c\text{-}Kit^+CD34^+$) in BM and; 3) release of neutrophils from myelopoietic niches by the expression of CXCR4 and CD62L in BM $Gr\text{-}1^+Ly6G^+Ly6C^-$ cells. The Cy group showed an impaired local innate immune response, a decrease of HSCs and MMPs, and an anchoring of myeloid precursors in BM. However, Lr05 and CW05 treatments were effective to significantly increase peritoneal neutrophils and macrophages, and allow an early HSCs and MMPs recovery with respect to the Cy group. These results were correlated with a decrease in the retention signals in the BM cells of Lr05 or CW05 groups compared to the Cy control. In conclusion, the postbiotic obtained from *L. rhamnosus* CRL1505 is capable of modulating a more efficient local innate immune response. This in turn could be correlated with the modulation of chemokines responsible for stimulating an adequate emergency myelopoietic response in BM against the pathogen.

251. (263) **SHIGA TOXIN (STX)-PRODUCING *ESCHERICHIA COLI* STIMULATES NEUTROPHIL INTERLEUKIN-1 BETA (IL-1B) SECRETION BY A NON-LYTIC CASPASE-1-DEPENDENT MECHANISM**

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) pathogen establishes non-invasive intestinal infections that can cause from diarrhea and hemorrhagic colitis to Hemolytic Uremic Syndrome (HUS); a disease that in Argentina is the most common cause of acute renal failure in early childhood. STEC release Stx in the gut, which upon translocation to the bloodstream reaches target organs like kidneys and brain, being responsible for HUS pathophysiology. This translocation can be facilitated by mucosal damage and promoted by inflammation.

We previously determined that neutrophils (N) isolated from human peripheral blood release Interleukin-1 beta (IL-1β) when exposed to STEC but not to bacterial supernatants. Here we investigated the requirements and mechanisms involved in N IL-1β secretion in response to STEC (serotype O157:H7) by analyzing IL-1β secretion at a multiplicity of infection of 0.5, at which the maximal secretion was previously observed. We found that the STEC, an isogenic mutant of STEC lacking the ability to produce Stx, and a non-pathogenic *E. coli* strain (C600) stimulated IL-1β release. However, IL-1β levels were significantly higher in response to the pathogenic strains independently of their ability to produce Stx ($p<0.05$; n=7). These differences were not due to variations in the capacity of each strain to promote N lytic death, because LDH levels in coculture supernatants were low and similar for all the strains (n=4). N IL-1β secretion required bacterial viability because none of the heat-killed bacterial strains induced IL-1β release ($p<0.05$; n=4). We also found that STEC stimulated N caspase-1 activation (n=4); and accordingly, the caspase-1 inhibitor Ac-YVAD-CMK significantly reduced IL-1β secretion ($p<0.05$; n=4). Altogether, our results suggest that N recruited into the STEC-infected gut might contribute to the inflammatory response triggered by the infection by secreting IL-1β through a mechanism that involves inflammasome activation.

252. (274) **HUMAN PLASMA EXTRACELLULAR VESICLES IMPAIR INFLAMMATORY RESPONSES TO A VIRAL PAMP IN M-CSF BUT NOT GM-CSF MONOCYTE- DERIVED MACROPHAGES**

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Extracellular vesicles are heterogeneous membrane structures, which can modulate normal and pathological conditions. The aim of this work was to study the role of healthy donors' plasma EVs (pEVs) in the modulation of the inflammatory response elicited by a viral PAMP in monocyte-derived macrophages with either inflammatory (GM-MDMs) or resolution (M-MDMs) profiles.
pEVs were isolated from plasma samples by size exclusion chromatography, followed by centrifugation. Human monocytes were isolated from healthy donors' buffy coats via density gradient centrifugation followed by positive isolation with CD14 magnetic beads, and differentiated with M-CSF or GM-CSF for 7 days. Macrophages were stimulated with the TLR 7/8 agonist resiquimod (RSQ) for 24 h in the presence or absence of pEVs. The production of cytokines in cell culture supernatants was evaluated by cytokine bead array and ELISA. Characterization and purity of EVs were assessed by western blotting. Several signaling pathways were studied by phospho array and western blot assays.
EV-treatment of M-MDM exposed to RSQ significantly reduced the secretion of IL-6 and TNF and increased the secretion of IL-10, as compared to only RSQ treated cells. In contrast, GM-MDMs co-stimulated with RSQ and EVs presented an increased IL6 response while no changes were observed in TNF or IL10 secretion. Signaling analyses further performed on M-MDMs indicated that EV-treatment reduced phosphorylation of activation pathways, such as MAPK and AKT.
pEVs appear to have an homeostatic role on M-MDMs exposed to a viral PAMP by reducing their inflammatory response. In contrast, pEVs are not able to modulate pro- inflammatory GM-MDMs. Further studies are needed to identify the cargo molecules responsible for these effects. Understanding in-vivo implications of these findings may lead to new therapies development for chronic inflammation.

253. (278) **HUMAN PLASMA EXTRACELLULAR VESICLES INDUCE AN ALTERNATIVE/GROWTH-PROMOTING PHENOTYPE IN M-CSF MACROPHAGES EXPOSED TO RESIQUIMOD VIA PGE2 SIGNALING**

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Extracellular Vesicles are cell-derived membranous structures important in cell communication, capable of modifying recipient cells' responses due to their different cargoes. We previously showed that plasma EVs (pEVs) dampen inflammatory cytokine response of M-CSF macrophages exposed to a viral PAMP, having a possible role in tissue homeostasis after an immune response.
Herein, we aim to characterize the phenotype of pEV-treated M-CSF macrophages exposed to a viral PAMP and to investigate whether PGE2, a known regulatory mediator, could be implicated in this phenotype.
pEVs were purified from healthy donor plasma by size-exclusion chromatography followed by ultracentrifugation, and characterized by western blotting (WB). Human monocytes were isolated from healthy donors' buffy coats via density gradient centrifugation followed by positive isolation with CD14 magnetic beads, and differentiated with M-CSF for 7 days. Resiquimod (RSQ), a TLR-7/8 agonist, was used as a viral PAMP. Apoptotic-cell phagocytosis was evaluated by flow cytometry. Expression of selected genes was assessed by qPCR. Phosphorylated signaling proteins were detected by WB. PGE2 was detected by a competitive assay.
Macrophages exposed to RSQ in the presence of pEVs showed increased apoptotic-cell phagocytosis and expression of VEGFa, CD300e, RGS2 and THBS1 transcripts at 4h and 24h. They also presented higher levels of PGE2 in 24h-supernatants, and augmented pCREB at 20 min post-stimuli.
To conclude, pEVs promote a growth-promoting and wound-healing phenotype on M- CSF macrophages exposed to RSQ, which may contribute to inflammation resolution. Although further studies are needed, we propose that by stimulating PGE2 production and CREB mediated transactivation, pEVs dampen macrophage activation following an encounter with a viral PAMP.

254. (281) **CHARACTERIZATION OF A NANOPARTICLE-BASED VACCINE**

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Nanotechnology plays an important role in vaccine development. It offers the opportunity to design different functional nanoparticles based on different compositions, sizes, shapes and surface properties and for applications in biomedicine. This work aimed to characterize nanoparticles as a safe vehicle and adjuvant for further use in immunotherapies.
Polycationic nanoparticles (Np) were characterized using human and murine antigen- presenting cells (APCs). Nanoparticle-driven cell interaction was evaluated by fluorescence microscopy (internalization and localization), flow cytometry (cell activation-MHCII and CD86 expression) and ELISA (IL-1β secretion). Furthermore, Balb/c mice were intraperitoneally and intranasally administered with Np-OVA and the pharmacokinetic was monitored using fluorescent Np. Finally, humoral (serum antibodies) and cellular immune responses (cell subsets and cytokines) were evaluated by ELISA and flow cytometry.
We found that Np were internalized and cells became activated, showing increased expression of CD86 and secretion of IL-1β. Experiments in cell lines showed that Np promoted a higher cell stimulation with enhanced IL-1β secretion than the positive control with an inflammasome activator ($P<0.05$). The IL-1β production was abrogated with different inflammasome inhibitors. In vivo experiments showed that Np protected OVA through the mucosa passage, with a significant induction of serum OVA-specific IgG, increased secretion of IFN-γ by spleen cells and high frequency of LT CD4+IFN-γ+ and LT CD8+IFN-γ+ cells ($p<0.05$).
In conclusion, we found that APC internalized Np and activated the inflammasome pathway with IL-1β secretion. Remarkably, this nanoparticle exhibited adjuvant properties for mucosal targeting to induce Th1-dependent immunity that could be exploited in preventive or therapeutic vaccine development for infectious and non- infectious diseases, respectively.

255. (285) **SENSITIVE ASSAYS FOR MONITORING NUCLEOLAR C23 IN THE CYTOSOL AND ENDOMEMBRANES OF MYCOBACTERIUM AVIUM-INFECTED MACROPHAGES AND TO STUDY ITS CLEAVAGE, KINASES AND INTERACTING PARTNERS**

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We had detected by cell-free in vitro proteome radiolabeling (RL) screening a decreased level of full-length nucleolar C23 but in the cytosol of M. avium-infected macrophages. C23 cytosolic roles were unknown in innate immunity, so, we searched alterations in proteins likely connected to C23 such as: a) CK2 and CDK kinases, b) p21waf1 (as partner of C23, CK2 and CDK), c) Pin1 as CDK target site phospho-dependent isomerase. We optimized assays to study CK2 and CDK as C23 kinases. Differentiated THP-1 cells ingested live or heat-killed M.avium. After time-courses, cells were harvested and cytosolic and membrane proteomes radiolabeled in reactions with or without inhibitors or substrates, then gel-resolved by 1D and 2D to analyze C23 RL level. Kinases activities were assayed by peptides containing C23 sites. Reproducible (in >95% replicates) significant time-dependent events were considered. The n for assays or for cell treatments was 5 to 9. We found that: 1) C23 downregulation quantitated sensitively by RL was likely caused by cleavage at 2 sites, not due to altered CK2 or CDKs and initially independent on bacterial viability. 2) cytosolic C23 was iv labeled by CK2 but not CDKs. 3) by WB, cytosolic p21 had cleavage. 4) C23 cleavage occurred also in endomembranes. 5) Pin1 was not controlling CDK sites in vitro. 6) C23 had different phospho-pattern in monocytes and macrophages. We conclude that the decreased C23 IV RL was due to cleavage but not kinases or Pin1. Since M. avium did not induce macrophage death, C23 cleavage might be a marker of sustained apoptosis signaling not resulting in death. Apoptotic bacteria should be compared. Our methods will allow finding protein complexes and innate receptors inducing p21 and C23 cleavage. C23 RNA-binding phosphoprotein might be a checkpoint hub integrating p21, CDK and CK2 with innate receptors and with pathways in nucleolus, membranes and cytosol. Infection cleaved C23, so, its fragments and other PTMs should be studied

256. (308) **DIRECT AND INDIRECT PROMOTION OF MYELOPOIESIS MECHANISMS BY POSTBIOTICS OBTAINED FROM LACTOBACILLUS RHAMNOSUS CRL1505**

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Many attempts have been made to find safer immunomodulatory agents that enhance the immune response and reduce the number and severity of infections in at-risk populations. Our previous studies have shown that *Lactobacillus rhamnosus* CRL1505 (Lr05) and its postbiotics, peptidoglycan (PG05) and cell wall (CW05), were able to improve bone marrow (BM) myelopiesis and to protect against respiratory pathogens in mice undergoing chemotherapy. However, the underlying mechanisms remain unknown. Hence, the role of TLR2 and G-CSF involved in the ability of Lr05, P05 and CW05 to induce basal myelopoiesis by direct or indirect interaction with BM hematopoietic stem and progenitor cells (HSPC) was evaluated. First, *in vitro* colony-forming unit assays were performed to assess whether the clonogenic capacity of BM cells responds to direct interaction with Lr05 and its postbiotics. For this, mouse BM cells were plated in the presence or absence of Lr05, PG05 or CW05 in culture medium for the granulocyte/macrophage forming unit (CFU-GM) (MethoCult™ GFM3534). The counts and the phenotypic characterization of the colonies obtained were determined. Besides, the effect of the addition of fibroblast supernatants conditioned by Lr05 or its postbiotics on the clonogenic activity of HSPC was investigated. Finally, the expression of TLR2 of CFU-GM and the levels of G-CSF in the culture medium on day 14 were determined by flow cytometry and ELISA, respectively. Lr05 significantly stimulated the TLR2 expression and secretion of G-CSF, and enhanced the clonogenic activity of HSPC and fibroblast. Interestingly, CW05 showed a strong stimulatory effect while PG05 showed immune effects that were more similar to Lr05. These results allow us to know, at least in part, the cellular and molecular mechanisms involved in the myelopoiesis-enhancing capacity of new safe products to be potentially used in patients undergoing chemotherapeutic treatment.

257. (313) **ZIKA VIRUS NS4B PROTEIN TARGETS TANK-BINDING KINASE 1 TO INHIBIT TYPE I INTERFERON PRODUCTION**

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Type I interferons (IFN I) play an essential role in antiviral innate immunity. During viral infections, cytosolic nucleic acids can be sensed by intracellular pattern recognition receptors, triggering TANK-binding kinase 1 (TBK1)- interferon regulatory factor 3 (IRF3) signaling axis to initiate IFN I transcription. However, many flavivirus use non-structural proteins to evade immune sensing favoring their survival. Here, we aimed to study the role of Zika virus (ZIKV) NS4b protein in the inhibition of IFN I induction and its interaction with host ligands.

For this purpose, we performed transfection assays with a plasmid encoding recombinant ZIKV NS4b or ZIKV NS4b C100S mutant. Using RAW-Lucia ISG cells, an IFN reporter cell-line, we showed that cells with ZIKV NS4b were able to reduce luciferase signals compared to empty vector. Interestingly, this reduction was abrogated with ZIKV NS4b C100S mutant (ANOVA+Tukey´s, $p<0.05$). Moreover, A549 cells transfected with plasmid encoding ZIKV NS4b and stimulated with poly(I:C) secreted less IFN-β levels (ELISA) compared to control (ANOVA+Tukey´s, $p<0.05$).

TBK1, a key component in IFN I production, has been proposed as a possible target of NS4b. Using transfection assays in Hela cells, we showed that TBK1 immunoprecipitated with ZIKV NS4b. Furthermore, we recombinantly produced N-terminal ZIKV NS4B in micelles and human TBK1. We performed Surface Plasmon Resonance (SPR) assays to further characterize this interaction. SPR assays showed that NS4b interacted with TBK1 with an equilibrium dissociation constant (KD) of 3.1 ± 0.2 μM.

Our results add evidence that ZIKV NS4b is involved in disrupting TBK1/IRF3 cascade and the conserved residue C100 is important for this function. Besides, this is the first report of biophysical interaction between N-terminal ZIKV NS4b and TBK1. Altogether, the information gathered herein can be of substantial use in the rational design of antiviral inhibitors.

258. (355) ***MINTHOSTACHYS VERTICILLATA* ESSENTIAL OIL ORALLY ADMINISTERED MODULATES GASTROINTESTINAL PROINFLAMMATORY PARAMETERS IN MICE**

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Farm animals are exposed to stressors that alter oxidative and immunological balance, affecting the gastrointestinal system. *Minthostachys verticillata* essential oil (EO) has shown antioxidant and immunomodulating activities and could be a natural alternative to improve animal health. The aim of this study was to evaluate the impact of EO oral administration on gastrointestinal proinflammatory parameters. For this purpose, three groups of male Balb/c mice (n=3) were orally administered with saline solution (control group) and EO (5 or 10 mg/kg/day) during 10 consecutive days. Subsequently, histological parameters, cytokines production and oxidative markers were evaluated. The results indicated that EO (5 mg/kg/day) improved mice growth performance compared to control and EO (10 mg/kg/day) groups ($p<0.05$). EO did not alter the morpho-physiology of intestine, however a moderate leukocyte infiltration in the small intestine could be observed in mice treated with EO (10 mg/kg/day). No differences in colon sections were observed between groups. EO decreases the IL-6 levels and increases the IL-4 and IL-10 concentrations compared to control group ($p<0.05$). EO improved total antioxidant capacity by decreasing malondialdehyde (MDA) concentrations, however also decreased the enzymatic activity of superoxide dismutase (SOD), compared to control group ($p<0.05$). Results indicate that *M. verticillata* EO modulate inflammatory and oxidative parameters constituting a natural alternative which could be applied as dietary supplement to improve gastrointestinal and immune functionality of farm animals.

259. (389) **REGULATION OF VIRULENCE FACTORS IN STAPHYLOCOCCUS AUREUS BY HOST INFLAMMATORY MEDIATORS**

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Staphylococcus aureus success as a human pathogen depends on the coordinated expression of several virulence factors and its ability to adapt to environmental changes. The microenvironment where the infection develops is rich in cytokines and chemokines produced by the host in response to the bacteria, however, the direct effect of these inflammatory mediators on *S. aureus* has not been elucidated. The aim of this study was to determine if *S. aureus* is able to sense host's IL-1β and IL-8 and the impact of these molecules in the expression of staphylococcal virulence factors. We first explored the binding of IL-1β or IL-8 to *S. aureus* by flow cytometry incubating the bacteria with the recombinant interleukins and ulterior labeling with specific antibodies. An increase in the mean fluorescence intensity was observed in a concentration-dependent manner, reaching maximum levels with 1ng of IL-1β and 1.5ng of IL-8 per $5x10^6$ CFU of *S. aureus*. We then evaluated the hemolytic and coagulase activity of *S. aureus* culture supernatants grown in the presence or absence of IL-1β or IL-8. For both, IL-1β ($p<0.01$) and IL-8 ($p=0.068$), a decrease in the hemolysis of rabbit red blood cells was observed compared with the control supernatant (Student *t* Test). IL-8 also induced a significant decrease in the coagulase activity of *S. aureus* ($p<0.05$, Student *t* Test). To determine the regulatory factors involved in the response to IL-1β and IL-8, we analyzed the expression levels of the global regulators RNAIII (effector of *agr*) and *sae*RS when bacteria was grown in the presence or absence of the IL-1β or IL-8. IL-1β induced a significant decrease in RNAIII ($p<0.05$) and *sae*RS ($p<0.001$) levels but, interestingly, no effect was observed in the presence of IL-8 (Student *t* Test). Taken together, our results indicate that IL-1β and IL-8 bind to *S. aureus* and induce different modifications in global regulators that affect bacterial toxicity and may contribute to adaptation during infection.

260. (391) **ANTI-INFLAMMATORY EFFECT OF RIFAMPICIN AND DEHYDROEPIANDROSTERONE ON A MACROPHAGIC HUMAN CELL LINE**

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Tuberculosis (TB) is a major health problem worldwide. The etiologic agent, *Mycobacterium tuberculosis* (Mtb) is transmitted by air and captured by lung macrophages (Mf). Mf activation along with an efficient cellular immune response is required for Mtb elimination, which at the same time can mediate tissue damage. We previously found that TB patients at the time of diagnosis showed an immune-endocrine imbalance: high plasma levels of pro- and anti-inflammatory mediators and cortisol, as well as lowered Dehydroepiandrosterone (DHEA) levels. During the specific anti-TB treatment, the proinflammatory mediators and DHEA levels reach values like those found in healthy controls. Rifampicin (R) is a potent antimicrobial agent and a major drug in TB treatment, its antibacterial activity is mediated by the inhibition of bacterial RNA polymerase. There is evidence that R also modulates the host immune response, influencing lymphocyte migration, cytokine production and phagocytosis. In a previous study on a Mf cell line, DHEA treatment decreased the colony-forming units of Mtb even in the presence of stressful and physiological doses of cortisol, an effect related to an increase in the number of autophagosomes. Given this background, we now investigated whether R, with or without DHEA, could affect the functional capacity of Mf (adherent human THP-1 cells, activated with PMA). TB patients under treatment show R plasma levels between 8–24 μg/ml. In a dose response study (5, 10, 15, 20, 25 μg/ml) the 15 μg/ml dose was selected. When Mf were treated with R or DHEA (10^{-6}M, 10^{-7}M, 10^{-8}M), there were no differences in supernatants levels of the pro-inflammatory cytokine IL-1β. However, the addition of R+DHEA significantly decreased IL-1β production regarding Mf untreated cultures ($p<0.05$ for all DHEA doses). Rifampicin, in addition to its antibacterial effect contributes with DHEA anti-inflammatory response.

261. (395) **SHIGA TOXIN INDUCES NETOSIS IN PMN THROUGH NOX-DEPENDENT MECHANISM**

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Hemolytic Uremic Syndrome (HUS), a vascular disease characterized by hemolytic anemia, thrombocytopenia and acute renal failure is caused by enterohemorrhagic Shiga toxins (Stx)-producing bacteria. Besides Stx, inflammatory response mediated by neutrophils (PMN) is essential to HUS outcome. PMN have the capacity to release "neutrophil extracellular traps" (NETs) which are involved in the pathogenesis of several diseases. Particularly, we have previously demonstrated that Stx type 2 (Stx2) is able to induce NETosis in PMN from healthy donors. The aim of this study was to evaluate the mechanisms involved in the NETosis triggered by Stx2 in PMN. To achieve this, PMN were purified from healthy blood donors and incubated for 4 h with RPMI medium alone (Basal), Stx2 (0.1 μg/mL) alone or with commercial inhibitors of NOX2 (DPI, 10 μM) or Elastase (EI, 10 μM). After incubation, supernatants were recovered and the DNA content was evaluated by a fluorometric assay by employing Sybr Gold and the Neutrophil Elastase (NE) activity was assessed by a spectrophotometric assay (absorbance at 405 nm).
We observed a 2-fold increase value of DNA over the control (Basal) after incubation with Stx2 which was impaired by the presence of both inhibitors, (Median (IQR)= Basal: 1; Stx2:1.91(1.46-2.40)*; Stx-2+DPI: 0.97 (0.75-2.36); Stx2+EI:1.16 (0.79-2.18); *p<0.05; n=8). Simultaneously, there was a similar increase in elastase activity upon treatment with Stx2 which was also reduced by both inhibitors (Median (IQR)= Basal:1;Stx:1.89* (1.34-2.63); Stx2+DPI:1.22 (0.57-2.11); Stx2+EI:0.64(0.30-1.03); *p<0,05;n=5.
Our results suggest that Stx2 is able to trigger NETosis by a mecha-

nism that involves the action of NOX2 besides the NE activity.

262. (402) **NEUTROPHIL CYTOKINE RESPONSES INDUCED BY SHIGA TOXIN (STX)-PRODUCING *ESCHERICHIA COLI***

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Infections produced by the enteric pathogen shiga toxin (Stx)-producing Escherichia coli (STEC) can cause from diarrhea and hemorrhagic colitis to Hemolytic Uremic Syndrome. STEC are non-invasive bacteria that colonize the intestine where they release the Stx, which then reaches the bloodstream; an event that appears to be facilitated by damages in the intestinal mucosa and promoted by inflammation. As neutrophils (N) are recruited to the intestine upon STEC infections, our aim was to evaluate whether they contribute to the gut inflammatory response by producing cytokines. We previously determined that STEC (E. coli O157:H7 strain), independently of its capacity to produce Stx, stimulates Interleukin-1β (IL-1β) secretion by human N. Here we evaluated the N cytokine responses when challenged with STEC within a range of multiplicities of infection (MOIs: 0.5-10). We found that IL-1β release was higher the lower the MOI was ($p<0.05$; n=6), indicating that IL-1β-secretory response behaves inversely to the number of bacteria that impact on N. This behavior was not due to N lytic death because low LDH levels were detected in culture supernatants at every MOI evaluated (n=6). TNF-α secretion also was higher at MOI 0.5 and 1 and declined at MOI 10 (n=6; $p<0.05$). Contrastingly, N released huge amounts of CXCL8 at every MOI evaluated, being the levels slightly lower at MOI 0.5 (N=6; $p<0.05$). On the other hand, N also exhibited serine proteases (NSPs) activation in response to STEC (n=4), and in agreement with the requirement of NSPs activation for IL-1β secretion we previously reported in N, the SP inhibitor AEBSF concentration-dependently reduced IL-1β secretion (n=3) without affecting pro-IL-1β synthesis (n=2).

Our results suggest that N recruited to the human gut upon STEC infection might contribute to the development of the inflammatory response that usually triggers the infection, by secreting the pro-inflammatory cytokines IL-1β and TNF-α, and the chemokine CXCL-8.

263. (432) **IMPACT OF SERINE PROTEASES IN HUMAN NEUTROPHIL INTERLEUKIN-1 BETA (IL-1B) SECRETION**

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Neutrophils (N) represent the first line of defense against bacterial and fungal infections. These granulocytes, which outnumber the rest of human immune cells found in circulation, are massively recruited to infectious foci where they contribute to the inflammatory responses by the release of cytokines. Among them is IL-1β, a leaderless protein which is synthesized in the cytosol as an inactive precursor, pro-IL-1β, that after processing is secreted by unconventional mechanisms. We previously determined that in response to LPS+ATP, human N release IL-1β by an autophagy-dependent mechanism and that both caspase-1- (C1) and N serine proteases (NSP)-activation are required for IL-1β secretion. Here we evaluated the role of both kinds of enzymes in pro-IL-1β processing in N isolated from healthy donors' peripheral blood. Our findings employing both ELISA and western blot indicated that inhibition of C1 with Ac-YVAD-CMK (50 μM) reduced IL-1β secretion but did not inhibit pro-IL-1β cleavage. By contrast, inhibition of NPS with AEBSF (0.35 mM) reduced IL-1β secretion and blocked pro-IL-1β processing ($p<0.05$; n=3-5). Supporting these data, LPS+ATP stimulation triggered NSP activation (n=9; $p<0.05$), evaluated by a fluorescent probe and flow cytometry, and their leakage to the cytosol. We also found by employing the fluorescent probe FLICA and flow cytometry, higher levels of active C1 upon NSPs inhibition, suggesting that these enzymes might also control active C1 stability.

In contrast to what is observed in other myeloid cells, these findings suggest that even though C1 is necessary for IL-1β secretion, in human N it is not required for the processing of its precursor. Instead, this role appears to be fulfilled by NSP. These results might have a potential impact on the design of new therapeutic strategies to selectively control inflammation in those scenarios where neutrophilic IL-1β plays a crucial role in the pathogenesis.

264. (442) **EPIDERMAL HOST DEFENSE (ANTIMICROBIAL) PEPTIDE GENE EXPRESSION IN EXPERIMENTAL DERMATOMYCOSIS**

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Host defense antimicrobial peptides (HDP) are small proteins that directly kill or inhibit pathogen growth, modulate inflammation and skin homeostasis. Previously, we demonstrated that type 17 response is crucial to inhibit fungal proliferation after dermatophyte infection in C57BL/6 mice and, in the absence of a functional IL-17 pathway in IL-17RAKO mice, fungal burden was significantly increased compared to WT mice. However, the IL-17-mediated effector mechanisms that inhibit dermatophyte growth and the role of HDP remain undefined.

The aim of this work was to evaluate the gene expression of the HDP β-defensins 2, 3 and 14 and calprotectin (S100A9) in the epidermis of C57BL/6 and IL-17RAKO mice at early time-points of *Nannizzia gypsea* infection.

C57BL/6 (WT) and IL-17RAKO mice were epicutaneously infected with a *N. gypsea* mycelia suspension (infected group) or treated with sterile saline solution (uninfected controls). On day 1 or 3 post-infection (dpi), skin was trypsinized, mRNA was extracted, cDNA was generated and used for quantitative PCR with primers for *mBD2*, *mBD3*, *mBD14*, *S100A9*, *GAPDH* and *β-actin* genes.

Dermatophyte-infected WT mice expressed lower levels of *mBD2* compared to uninfected controls by 1 dpi ($p<0.01$) and remained decreased by 3 dpi ($p<0.05$) along with diminished *mBD3* and *mBD14* expression ($p<0.0001$, $p<0.001$, 3-day infected mice vs controls). On the contrary, infected IL-17RAKO mice early expressed *mBD2* and *mBD14* mRNA by a 5- and 6-fold change respectively ($p<0.0007$, $p<0.0012$, compared to controls) and continued to be upregulated by 3 dpi ($p<0.0001$, $p<0.0007$, respectively) along with an increased expression of *mBD3* ($p>0.003$) and *S100A9* ($p>0.0001$), compared to controls.

These data suggest that, in our model, the susceptibility to fungal skin infection in IL-17RAKO mice is uncoupled from *mBD2*, *mBD3*, *mBD14 and S100A9* expression. Probably, the elevated fungal burden observed in IL-17RAKO induces HDP through IL-17 signalling-independent mechanisms.

265. (455) **PARTICIPATION OF INTERFERON GAMMA IN THE LEUKOCYTE RECRUITMENT TO THE CENTRAL NERVOUS SYSTEM IN LPS-INDUCED NEUROINFLAMMATION**

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Introduction: microglial cells are brain immune components in charge of maintaining the homeostasis and protecting the brain parenchyma against injuries.
Peripheral leucocytes are recruited to the central nervous system (CNS) in response to cytokines and chemokines during neuroinflammatory processes. If the response is not properly controlled, that recruitment could be harmful and may contribute to the progression of neuroinflammation.
We previously found that systemic lipopolysaccharide (LPS) challenge induced glial activation and recruitment of CD45hi leukocytes to brain blood vessels, in circumventricular organs. During systemic inflammation interferon-gamma (IFN-**γ) is induced and participate in the inflammation observed in different target organs.** However, little is known about the impact of this cytokine in the CNS immune cells during this process.
Methods: we injected PBS or LPS (1.6 mg/kg, i.p.) to wild type (WT) C57BL/6 strain and IFN-**γ** knockout (KO) mice (n=4). After perfusion, we processed the brains to isolate the immune cells for staining and measuring them by flow cytometry.
We performed conventional and high dimensional flow cytometry analysis to study the different immune cells populations.
Results: LPS systemic stimuli increased the monocyte recruitment to WT mice brains compared to PBS-injected control group ($p<0.05$). However, this effect was not observed in the IFN-**γ** KO mice (p NS), indicating that the presence of type II interferon participates in the response that results in the arrival of monocytes to the brain.
Our preliminary results also showed increased lymphocytes recruitment to the brains of LPS-stimulated WT mice, compared to controls ($p<0.05$). Nevertheless, that increase was impaired in the absence of IFN-**γ** (p NS).
Conclusion: during mice systemic inflammation, leukocyte recruitment to CNS take place in a type II interferon-dependent manner, suggesting that this cytokine might be an important mediator of neuroinflammatory responses.
Keywords: neuroinflammation, microglia, monocytes, type II interferon, LPS

266. (469) **PHAGOCYTOSIS OF TUMOR CELLS BY MONOCYTES TRIGGERS IL-18 SECRETION WHICH CONTRIBUTES TO PD-L1 UP-REGULATION ON NK CELLS**

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NK cells are key effectors against tumor and virus-infected cells. However, evidence of a regulatory role during autoimmunity and viral infections is emerging. We have identified a subset of immunoregulatory, PD-L1-expressing tumor-infiltrating NK cells in tumor-bearing mice and in patients with renal cell carcinoma (RCC). *In vitro*, PD-L1 expression was induced on NK cells from healthy donors (HD) upon tumor cell recognition through NKG2D and was further up-regulated by monocyte (Mo)-derived IL-18. To additionally explore this circuit, the aim of this work was to characterize the underlying mechanisms that control IL-18 production by Mo. To this end, peripheral blood mononuclear cells (PBMC) from HD were cultured with K562 cells in the absence or in the presence of different pharmacological inhibitors of pathways that could lead to IL-18 production. While an ATP receptor antagonist (A740003) or inhibitors of ROS (catalase), NOS (L-NAME), or autophagy (Bafilomycin) had no effect, the inhibition of phagocytosis using cytochalasin D abolished IL-18 secretion (assessed by ELISA, $p<0.0001$). Moreover, cytochalasin D completely abrogated PD-L1 up-regulation on NK cells ($p<0.01$). When PBMC were separated from K562 and NK cells using transwells, IL-18 secretion was totally abolished ($p<0.01$), confirming that K562-driven IL-18 production by PBMC required Mo contact with tumor cells. Also, culture of isolated Mo ($CD14^+$ cells) with eFluorDye 670-labeled K562 cells for 15 min resulted in 67.7±5.6% of phagocytosis (evaluated as frequency of eFluorDye 670^+ $CD14^+$ cells by flow cytometry) that was significantly reduced by cytochalasin D (13.4±5.7%, $p<0.001$). Moreover, IL-18 secretion was induced when isolated Mo were cultured with K562 cells ($p<0.05$), and such effect was also blocked by cytochalasin D ($p<0.05$). Altogether, our results show that recognition and phagocytosis of tumor cells by Mo triggers IL-18 secretion that subsequently contributes to PD-L1 up-regulation on NK cells.

267. (478) **PEPTIDES FROM AMARANTH SUPPRESSED INTESTINAL INFLAMMATION IN A MOUSE MODEL**

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Amaranth is a pseudocereal with a high content of proteins with good nutritional and health quality (anti-hypertensive, anti-oxidant, anti-thrombotic and anti-proliferative and anti-inflammatory effects). This study aimed to broaden the anti-inflammatory effect of peptides from Amaranth on mucosal inflammation in a colitis model.
Colitis was induced in Balb/c mice with an intrarectal administration of TNBS in ethanol on day 0. Then mice were daily given a formulation containing a peptide of Amaranth (PA) during a week through the oral route. As control of colitis, mice received PBS or ETOH, or PBS as treatment. Corporal weight and disease activity index were monitored, and on day 7 mice were sacrificed. The colonic inflammatory response was analyzed (weight, length, histology, cytokine gene expression by qPCR).
We found that PA reversed the weight loss of mice with TNBS-induced colitis ($p<0.05$). The colon of PA-treated mice showed a decreased histological score, with less edema and cellular infiltration than those from untreated TNBS-treated mice. Mice treated with PA showed a colon with a lower weight/length ratio than mice from the TNBS group (21.56±0.46 vs 32.46±2.26; $p<0.05$). In concordance, we found a significantly decreased expression and production of proinflammatory cytokines (Ccl20, IL-1b, TNF and IFN-γ, $p<0.01$) and lower myeloperoxidase activity in the peptide-treated group than in TNBS-mice ($p<0.05$). Moreover, we found augmented NF-κB p65 levels along with a significantly higher transcript level of the peptide transporter PepT1 in mice with colitis than in PA-treated mice ($p<0.05$).
In conclusion, our findings indicated that peptides from Amaranth exert a mucosal anti-inflammatory effect that suppressed the TNBS-driven NF-kB-mediated intestinal inflammation that promoted a Th1-immunity. These findings led us to propose using the amaranth peptide in a functional food with an anti-inflammatory protective effect.

268. (485) ***CLOSTRIDIOIDES DIFFICILE* UPTAKE BY HUMAN MACROPHAGES OCCURS IN A SLAMF1-INDEPENDENT MANNER**

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C. difficile (*CD*), a Gram+ spore-forming anaerobic bacteria, is the major cause of nosocomial infectious diarrhea that generally develops after antibiotic treatment. SLAMF1 (Signaling Lymphocytic Activation Molecule) is expressed on macrophages and triggers not only phagosome-related functions, but can also recognize and internalize different pathogens. *CD* components regulate macrophages functions and both toxins and spores invade host cells. However, the endocytosis of the bacteria has not been explored. Here, we address the internalization of *CD* and the role of SLAMF1 during this process.

Monocyte-derived macrophages from healthy donors were cultured in the presence or absence of *CD* (NAP1/BI/027 strain) inactivated by heat or formalin treatment (*CD*H or *CD*F). For some experiments *CD* was coupled with FITC and an agonistic antibody for SLAMF1 was added to the cell culture.

Our results show that SLAMF1 expression is not modulated on human macrophages surface by either *CD*H or *CD*F, even in the presence of increasing amounts of bacteria as shown by flow cytometry. Biochemical assays were conducted and SLAMF1 detection with a specific antibody indicated that there is no interaction between SLAMF1 and *CD*H or *CD*F. Nevertheless, we did detect interaction of macrophages with *CD*. SLAMF1 is also a costimulatory molecule, but SLAMF1 costimulation through an agonistic antibody had no effect on the *CD*-macrophage interaction as measured by flow cytometry and fluorescence microscopy. Moreover, most of the interacting macrophages were SLAMF1 negative. Finally, we confirmed intracellular localization of *C. difficile* in macrophages and a partial colocalization with LAMP2 by confocal microscopy.

In conclusion, SLAMF1 does not promote the interaction between human macrophages and *C. difficile* and does not participate in the internalization process of the bacterium. Further studies are needed to elucidate the molecules involved in macrophage entry mechanisms of *C. difficile*.

269. (489) ***CANDIDA ALBICANS* MODULATES ANTIMICROBIAL PEPTIDES OF B–DEFENSINS FAMILY DURING VAGINAL INFECTIONS**

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Vulvovaginal Candidiasis (VVC) is an acute inflammatory disease caused by *Candida* species that affects up to 75% of women of childbearing age once in their life. β-defensins (BDs) are one of the most important families of antimicrobial peptides in the female genital tract with relevant local functions such as antimicrobial and chemoattractant of PMNs, but its role during VVC is limited. Our aim was to study the role of BD1 (constitutive) and BD3 (inducible) in the pathogenesis of VVC using a murine model. Females (8-10-week-old C57BL/6J) were treated subcutaneously with β-estradiol-17–valerate in sesame oil (0.2mg/100µl) on days (D) -6,-3,2 and 4 post infection(pi). On D0 mice were inoculated intravaginally with *C. albicans SC5314* suspension (5.10^6 yeasts/PBS)(infected group), or uninfected (No-infected group). Animals without any treatment were used as controls (untreated). Cervicovaginal lavages (VL) were obtained for the study of different parameters. Infection was evaluated by local fungal load (CFU) and PMNs recruited to vaginal lumen on D2,4 and 8 pi. Fungal burden remained stable from D2 to D4, with a significant decrease at D8($p<0.001$), and the PMNs recruitment to vaginal lumen reached a peak at D2. Regarding BD1 and BD3 study, the mRNA (qPCR) and protein expression (immunofluorescence assay) were evaluated in cells recovered from VL. At early times(D2) a significant increase in mRNA of BD1($p<0.05$ vs untreated group), and BD3($p<0.05$ vs No-infected and untreated groups) was observed. Corrected total fluorescence analysis of cytoextended showed a significant increase of protein expression for both BDs in the cytoplasm of epithelial cells (BD1$p<0.001$;BD3$p<0.05$). Due to the relevant role of BDs in the protection against pathogens and in the modulation of the local innate immune response, the knowledge about their behavior during VVC is relevant to contribute to the understanding of the pathogenesis of this mycosis with elevated incidence and difficult treatment.

270. (520) **TRYPANOSOMA CRUZI INFECTION: ROLE OF WNT SIGNALING IN CARDIAC MACROPHAGES POLARIZATION**

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Chagas cardiomyopathy represents the most frequent and serious complication of chronic Chagas disease, affecting about 20-30% of patients. Cardiac inflammation and tissue damage is orchestrated by the infiltration and activation of immune cells including macrophages (Mo) into the myocardium. Mo with M1 phenotype (F4/80+ CD11b+ CD86+ CD206–) predominate at short times post infection (pi) and then they are rapidly polarized toward M2 phenotype (F4/80+ CD11b+ CD86– CD206+) which remains sustained during the infection. We have showed that in vivo inhibition of Wnt signaling by treatment with IWP-L6 (an inhibitor of Wnt proteins secretion) during the acute phase of *T. cruzi* infection controls the parasite replication, inhibits the development of parasite-prone and fibrosis-prone Th2-type immune response, and prevents the development of chronic Chagas disease's cardiac abnormalities. To investigate the role of Wnt signaling in the modulation of cardiac infiltrating Mo phenotype, BALB/c mice infected with 1,000 trypomastigotes of *T. cruzi* and treated with IWP-L6 (7.5 mg/kg) or vehicle (control) on days 5, 8, 11 and 14 pi were sacrificed at day 17 pi. Heart Mo isolated by a sequential combination of **w**ashing, **m**echanical disruption, **e**nzymatic digestion, and density centrifugation using **P**ercoll were stained with appropriate antibodies to determine the phenotype by flow cytometry. Results were analyzed with FlowJo (V10.7) using One-way ANOVA and Tukey's test for multiple comparison. We found that *T. cruzi* infection induced an increase of heart Mo (F4/80+ CD11b+) infiltration that was reversed by IWP-L6 treatment ($P<0.05$). Compared to non-infected mice, heart of control mice showed increased frequency of M1 (F4/80+ CD11b+ CD86+ CD206–) and M2 (F4/80+ CD11b+ CD86– CD206+) Mo populations, while cardiac tissue of IWP-L6-treated mice only showed M1 Mo infiltrate. Thus, these results suggest that Wnt signaling participate in the polarization of cardiac Mo towards M2 phenotype.

271. (523) **WNT SIGNALING CONTRIBUTES TO INSTALL M2-PHENOTYPIC PROGRAM IN *T CRUZI*-INFECTED MACROPHAGES**

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Trypanosoma cruzi infection boost a complex immune response in the host, which involve both innate and adaptative immune cells. In this context, macrophages (Mo) represent one of the main targets for the parasite. Mo are extremely plastic and respond to environmental signals adopting at least two extreme profiles: Classical M1 Mo which respond to inflammatory environment increasing CD86 expression, Nitric Oxide (NO) and proinflammatory cytokines production, plays a key role in the control of intracellular parasite growth; whereas Alternative M2 Mo which respond to IL-4 with increasing CD206 and Arg1 expression and anti-inflammatory cytokines release, promotes parasite replication. We have reported that *T. cruzi* infection induces Wnt signaling activation in Mo, with the inhibition of Wnt proteins secretion (with IWP-L6) modulating the Mo activation status to a more microbicidal phenotype. So, infected Mo that

were previously treated with IWP-L6, are transcriptionally similar to infected M1 Mo, although does not fully fit the classical M1 transcriptional pattern. Here, we aim to evaluate the effect of Wnt signaling inhibition on the phenotype of infected Mo. For that, bone marrow derived Mo were treated with IWP-L6 or Vehicle (V) for 24 h and infected with *T cruzi* trypomastigotes (Tps) (Mo:Tps=1:3). Non-infected, LPS+IFN-γ- (M1) and IL4-treated (M2) Mo were used as controls. Antibody panel for flow cytometry included F4/80, CD11b, CD86, CD206, iNOS and Arg1. We observed that while infection of Mo induces a reduction in the % of CD86+CD206- cells, this effect was not evident in IWP-L6-treated cultures. In addition, cultures of infected IWP-L6-Mo showed lower frequency of total CD206+ cells ($p<0.05$) with lower expression of CD206 in the CD206+CD86+ population ($p<0.05$) when compared with V-treated ones, with not significant differences in CD86 and iNOS expression. In summary, Wnt signaling contributes to install M2-phenotypic program in *T cruzi*-infected Mo.

272. (535) ***Lsp1-/-* DENDRITIC CELLS HAVE SIMILAR MHC II KINETICS DESPITE THEIR IMPAIRED ABILITY TO PRESENT ANTIGENS TO CD4+ LYMPHOCYTES.**

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Leukocyte-specific protein 1 (LSP1) is a 52kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes and endothelial cells. LSP1 is an important regulator of actin cytoskeleton remodelling. We have previously shown that *Lsp1-/-* dendritic cells (DCs) have a defective antigen presentation to CD4+ T cells compared to DCs from wild type (WT) mice. In order to study whether defective antigen presentation in *Lsp1-/-* mice is due to alteration in MHC class II dynamics, we evaluated I-Ab kinetics expression on cell surface and intracellulary in *Lsp1-/-* DCs upon activation with CpG-ODN. DCs were *in vitro* derived from bone marrow precursors with Flt3-L and stimulated with CpG-ODN 1826, at different times (1-2-3-4-8-12 and 18h) they were collected and stained with anti-I-Ab antibody (Ab) either permeabilized or not, to measure total or cell surface content of I-Ab and analyzed by flow cytometry. We found that total and cell surface I-Ab molecules increases in *Lsp1-/-* DCs upon stimulation similar than DCs from *Lsp1+/+* mice, with a peak in both cases at 3h. Intracellular content of I- Ab increased more than cell surface expression in both groups and remained high for at least 18h. Analyzing the kinetics of peptide-I-Ab complexes on DC surface by incubating DCs with the Ea52-68 peptide (which binds to I-Ab) and then labeling them with Y-Ae Ab (which recognizes I-Ab-Ea52-68 complex) by flow cytometry, we observed that these complexes remained stable up to 24h after on surface in *Lsp1-/-* and *Lsp1+/+* stimulated DCs at similar levels. These results suggests that the altered antigen presentation in *Lsp1-/-* DCs could be related to other steps in Ag processing and not to MHC II dynamics.

273. (538) **EXACERBATED ENDOPLASMIC RETICULUM STRESS SUFFERED BY ENDOMETRIAL STROMAL CELLS ALTERS THE CONDITIONING OF MATERNAL MONOCYTES TO A TOLEROGENIC DENDRITIC CELL PROFILE**

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Endometrial stromal cells undergo physiological endoplasmic reticulum stress (ERS) and unfolded protein response (UPR) allowing the secretion of immunoregulators and associated with a sterile inflammatory response to achieve a successful implantation. However, an exacerbated ERS/UPR was found in endometria of women with recurrent pregnancy loss, suggesting that a rigorous balance is required. Considering that stromal cells condition maternal monocytes to a tolerogenic dendritic cell profile, here we evaluated the impact of ERS/UPR triggered on stromal cells on monocyte differentiation. Thus, human endometrial stromal cell line (HESC) were treated: +/- Thapsigargin (an ERS-inducer Tg, 4h) or +/- decidualization stimuli (MPA+db-cAMP for 8 days). Conditioned media (CM) were collected after 48h. Then, isolated monocytes from peripheral blood mononuclear cells from healthy women were cultured with rhGM-CSF+rhIL-4 for 5 days in the absence/presence of CM. Monocyte-derived cultures differentiated with HESC+Tg CM showed a lower frequency of CD1a+CD14- cells compared with HESC CM, quantified by FACS analysis ($p<0.05$, Wilcoxon Test). Also, this CM induced a higher IL-1b ($p<0.05$) and a lower IL-10 secretion on monocyte-derived cells accompanied with a higher frequency of $CD86^{hi}$ cells ($p<0.05$). Also, we observed a higher frequency of apoptotic cells (Annexin V+ Propidium Iodide-) in these cultures. Finally, we evaluated the expression of ERS-sensors on monocyte-derived cultures. Whereas IRE1a expression was increased, a significant lower expression of ATF6 was observed on HESC+Tg CM cultures ($p<0.05$). Surprisingly, this effect was also observed when monocytes were differentiated in the presence of CM from decidualized cells, suggesting that it operates during the decidualization program. These results suggest that ERS might be transmitted from stromal cells to monocytes and its fine balance is required to maintain the tolerance needed for successful implantation.

274. (541) **IL-13 PROMOTED THE PHOSPHORYLATION OF STAT3 AND STAT6 ON COLONIC EPITHELIAL CELLS WITH THE SECRETION OF TSLP AND CCL26**

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Food allergy is rapidly increasing in prevalence worldwide and is one of the main triggers of anaphylaxis. Eosinophilic inflammatory responses can be associated with food allergy and the underlying mechanisms in human colonic mucosa are poorly understood. We previously reported that allergic sensitized pediatric patients with juvenile polyps (JP) showed local IgE class-switch recombination with high levels of TSLP, IL-33 and CCL26 (the main chemoattractant for eosinophils), that might be related with the hypereosinophilia. This study aimed to investigate the intestinal epithelial cells as a source for these soluble mediators under different inflammatory settings.
Caco-2 cells were stimulated with IL-13 (10 ng/ml) or IFN-γ (10 ng/ml) and CCL26, TSLP and IL-33 were evaluated by ELISA in culture supernatants. Cell activation was analyzed with a tyrosine-kinase phosphorylation array and finally, kinase activation was confirmed by immunoblotting.
We found that TSLP and CCL-26 levels were significantly increased in Caco-2 cells in response to IL-13, whereas it remained unchanged following the stimulation with IFN-γ. Cell activation was also demonstrated by analyzing the tyrosine-kinase activation, and we found that STAT-3, STAT-6, Lck, Akt, IGF-IR were higher phosphorylated than cells exposed to IFN-γ or medium. No effect was observed on IL-33 under the conditions studied.
In conclusion, our findings show that colonic epithelial cells respond to a Th2 environment by secreting TSLP, which may promote mucosal type-2 inflammation through ILC2 activation, and CCL26, which is probably involved in the eosinophil chemoattraction. The IL-13-driven cell activation involves the STAT3, STAT6 pathways, and other kinases. These findings may pave the way to identify the mucosal epithelial cells as the triggers of inflammation and eosinophil-rich cell infiltration, and the tyrosine phosphorylation as a therapeutic

target.

275. (552) **PROSTAGLANDIN E2 PROMOTES A UNIQUE RESOLUTIVE PROFILE IN HUMAN MACROPHAGES**

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Prostaglandin E2 (PGE2) is an immune mediator with recognized inflammatory properties, but recent studies have shown that it is also required for the initiation of the resolution process. One key feature in the resolution of inflammation is the phagocytosis of apoptotic neutrophils by macrophages. Here, we aim to assess the role of PGE2 in the acquisition of a resolutive profile by monocyte-derived macrophages.

Human monocytes isolated from peripheral blood were cultured during 7 days in the presence of M-CSF (50 ng/ml). PGE2 (1 µM) was added at culture days 3, 5 or 7 (4 hours before cell collection). Control macrophages were cultured without PGE2. For comparison, M2-macrophages were generated adding IL-4 (20 ng/ml) for the last 48 hours of culture.

To begin with, we could not detect endogenous production of PGE2 (detection limit 10^{-11} M) at days 1,3, 5 or 7 of macrophage culture. Treatment with PGE2 at day 3 or 5, but not at day 7, resulted in macrophages characterized by higher expression of CD14, CD163, CD16 and MerTK. (n=5, $p<0.05$ against control macrophages). Presence of PGE2 during differentiation did not modulate the expression of CD209, CD36 or HLA-DR. In contrast, IL-4 led to a distinct phenotype characterized by increased expression of CD209 and CD36 and lower CD14.

PGE2 promoted macrophage capacity to engulf apoptotic neutrophils, as demonstrated by an average 30% increase of phagocytic macrophages (treated vs control, n=6, $p<0,01$). While addition of IL-4 decreased phagocytosis by 20% in control macrophages (n=6, $p<0,05$), it did not impact on the phagocytic capacity of PGE2-derived macrophages.

In summary, presence of PGE2 led to macrophages characterized by a resolutive profile distinct from the phenotype of macrophages obtained with M-CSF alone or M-CSF plus IL-4. Our results highlight the relevance of PGE2 acting on macrophages during the resolution process.

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276. (062) **PHENOTYPIC AND FUNCTIONAL IMMUNE ALTERATIONS IN A PATIENT WITH HYPERORNITHINEMIA-HYPERAMMONEMIA-HOMOCITRULLINURIA SYNDROME**

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Background. The urea cycle disorder hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome is caused by *SLC25A15* gene mutations that lead to deficiency of the ORC1 transporter. Interestingly, SLC25A15 mRNA is highly expressed in CD4 T lymphocytes. However, immune alterations have not been reported in HHH patients up to date.

Methods. Biochemical and molecular diagnosis of HHH syndrome. Assessment of phenotypic and functional immune parameters: lymphoproliferation against polyclonal and memory antigens, T helper cell subsets, cytokine secretion in culture supernatants, immunoglobulin serum levels, and glycosylation of leukocyte surface and serum proteins.

Results. A 3-years-old Argentinian female patient was admitted to the hospital with an episode of recurrent otitis, somnolence, confusion and lethargy. Laboratory tests revealed hyperammonemia, metabolic alkalosis, elevated transaminases, haemostasis alterations and increased urinary orotic acid excretion. Noteworthy, serum proteinogram showed a reduction in the gamma globulins. Direct sequencing of the *SLC25A15* gene revealed two novel heterozygous non-conservative substitutions in the exon 6: c.649G>A (p.Gly217Arg) and c.706A>G (p.Arg236Gly) confirming the diagnosis of HHH syndrome. *In silico* analysis indicated that the mutations inhibit ornithine transport almost completely. Interestingly, immune analysis revealed striking phenotypic and functional alterations in the T and B cell compartments and in the glycosylation of serum immunoglobulins.

Conclusions. Our study identified two non-previously described mutations in the *SLC25A15* gene underlying the HHH syndrome associated to functional and phenotypic immunologic alterations that would render patients more susceptible to infections. Our results highlight the importance of a comprehensive analysis to gain further insights in the underlying pathophysiology of the HHH syndrome.

277. (102) **GRK2 (G PROTEIN-COUPLED RECEPTOR KINASE-2) EXPRESSION AND FUNCTION ON LEUKEMIC CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS**

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Leukemic B cells from CLL patients proliferate and resist therapeutic agents within lymphoid tissues in close contact with the tumor microenvironment. GRK2 plays a central role in B cell homing by inducing S1PR1 (Sphingosine-1 phosphate receptor-1) downregulation, which allows the lymphocyte to overcome the S1P-mediated retention in the blood and to enter into the lymphoid organs guided by chemokines. GRK2 also modulates cellular functions such as proliferation and survival in different cancer cells. Here we aimed to evaluate GRK2 expression in CLL cells and the effect of a GRK2 inhibitor, CMPD101 (CMP), on leukemic cell survival, activation and migration.

Leukemic B cells were obtained from CLL patients´ peripheral blood. GRK2 expression was evaluated by western blot and cell viability by flow cytometry (FC) and LDH activity assay. B cell activation, induced by immobilized anti-IgM mAb, was evaluated by FC. Chemotaxis assay toward S1P or CXCL12 was carried out using Transwell migration assay. Venetoclax, a Bcl-2 inhibitor was used to evaluate drug-induced apoptosis. Statistical significance was determined using non-parametric tests with the GraphPad Prism software v7.

We found that leukemic cells from CLL patients express GRK2 at levels comparable to B cells from healthy donors (n=10). Regarding the effect of GRK2 inhibition on cell viability, we found that CMP used in the concentration range of 0.3-100 uM did not affect spontaneous or venetoclax-induced apoptosis of leukemic cells (n=10). Furthermore, CMP did not affect the up-regulation of CD69 in BCR-stimulated leukemic cells (n=7). Interestingly, we found that CMP significantly increased leukemic and T cell migration towards S1P, while it did not affect migration towards CXCL12 ($p<0.05$, n=10).

Our results suggest that GKR2 inhibition could be used to increase CLL cell migration towards S1P and open the possibility to study GRK2 as a potential target to induce CLL cell mobilization from lymphoid tissues.

278. (131) **SUPPRESSOR OF CYTOKINE SIGNALING 1 (SOCS1) AND IMMUNE DYSREGULATION IN INBORN ERRORS OF IMMUNITY**

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Introduction: Suppressor of cytokine signaling (SOCS) proteins function as important negative regulators of cytokine signaling, which impacts on multiple immune pathways, thus modulating cell functions. SOCS proteins exert their action by interacting with Janus kinases (JAKs), tyrosine kinase-2 (TYK2) and certain surface cytokine receptors. SOCS1 binds and inhibits the phosphorylation of JAK1/2 and TYK2, thus acting as a negative regulator of type I and II Interferon mediated signals. **Aim:** describe clinical features and laboratory findings of a patient with a novel *SOCS1* variant. **Results:** 8-year-old male, from non-consanguineous parents with no relevant perinatal history. His background includes vitiligo, asthma, and splenomegaly with cytopenias. Lung images showed multiple bilateral nodules. Lung biopsy: granulomatous-lymphocytic interstitial lung disease. Malignancies and infectious diseases were ruled out. Immunological findings revealed panhypogammaglobulinemia and poor polysaccharide response. Low naive T-cells with high expression of activation markers, increased circulating follicular T cells with a skew towards a Tfh1 profile and elevated double-negative T cells with normal $FOXp3^+$ and reduced $Th17^+$ $CD4^+$ T cells. Impaired B-cell subsets showed low post-switch memory cells with high frequencies of $CD21^{low}$ B cells. Normal lymphoprolipheration assay. Therefore, he was diagnosed with Common Variable Immunodeficiency with dysregulation and started immunoglobulin replacement. Whole exome sequence revealed c.368C>A and c.365G>A variants in the same allele of *SOCS1* gene. Enhanced phospho-STAT1 kinetic assay confirmed the pathogenic role of these variants. **Conclusion:** In humans, mutation in SOCS1 impacts the STAT1 signaling pathway thus affecting multiple JAK/STAT signaling pathways. Only a few patients with SOCS1 mutation have been recently reported worldwide with a broad phenotypic spectrum overlapping other inborn errors of immunity.

279. (154) **INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELL RESPONSE TO BACTERIAL LIPOPOLYSACCARIDE AND SHIGA TOXIN**

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Mesenchymal Stem Cells can be activated and respond to different bacterial toxins. Lipopolysaccharides (LPS) and Shiga Toxin (Stx) are the two main bacterial toxins present in Hemolytic Uremic Syndrome (HUS) that cause endothelial damage. In this work we aimed to study the response of induced Pluripotent Stem Cells derived Mesenchymal Stem Cells (iPSC-MSC) to LPS and/or Stx and its effect on the restoration of injured endothelial cells. For this purpose, we stimulate iPSC-MSC with LPS and/or Stx for 24 h. using Polymyxin B in the Stx treatments in order to avoid LPS contamination. The results obtained showed that LPS induced a pro-inflammatory profile on iPSC-MSC with an increment of IL-8 and TNF-α, but not Stx when we measure with ELISA kit, (pg/ml IL-8 Control: 1988±299; LPS: 20876±1233*#, Stx: 1801±137; LPS+Stx: 17935±213* and pg/ml of TNF-α Control:880±32; LPS:3291±116*#; Stx:627± 8; LPS+Stx:2092 ±59*, *vs. Control, #vs Stx $p<0,05$). Moreover, LPS induced on iPSC-MSC an increment on the migratory capacity of these cells (percentage of migration Control: 44±10; LPS:69±11*; Stx: 42±10; LPS+Stx: 72±10, *vs. Control $p<0,05$) and adhesion to gelatin substrate (number of cells adhere to gelatin Control: 533±37; LPS: 769±114*; Stx:702±102*; LPS+Stx:976±142, *vs. Control $p<0,05$). Finally, the addition of conditioned media of iPSC-MSC treated with LPS+Stx to HMEC-1 (Human Microvascular Endothelial Cells-1), decreased the capacity to close a wound in an endothelial monolayer (percentage of wound closure Control: 38±4; LPS:32±4; Stx:24±8; LPS+Stx: 18±6*, *vs. Control $p<0,05$). In conclusion, these results suggest that iPSC-MSC activated by LPS acquired a pro-inflammatory profile that induces migration and adhesion to extracellular matrix proteins, but the combination of both toxin decreased the repair of endothelial damage.

280. (312) **AIRWAY INFLAMMATION IN A *SALSOLA KALI* POLLEN-INDUCED MURINE MODEL OF ALLERGY**

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Allergic rhinoconjunctivitis and asthma are diseases with an increasing worldwide prevalence. In our region, common weeds, e.g. *Salsola kali*, are one of the major causes of pollinosis. Murine models are useful for studying the mechanism of allergic disease. Regarding the model antigen, the majority of studies have been performed using ovalbumin. The aim of this work were to develop an experimental animal model of allergy based on relevant human aeroallergens, such as *S. kali* pollen, and to define the immunological and cellular airway features of the allergic response. BALB/c mice (n = 5/group) were administrated with PBS or *S. kali* pollen extract through i.p. route and later challenged by nasal instillation of PBS or *S. kali* pollen respectively for 3 consecutive days. *S. kali*-specific IgE were measured by ELISA. After sacrifice, the noses and lungs were fixed and paraffin embedded for histological analysis (H&E, toluidine blue and periodic acid-Schiff). After nasal challenge with *S. kali* pollen, sensitized mice manifested early-phase (sneezing) and late-phase (eosinophilic and basophilic accumulation) response compared with the control group. Frequency of sneezing in sensitized mice were higher than the control throughout the challenge phase ($p < 0,01$). The histology showed goblet cell hyperplasia and eosinophil infiltration in nasal lateral mucosa (135 ± 58 in sensitized mice vs. 8 ± 1 in control group) and septum (52 ± 36 vs. 1 ± 1 respectively). Also, sensitization induced moderate to severe inflammatory infiltration in lungs. *S. kali*-specific IgE value was not increased in all sensitized mice. Our results confirm upper airway inflammation correlated with lower airway inflammation in response to allergen exposure. The symptoms and histology observed encourages us to think about the establishment of an alternative murine model based on relevant human allergens that allow to understanding the disease and exploring therapeutic approaches.

281. (343) **PLATELETS MODULATES $CD4^+$ T CELL FUNCTION IN COVID-19 THROUGH A PD-L1 DEPENDENT MECHANISM**

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Severe COVID-19 is associated with a systemic inflammatory response and a progressive CD4⁺ T cell lymphopenia and dysfunction. Here, we analyzed whether platelets might contribute to CD4⁺T cell dysfunction in COVID19.
Blood samples were obtained from healthy donors (HD) n=30 or COVID19 patients, n=60. Patients were classified into mild, moderate and severe according to WHO criteria. Each participant provided written informed consent. Oncologic and vaccinated patients were excluded from the study. Proportion of CD4+T Cells–platelets aggregates was measured by flow cytometry (CD4+CD62p+ cells). CD4+T cells were isolated from HD and cultured with platelets from a single HD or a COVID19 patient (1:100 ratio). CD25 was evaluated by flow cytometry and cytokine production was measured by ELISA.
We observed a high frequency of CD4⁺ T cell-platelet aggregates in COVID19 (n=30-60, p<0.0001) that inversely correlated with lymphocyte counts (n=60, p=0.0267). Platelets from COVID19 but not from HD inhibited the up-regulation of CD25 expression (n=7, p=0.002) and TNF-α production by CD4⁺T cells (n=7-13, p=0.0236). IFN-γ production was increased by platelets from HD but not from COVID19 (n=19-33, p=0.0016). An available RNAseq from purified platelets showed that COVID19 patients presented higher expression of PD-L1 than HD (n=5-9, p=0.02), and the same was observed by flow cytometry (n=26-30, p<0.0001). The proportion of PD-L1+platetelets inversely correlated with IFN-γ production by activated CD4⁺T cells cocultured with platelets (n=43, p=0.0009). Furthermore, supporting a role for PD-L1 in the immunomodulatory activity of platelets, we found that a blocking antibody directed to PD-L1 significantly restored COVID19 platelet-ability to stimulate IFN-γ production by CD4⁺T cells (n=12, p=0.005). Our study suggests that platelets might contribute to disease progression in severe COVID19 not only by promoting thrombotic and inflammatory events, but also by suppressing T cell response.

282. (363) **THE ACTION OF SLPI ON TUBULAR EPITHELIAL CELLS AND IMMUNE RESPONSE PROTECS THE KIDNEY OF TRANSPLANTNT PATIENTES.**

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Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor with pleitropic activities on immune and non-immune cells. Lately, it has been proposed SLPI as a biomarker of acute kidney injury with a protective role on tubular epithelial cells. The aim of the present work was to unravel whether SLPI action on kidney is an immune mediated effect. We found that the administration of SLPI reduced the kidney damage in a rat model of ischemia reperfusion injury. However, SLPI was also able to reduce the damage in a gentamicin-induced nephrotoxicity rat model. In *in vitro* experiments, the HK-2 human epithelial cells-treatment with SLPI reduced the cell apoptosis induced by serum starving conditions or calcineurin inhibitors. A real-time PCR array data with HK-2 cells revealed that SLPI modulated transcript levels of genes mainly involved in DNA repair, response to misfolded proteins and inflammatory response pathways. Furthermore, SLPI favors the proliferation and migration of HK-2 cells. In kidney transplant patients, plasma levels of SLPI were high and indirectly associated with kidney function but also with leukocyte proliferation index. However, the levels of SLPI transcripts found in the renal biopsies were indirectly associated with plasma creatinine and TGFβ but directly associated with HMOX-1. An indirect association was found between plasma levels of FK506 and SLPI. These results confirm that local SLPI exert a protective role in kidney transplant patients which can be mediated directly on epithelial cells and indirectly by affecting the immune response. Furthermore, it suggests that immunosuppression treatment can reduce the expression of this nephropotective factor.

283. (446) **IMPACT OF TREATMENT WITH DIRECT ANTIVIRAL AGENTS ON HCV SPECIFIC IMMUNE RESPONSE IN HIV/HCV COINFECTED INDIVIDUALS**

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Purpose: The aim of this research was to evaluate the changes in the HCV specific immune response and exhaustion phenotype secondary to HCV clearance by direct antiviral agents (DAA) in HIV/HCV coinfected individuals.
Methods: prospective longitudinal study. 22 HIV/HCV coinfected individuals were enrolled (12 with METAVIR F4 and 10 with F0/F1). All individuals were on successful antiretroviral therapy and began HCV treatment with different DAA combinations. Peripheral blood samples (PBMC) were taken before DAA (BSL) and 3-12 months post-treatment (3-12MPT). PBMC were incubated for 4 days with peptides spanning structural and non-structural (NS4a, NS5a and NS4b) HCV proteins. IFN-γ was measured in cell culture supernatants by ELISA after stimulation. PD-1 expression on T-cells was evaluated by flow cytometry. Nonparametric tests were used for statistical analysis.
Results: Significant increments in the specific response to NS4a/NS4b (1,5±3,6 vs 17,9±17,2pg/ml, p=0,0039) and NS5a/NS4b antigens (1,0±2,4 vs 8,6±9,4pg/ml, p=0,0234) were observed only in F4 HIV/HCV between BSL and 3-12MPT. In both groups, absolute CD4+ T cell counts at BSL positively correlated with the magnitude of the specific response to structural proteins at BSL (p<0,0001, r=0,8006). PD-1 expression was higher in F4 than in F0/F1 group at BSL (36,1±9,4% vs 48,8±11,8%, p=0,0232) and 3-12MPT (35,3±16,9%vs 50,3±10,8%, p=0,0300). No differences in PD-1 were observed after DAA treatment in either of the groups.
Conclusions: HCV-specific cellular response is improved after HCV viral clearance despite the lack of improvement in the immune exhaustion profile. This might be due to the elimination of HCV-antigen chronic stimulation, which would reduce the depletion of cells and allow the proliferation of specific cells upon re-stimulation in vitro. A better immunological status before DAA initiation correlates with a higher recovery of specific cellular response.

284. (491) **THE FREQUENCY OF FOLLICULAR HELPER T CELLS (TFH) IS DIMINISHED IN TONSILS FROM CHILDREN WITH TRISOMY 21 WHEREAS THE FRACTION OF PRE-TFH IS INCREASED AND BIASED TOWARDS A TH1 PROFILE**

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Down Syndrome, which is caused by trisomy 21 (T21), is characterized by immune dysregulation, anatomical differences in the upper respiratory tract and higher rate of comorbidities. Since tonsils are the first barrier against airborne pathogens, we characterized the T cell compartment obtained from hypertrophied tonsils in T21 children and age-matched controls (n=5 per group). We studied the frequency of activated non-Tfh cells (CD3+ CD4+ CD45RA-CXCR5-

PD1+), Tregs (CD3+ CD4+ CD45RA-Foxp3+), pre-Tfh (CD3+ CD4+ CD45RA-CXCR5^{low} PD1^{int}) and Tfh (CD3+ CD4+ CD45RA-CXCR5^{hi} PD1^{hi}) by multiparametric flow cytometry. Also, the expression of CXCR3 (related to a Th1 profile) and the cytokines IL21, IFNγ and IL17 were analyzed. The frequencies of CD3+, CD4+, CD8+, Tregs and CD19+ cells were not altered in T21 tonsils, but a diminution in Ki67+ CXCR5+ CD19+ cells was observed ($p<0.05$). There is a 40% increase in the fraction of activated non-Tfh cells ($p<0.05$) whereas the ratio of Tfh decreases ($p<0.3$). There seems to be an arrest in the pre-Tfh population which shows an increase in their proportion ($p=0.08$). Interestingly, a higher percentage of CXCR3+ pre-Tfh population ($p<0.01$) is observed. Moreover, the mean fluorescent intensity of CXCR3 is enhanced in all the T cell populations analyzed. When cytokines were studied, an increase in the fraction of Tfh IFNγ+, IL21+ and IFNγ+IL21+ was observed. To explore if dendritic cells (DC) could be involved in promoting a Th1 bias among the non- and pre Tfh cells, we phenotypically analyzed the different DC population using the following markers (CD45, CD3, CD19, CD56, CD11c, HLADR, CD304, CD1c, CD141, CD86, FcεRI, PDL1). No significant changes in the frequencies of the different DCs populations were found. Our results suggest that T21 children have an altered tonsil T cell compartment, with a skewed Tfh differentiation and a Th1 profile among the pre-Tfh population. Further research should be done to understand the mechanisms involved.

285. (524) **HLA EPLET MISMATCH IDENTIFIES PEDIATRIC LIVER TRANSPLANT PATIENTS WITH HIGHER RISK OF DEVELOPING ACUTE REJECTION**

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The quantification of HLA eplet mismatch (eMM) has demonstrated its superiority as a biomarker for the immunological risk stratification in kidney, heart and lung transplantation. The aim of this study is to evaluate the association between HLA eMM and the development of biopsy-proven acute rejection (BPAR).
Patients that received liver transplant between 2018 and 2020 were included and prospectively followed for 9 months. Recipients and donor pairs were HLA typed by NGS and the number of eMM was quantified. Risk loci for BPAR were identified by Cox regression models. ROC analysis was performed to identify specific eMM thresholds for BPAR development and Kaplan-meier curves for the BPAR-free survival according to high or low eMM load were constructed.
Thirteen of our 37 patients included in the study developed BPAR during follow up. The number of antibody verified (ab) eMM in HLA-A (HR: 1.10, CI95% 1.00-1.20; p= 0.045) and HLA-DQ (HR: 1.21, CI95% 0.99-1.47; p= 0.063) were significantly associated with BPAR. Having ≥ 2 ab HLA-DQ eMM and ≥ 9 ab HLA-A eMM were considered as high loads. At 9 months post-transplant, the BPAR-free survival for patients with low and high ab HLA-DQ eMM load was 81.0% (CI95% 65.8-99.6) and 43.8% (CI95% 25.1-76.3), respectively, while for patients with low and high ab HLA-A eMM load was 75.0% (CI95% 60.6-92.9) and 33.3% (CI95% 13.2-84.0), respectively.
The measurement of the ab eMM load for the loci HLA-A and HLA-DQ between donor and recipient prior liver transplantation could identify those at risk of developing BPAR. Individualized immunosuppression protocols and closer surveillance could be performed if the immunological risk is assessed precisely. Further studies in our population are being performed to confirm these results.

286. **(528) DYNAMICS OF SOLUBLE IMMUNE MEDIATORS IN COVID-19 PATIENTS FROM AN ARGENTINEAN COHORT WITH MODERATE AND SEVERE SYMPTOMS**

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The cytokine storm, a form of systemic inflammatory response syndrome, is one of the most dreadful complications that can occur during COVID-19. In this work, we aimed at studying the occurrence of a cytokine storm in a cohort of COVID-19 patients (Cpts) from Córdoba (Argentine). During first wave, we collected sera from individuals with RT-PCR+ for SARS-CoV2 hospitalized in Hospital Privado with moderate (MOD) and severe (SEV) disease (n=62, aged 21-80 years) as well as healthy controls (HC n=24, age matched), to determine the concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12, IL-28A/B, IL-29, TNF, IP-10, IFNα2, IFNβ, IFNγ and GM-CSF by LEGENDplexTM. Males represented 74% of MOD and 67% of SEV Cpts. Hypertension (HT, 48%), obesity (31%), dyslipidemia (DL, 24%), and diabetes (24%) were the most frequent comorbidities. All cytokines, except IL-28A/B, were significantly increased in total Cpts in comparison with HC ($p<0.01$). Elevated levels of IL-6 and CRP ($p<0.01$) between 4-7 day after hospitalization were found in all Cpts who died, but the cytokine profiles were different in deceased SEV than in MOD Cpts. Mortality in SEV group was associated with high levels of IL-6 ($p<0.001$), GM-CSF ($p<0.01$), IL-8, CRP, leukocytosis and decreased platelets ($p<0.0001$ for all). Comorbidities were linked to particular patterns of immune mediators on admission but not afterwards during infection, with HT Cpts exhibiting increased IP-10 levels but DL Cpts showing lower concentration of IL-1β, GM-CSF and IL-10 compared to no-HT and no-DL Cpts, respectively ($p<0.05$). The frequency of Cpts who required O2 support was higher in HT (84%) vs DL (67%). Although our data have similarities with those in international reports, the complete profiling of different parameters (cytokine/chemokines, risk factors, epidemiological and clinical characteristics) in the local cases add value by identifying particularities that may be relevant for the management and prognosis during SARS-CoV2 infection.

287. (533) **ALTERATIONS OF BLOOD IMMUNE CELLS IN COVID-19 ELDERLY PATIENTS WITH OR WITHOUT TYPE 2 DIABETES**

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Elderly individuals, especially those with pre-existing conditions like diabetes mellitus (DM), have a high risk for developing severe cases of COVID-19. The aim of this work was to characterize the alterations of blood immune cells (BIC) in patients with symptomatic COVID-19 and confirmed SARS-CoV-2 infection, ≥ 60 years and who needed hospitalization in the Centro de Salud Hospital of Tucuman during the second peak of the pandemic. Blood samples

were taken at the time of admission (d0) and five days after (d5) for routine laboratory tests and the characterization of BIC by flow cytometry. Most of the patients were men (70%) aged between 60 and 78 years. The 70% of patients had DM while 50% had arterial hypertension. At d0, all the patients had increased neutrophils and inflammatory markers (C reactive protein and D-dimers) and reduced numbers of lymphocytes, HLA-DR^{hi} monocytes, CD16^{+}CD56^{+} NK cells, CD3^{+}HLA-DR^{+}CD25^{+} cells, CD4^{+} and CD8^{+} T cells in blood. Patients received a standard treatment for COVID-19 care (O_2, corticosteroids and antibiotics). The treatment normalized the levels of BIC (d5) in 30% of patients who were those with no comorbidities. In patients with DM, BIC recovery was variable. In DM patients who required administration of plasma (30%), prolonged O_2 therapy (40%) or referral to the intensive care unit (10%) significant reductions of CD16^{+}CD56^{+}, CD3^{+}HLA-DR^{+}CD25^{+}, CD4^{+} and CD8^{+} cells were observed between d0 and d5. In line with previous studies, our results shows that absolute counts of major lymphocyte subsets in blood are significantly and substantially decreased during the course of severe COVID-19 disease in elderly patients. These BIC alterations may persist despite clinical care in elderly patients with DM. Further studies are needed to investigate the utility of early lymphocyte subset measurements as prognostic biomarkers of disease severity, mortality, and response to treatment in COVID-19 elderly patients with DM.

288. (543) **MOLECULAR DETECTION OF *CLOSTRIDIOIDES DIFFICILE* BY DIRECT PCR: NEW TOOLS FOR THE DIAGNOSIS OF *C. DIFFICILE* INFECTION**

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Clostridioides difficile infection (CDI) is the major cause of hospital-acquired diarrhea associated to antibiotics treatment in developed countries. CDI has become a health security threat and a considerable challenge to public health worldwide. The increased incidence and the severity of disease have been linked to the emergence and fast spread of hypervirulent epidemic strains. Moreover, a further increase in community-acquired infections and the zoonotic potential of *C. difficile* lead to a highly dynamic epidemiology of CDI. Enzyme immunoassay (EIA), a technique with high specificity but low sensitivity, is widely used as a diagnostic tool for *C. difficile* nowadays. To optimize the diagnosis and provide information for epidemiological surveillance strategies, the expression of glutamate dehydrogenase and toxin B (TcdB) of *C. difficile* was determined by EIA, direct PCR of stool samples and colony PCR of anaerobic culture. We also conducted comparative analysis to determine the performance of the direct PCR for *C. difficile*.

Faecal samples from 81 hospitalized individuals with diarrhea were collected. Clinical and demographic data were analyzed. We found a frequency of 18.5% for toxigenic strains. Treatment with antibiotics or proton-pump inhibitors were the main risk factors for CDI present in our cohort. No differences were observed between CDI^{+} and CDI^{-} individuals for the aforementioned risk factors, nor comorbidities or age distribution. However, we did detect an increase in leukocytes, lymphocytes and monocytes counts in CDI patients ($p<0.05$).

To validate our direct PCR method we used the EIA as the reference test. Our results showed a sensitivity of 1.0 and a Negative Predictive Value of 0.85 compared to EIA. Although a larger number of samples is needed to validate the method and determine specificity, this technique could be a useful method for *C. difficile* infection screening.

289. (546) **EFFECT OF TOFACITINIB ON THE ACTIVATION OF T LYMPHOCYTES IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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Tofacitinib (Tofa) is a Jak1/3 inhibitor that blocks the intracellular signaling of inflammatory cytokines and is used as 3rd line of treatment in Rheumatoid Arthritis (RA). Tofa is very effective to achieve disease remission but it is associated to higher incidence of herpes zoster reactivation likely due to alterations in cellular immunity. While several studies have evaluated on the effects of Tofa on the immune system in the context of RA, knowledge about its impact on the activation and differentiation of T lymphocytes (TL) is scarce. We aimed to study this aspect in vivo and in vitro by determining the functional status of TL in different groups of treated RA patients (Tx RA) and the effect of Tofa in the activation of T cells from healthy donors (HD), respectively. Thirty-one HD and 106 RA patients were recruited in the Rheumatology Service (HNC) to evaluate numerous biochemical and immunological parameters. Principal component analysis showed that 82 of these variables explain around 70% of the variance, with variables related to the activation and differentiation of TL as the main difference between HD and different groups of Tx RA. Compared to HD, Tofa Tx RA patients presented a significant increase in the % of populations with terminal differentiation characteristics including CD27-CD28- of CD4+ TL ($p<0.01$) and KLRG1+CD57+ CD4+ and CD8+ TL ($p<0,05$). In addition, in vitro studies showed that Tofa reduced the activation of purified CD4+ and CD8+ TL as evidenced by a decrease in the upregulation of CD25, T-bet and the frequency of Ki-67+ cells. These effect were a dose-dependent and observed in total, naïve and, mainly, memory TL. Interestingly, Tofa increased the expression of senescent marker p21 in memory CD8+ TL. Altogether, our findings suggest that Tofa-induced replicative immunosenescence could underlie the biological effects of this drug in RA and be also involved in side effects, restraining the activity of memory TL involved in viral control.

290. (558) **CHEDIAK HIGASHI SYNDROME: CASE REPORT**

Introduction: Chediak Higashi Syndrome (CHS) is a rare autosomal recessive disorder, characterized by partial oculocutaneous albinism, prolonged bleeding, immune and neurologic dysfunction, and risk for the development of hemophagocytic lympohistiocytosis. The presence of giant secretory granules in leukocytes is the classical diagnostic feature, which distinguishes CHS from closely related Griscelli and Hermansky Pudlak syndromes.

The accelerated phase or HLH, is the primary cause of mortality in CHS and can occur at any age

Objective: Present patient with late diagnosis without development of accelerated phase

Clinical case: 5 year old male referred by Hematology due to the presence of intracytoplasmic granulations in neutrophils and gray hair

First child of healthy parents, not consanguineous. Recurrent obstructive bronchitis treated with budesonide with good response. No relevant infections. Difficult management of epistaxis and mild neurocognitive delay. Physical examination only shows gray hair and nystagnmus.

In laboratory, moderate neutropenia and mild anemia. Negative EBV and CMV serologies

Hair´s microscopic evaluation detects dispersal of pigment clumps throughout the hair shaft.

Normal abdominal ultrasound.

Conclusions: CHS is a rare disease. The diagnosis is suggested by characteristic findings on hair microscopy and pathognomonic giant cytoplasmic granules in leukocytes on a peripheral smear. Confirmation is made by the identification of a pathogenic variant in the CHS1/LYST gene The prognosis of the HLH phase is poor and hence early diagnosis on the basis of characteristic clinical findings and diagnostic laboratory examinations is critical to facilitate timely bone marrow transplantation before the development of accelerated phase.

291. (567) **DETECTION OF INTESTINAL MICROBIOTA COMPONENTS IN THE SYNOVIAL MICROENVIRONMENT OF PERIPHERAL SPONDYLOARHRITIS AND ITS ASSOCIATION TO IMMUNOPATHOGENIC MECHANISMS**

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A role for intestinal microbes in arthritis is being considered. We analyzed intra-articular microbiota components and their association with immunopathogenic mechanisms of Spondyloarthritis (SpA). Synovial fluid (SF) of peripheral SpA patients were pooled (n=9): 6 psoriatic arthritis, 2 reactive arthritis, 1 undifferentiated SpA (Protocol approved CE002-2017). IL-17, IL-6, IL-23 and TGF-β levels were quantified in each SF sample by ELISA and compared with SF from osteoarthritis (OA). Moreover, SW982 cells (human synovial fibroblasts) were incubated with the SpA SF pool in absence or presence of polymyxin B (LPS inhibitor) and 48 h later the IL-6 levels in the supernatant were measured by ELISA. Furthermore, intestinal microbiota proteins were analyzed in the SpA SF pool through a Gel-LC bottom-up mass spectrometry-based proteomic study. Higher levels of IL-17, IL-6, IL-23 and TGF-β were detected in SpA compared with OA SF ($p<0.001$, $p<0.01$, $p<0.001$, and $p<0.01$, respectively). Since segmented filamentous bacteria (SFB) have a cardinal feature to induce Th17 cell differentiation in gut immunity, we searched for SFB peptides in the SF of SpA. We found 54 peptides of SFB with abundance estimated with an exponentially modified Protein Abundance Index (emPAI) from 0.01 to 0.28. In addition, LPS is present in synovial microenvironment of SpA since polymyxin B treatment significantly reduced IL-6 secretion by synovial fibroblasts stimulated with SpA SF ($p<0.05$). Therefore, we explored the synovial presence of peptides of microbiota Gram-negative bacteria, particularly genus *Dialister* because it was described as a potential intestinal microbial marker of disease activity in SpA. Accordingly, we found 55 peptides of genus *Dialister* (emPAI 0.02-0.33). Our findings show the presence of LPS and microbiota bacterial proteins in the synovial microenvironment of peripheral SpA and suggest their potential association with immunopathogenic mechanisms of this inflammatory arthropathy.

292. (570) **YAO SYNDROME (YAOS). FIRST REPORT OF A CASE IN ARGENTINA**

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Autoinflammatory syndromes (AIS) are characterized by apparently unprovoked episodes of inflammation, in the absence of autoantibodies or antigen-specific T cells, and result from genetic variants of the innate immune system.

Yao syndrome (YAOS), a NOD2-associated autoinflammatory disease, (first described in 2011) appears as episodic attacks of fever, dermatitis, polyarthritis, gastrointestinal and sometimes sicca symptoms associated with specific NOD2 sequence variants.

A 20 years-old previously healthy female, started 6 months previous to first visit, with **urticaria, angioedema, high grade fever, arthralgia, abdominal pain and diarrhea**. She was treated as chronic urticaria, **with poor response to anti-histaminics and NSAIDS.** Ambulatory evaluation by rheumatology and internal medicine showed no association to autoimmune nor infectious disease. Laboratory evaluation depicted **elevated acute phase reactants: ESR, CRP, fibrinogen, platelets, ferritin, together with granulocyte predominant leukocytosis.** MRI showed a mild splenomegaly with small scattered lymph nodes in abdomen and mediastine, which were metabolically active in a PET-CT.

Bone marrow aspiration and biopsy were hypercellular, with normal phenotype. Lymph node biopsy depicted chronic lymphadenitis with follicular hyperplasia, negative for neoplasia. Screening for PIDs was negative. Upper endoscopy and colonoscopy showed normal findings.

Inflammatory episodes continued lasting a few days to a week, with response to high dose steroids. Next generation sequencing for AIS and PID target genes was run (saliva sample, Illumina© sequencing and Sanger, MLPA, MLPA-seq, Array CGH confirmation) and detected the **NOD2 variant c.2104C>T (p.Arg702Trp).**

YAOS was diagnosed according to 2015 criteria, with the patient fulfilling 2 major, three minor and the molecular criteria. She is on moderate dose meprednisone regimen with good response to date.

This is the first description of a case of YAOS in Argentina.

293. (603) **PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF PERIPHERAL T CELL POPULATIONS FROM COVID-19 PATIENTS HOSPITALIZED IN HOSPITAL PRIVADO UNIVERSITARIO CÓRDOBA- ARGENTINA.**

Luisina Onofrio*, Jeremías Dutto*, Sabrina Bossio*, Ruth E. Baigorri*, María Belén Brugo*, Laura Almada**, Constanza Marín**, Federico Ruiz Moreno**, Ximena Volpini**, Mariel Almeida, Carolina Olivera, Nicolás Ponce, Juan Nahuel Quiroz, Silene Silvera Ruiz, Lucia Bofelli, Eva Acosta Rodríguez, Carolina Amezcua Vesely, Daniela Arroyo, Fabio Cerbán, Laura Cervi, Laura Fozzatti, Paula Icely, Pablo Iribarren, Belkys Maletto, Gabriel Morón, Cecilia Rodríguez Galán, Cinthia Stempin, Claudio Abiega#, Daina Escudero#, Adrian Kahn#, Juan Pablo Caeiro#, Mariana Maccioni, Cristina Motrán, Laura Chiapello, Carolina Montes, Adriana Gruppi, Claudia E Sotomayor. **Grupo ImmunoCovidCBA**

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SARS-CoV-2 infection results in asymptomatic, mild or severe disease. T cells could contribute to these different outcomes, but it remains unclear whether T cell response is dysfunctional or excessive. Here, we evaluated the phenotypic and functional features of circulating T cells from a cohort of 40 COVID-19 patients (Cpts) with moderate (MOD) and severe (SEV) clinical disease (aged 21-80 years) and 14 aged matched healthy controls (HC) by FACS. All Cpts exhibited a reduced frequency of CD3+T and Tregs cells compare to HC ($p< 0.05$). When exhaustion was evaluated, SEV Cpts showed higher % of PD-1+ and CD39+ in T conv cells ($p<0.05$), whereas no differences were found in BTLA or TIGIT expression. Even though, no differences in cytokine production (INF-γ, IL-2 and TNF) were observed, T conv cells from SEV Cpts showed a higher % of GZMB+ and CD107+ cells than MOD Cpts or HC ($p<0.05$). Circulating CD8+ T cells express different levels of CD8, where CD8lo cells represent highly activated cytotoxic T cells. COVID-19 patients presented a higher % of CD8lo T cells than controls and this increment was even more pronounced in SEV Cpts (p=0.04, MD vs HD; p=0.0012, SD vs HD). CD8lo T cells exhibited impaired cytokine production and CD107a expression compared to CD8hi T cells, al-

though GZMB levels were similar among both CD8+ subsets. CD8lo population from HC showed higher % of naïve T cells than effector memory (EM)(p=0.04) or EMRA (p=0.008) subsets, but this distribution was not seen in MOD or SEV Cpts. Indeed, MOD or SEV Cpts showed higher % of EM cells than HC.
Conclusion: the disease severity impacts on the phenotype and functional features of CD4+ and CD8+ T cells, with a pronounced increment in the % of CD8lo T cells as the disease worsens. These cells appear to have dysfunctional phenotype with an impairment of effector cytokines production but maintaining cytotoxic potential. The CD8hi/CD8lo ratio might be a useful parameter to predict the disease outcome.

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294. (145) **EXTRACELLULAR DNA TRAPS: NEW POTENTIAL BIOMARKERS AND THERAPEUTIC TARGETS FOR CHRONIC HEMOPHILIC SYNOVITIS IN PATIENTS TREATED WITH PRP**

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Introduction & aims: Hemarthroses in hemophilia cause chronic hemophilic synovitis (CHS) and the role of neutrophils infiltration on the pathophysiology of CHS is unknown. Neutrophils release extracellular DNA traps (ETs), structures containing DNA fibers and Elastase, which are associated with chronic inflammation. We aim to evaluate the association of ETs with join damage in CHS, the protective effect of platelet rich plasma (PRP) against ETs formation and after intraarticular injections in patients with CHS. **Methods:** Synovial Fluid (SF), PRP and platelet-poor plasma were obtained from 21 patients with CHS. Joint damage was evaluated with the Haemophilia Joint Health Score and monthly bleeding episodes. Synovial and plasmatic ETs were quantified by fluorometry (DNA level), ELISA, and microscopy (DNA-Elastase complex) and correlated with clinical parameters by Spearman's test.
Results: Patients with CHS showed DNA level of 0.37±0.06µg/ml and DNA-Elastase of 0.27±0.03OD in SF. Furthermore, the plasma of patients with CHS also showed significant DNA levels (0.17±0.01µg/ml) and DNA-Elastase (0.16±0.03DO) which positively correlated with the synovial level (r=0.7, p>0.05). ETs in plasma of healthy donors were undetectable. Correlations between synovial and plasma DNA-Elastase with joint damage parameters were significant (r=0-5-0.7;p<0.05). Moreover, incubation of SF with neutrophils resulted in the release of ETs and this phenomenon was impaired by the presence of plasma or PRP. Finally, preliminary data of 5 patients with CHS showed that joint damage and synovial/plasma DNA-Elastase levels decreased after 1 month of receiving PRP. **Conclusion:** The correlation of synovial/plasma levels of ETs with articular damage suggests synovial ETs as potential biomarker and therapeutic target for CHS. The protective effect of plasma on reducing ETs formation could be attributed in part to plasma DNAses as potential mechanism underlying the therapeutic action of PRP in CHS.

295. (166) **THE WNT/BETA-CATENIN SIGNALLING INVOLVEMENT DURING HEPATIC DIFFERENTIATION OF HUMAN AMNIOTIC EPITHELIAL STEM CELLS**

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The amniotic membrane from the human placenta at term is an important stem cell source, including human amniotic epithelial cells (hAECs). They express embryonic stem cells markers, and they are pluripotent. These characteristics position hAECs as ideal candidates for regenerative medicine. Hepatic failure is one of the major causes of morbidity and mortality worldwide. The available treatments have several obstacles. Recently, hAECs have been spotlighted as an alternative source of hepatocytes because of their potential for hepatogenic differentiation. The Wnt/beta-catenin pathway is an evolutionarily conserved signalling cascade that is important for stem cell renewal, cell proliferation, and cell differentiation, including hepatogenesis. This work aimed to assess the Wnt/beta-catenin pathway participation during hepatic differentiation of hAECs. Previously, we have demonstrated that hAECs efficiently differentiate to hepatic-like cells, by applying a specific hepatic differentiation (HD) protocol. We have found that HD medium significantly induced an increment in Wnt-1, beta-catenin, and E-cadherin expression in hAECs, measured by qRT-PCR and Western blot. We have also observed a significant increment in E-cadherin nuclear localization during hAECs HD, evaluated by immunofluorescence. Moreover, HD decreased GSK3-B expression. Treatment of hAECs with XAV939 (a beta-catenin pathway inhibitor) caused the inhibition of HD, as albumin and CYP7A1 expression were reduced. Additionally, beta-catenin pathway inhibition during HD, diminished the expression of miRNAs involved in liver development (mir-122, mir-221, and mir-194). These results suggest that the Wnt/beta-catenin pathway activation may be responsible for successful hepatic differentiation of hAECs. Understanding the molecular mechanisms regulating hepatocyte differentiation will significantly facilitate the development of stem cell-based therapy to treat liver diseases.

296. (178) **THREE-DIMENSIONAL CULTURE OF DPC CELLS IMPROVES HUVEC ENDOTHELIAL CELLS ANGIOGENIC CAPABILITIES**

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Tissue-engineered skin represents a useful strategy for the treatment of deep skin injuries. However, its correct vascularization remains a major challenge. We have shown that the presence of dermal papilla cells (DPCs) in these constructs favors the vascularization process, resulting in a better wound healing and graft take. We also have seen DPC-spheres culture increase the expression of angiogenic genes as VEGF, angiogenin and FGF. Since angiogenesis in scaffolds is essential for grafts to survive and integrate with existing host tissue, our aim was to compare the neovascularization abilty of monolayer and spheres cultures of DPC by using two adapted angiogenic models
To be able to compare the inductive molecules secretion between monolayer and spheres, we looked for the condition in which both systems contained the same amount of metabolically active cells. In our culture conditions, 45 spheres were metabolically equivalent to 10^4 cells/cm^2 monolayer seeded cells.
We observed that serum concentration is critical to sphere formation, when generated by the hanging drop technique. As at least 5% was required for DPC to aggregate, the same conditions were used for monolayer culture, in order to compare the angiogenic effects.
For the migration assay, conditioned media by monolayer or spheres were transferred to the bottom of a transwell plate and the HUVEC were seeded on the insert and placed into wells. We obtained ~10% more HUVEC cells migrated in the culture medium conditioned by the spheres than in the culture medium conditioned by the monolayer.
In conclusion, three-dimensional culture of DPCs improved the migration ability of HUCE *in vitro*. In this way, the use of DPC spheres in skin substitutes may favor the vascularization of the grafts, favoring the closure of the wound and the graft take.

297. (233) **AMNIOTIC MEMBRANE CONDITIONED MEDIUM MODULATES PRO- AND ANTIAPOPTOTIC PROTEINS EXPRESSION IN HEPATOCARCINOMA CELLS**

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The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Stem cells derived from amniotic membrane offer considerable advantages over other stem cells because of the ease of collection, their low immunogenicity and minimal ethical and legal barriers are associated with their use. Amniotic epithelial cells isolated from the amnion express embryonic stem cells markers and are pluripotent. Not only are amnion-derived stem cells applicable in regenerative medicine, but also have antitumoral properties. Hepatic failure is one of the major causes of morbidity and mortality. In particular, the hepatocarcinoma is the fifth leading cause of death worldwide. A few studies have demonstrated the antitumoral effects of the amniotic membrane and their cells, but little is known about the molecular and cellular mechanisms involved. The aim of this work was to analyze the expression of some important pro and antiapoptotic proteins, in hepatocarcinoma cells treated with amniotic membrane conditioned medium (AM-CM). Previous results showed that AM-CM inhibits proliferation of HepG2 and HuH-7 cells. We have analyzed the expression of the proapoptotic proteins Bax, Bad and Bak and the antiapoptotic proteins Bcl-2, Bcl-XL and Mcl-1, by *q*RT-PCR and Western blot, in HepG2 and HuH-7 hepatocarcinoma cells, treated with AM-CM. We observed a significant increment in proapoptotic mRNAs and proteins expression and a decrease in antiapoptotic genes and proteins expression, after 24 and 72 h of treatment with AM-CM. Moreover, AM-CM treatment increase the presence of apoptotic nucleus, measured by DAPI staining. Our results position amnion-derived stem cells as emerging candidates in anticancer therapy.

298. (304) **FUNCTIONAL PATCHES WITH MESENCHYMAL STEM CELLS FOR REGENERATIVE MEDICINE**

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Mesenchymal/Stromal Stem Cells (MSCs) are multipotent cells present in different adult or fetal tissues mainly bone marrow, adipose tissue or umbilical cord. In recent years biotechnological research with MSCs has grown steadily based on the ability to integrate them into damaged tissue and to immunomodulate the inflammatory response in vivo, helping in tissue regeneration. Here we evaluated three-dimensional pieces with biocompatible materials containing Mesenchymal Stem Cells, i) functionalizing a hydrogel-based bioink by adding to the formulation extracellular matrix proteins, iii) formulating a PEG-based bioink ii) evaluating the viability and behavior of bioprinted MSCs over time post-printed in the ink variants.
MSCs were obtained from the differentiation of Pluripotent Stem Cells as previously reported. We added in different proportions, hyaluronic acid to the alginate-based bioink that we develop previously. We also tested in different proportions PEGDA (Poly (ethylene glycol) diacrylate) combined with a photoinitiator. The optimal combination of the components was given by the evaluation of the inks based on its ability to: print pieces with good height; be sterilizable, without destroying its components; good swelling capacity, for the correct distribution of oxygen and nutrients; and ability to maintain cell viability. Cell viability was evaluated with the CellEvent Caspase-3/7 Green Detection Reagent Kit.
We found that the combination of hyaluronic acid with alginate-based bioink increases post-print cell viability and we also found patches with PEGDA did not modify cell viability significantly.
In conclusion, we found that the combination of hyaluronic acid with alginate-based bioink improves the extracellular environment increasing cell viability and swelling of the patch. Our results also suggest that PEGDA formulation is one more possible alternative for our experiments.

299. (369) **SELECTIVE DECELLULARIZATION AND ENDOTHELIAL REPLACEMENT AS A STRATEGY TO IMPROVE IMMUNOCOMPATIBILITY IN TRASPLANTED ORGANS**

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The number of kidney transplants performed each year cannot meet the increasing demand of these organs. Several strategies aim to increasing the offer of immune compatible organs, such as xenotransplantation of genetically modified pig organs and the generation of bioartificial organs. Herein we described a new technique that allows us to generate a rat bioartificial kidney whose endothelial cells have been replaced with human endothelial cells.
Two different protocols were used in order to maintain parenchyma epithelial cells and remove selectively rat endothelial cells. In the first one, 2ml of a solution of 0.01% of SDS was perfused via renal artery at 4°C. For the second protocol, the rat kidney was perfused with increasing osmolarity solutions of saccharose and EDTA at room temperature for 15 minutes. The effectiveness of partial decellularization was calculated by comparing those endothelial cell decellularized kidneys with a kidney that has been decellularized with collagenase (positive control). Albumin conjugated to Evans blue dye was perfused through the artery in order to evaluate vascular permeability. Both protocols achieved a similar vascular permeability while the second group maintained a better epithelial cell viability assessed with TUNEL assay (65 % vs 40 %).
Afterwards, partially decellularized kidneys were recellularized (2 hours at 37°C) with 7x106 human endothelial cells transfected with GFP protein. Histological analysis showed HMEC-GFP attached in almost all the vascular bed. Finally, recellularized kidneys were transplanted in an anesthetized rat. After surgery, complete organ perfusion was achieved and also urine production for at least 30 min pos-transplant.
These results suggest that endothelial decellularization and recellularization with human cells can be feasible methods in order to generate a more immune compatible bioartificial organs.

300. (381) **A CRITICAL PATHWAY IN HUMAN DEVELOPMENT: C19MC MICRORNAS REGULATES FGF2 RESPONSE IN A MODEL OF HUMAN PLURIPOTENT STEM CELLS CARDIAC DIFFERENTIATION**

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Human pluripotent stem cells (hPSC) have the capacity to self-renew and differentiate *in vitro* into all cell types of the organism, and it is an established model for early human embryo development. Recently, we found a 56-miRNA-cluster located at human chromosome 19 (C19MC) that downregulates during hPSC cardiac differentiation (CD). To ascertain the role of this primate-specific microRNA cluster, a hPSC-C19MC$^{(-/-)}$ line was generated with CRISPR/Cas9. C19MC$^{(-/-)}$ cells displayed no evident changes in the cell cycle, apoptosis or differentiation markers compared to wild type. Contrarily, C19MC$^{(-/-)}$ cells were significantly impaired to differentiate into cardiomyocytes. Early mesoderm and cardiac RNA markers, like EOMES, TBX6, MESP1, were found altered. In order to further explore the early steps of differentiation, we performed RNA-seq of the cells at the gastrulation stage (0 and 24hs after CHIR99021 incubation). Gene ontology analysis revealed altered signaling pathways, including PI3K-Akt, MAPK and Wnt, and FGF2. As FGF2 is a key pathway in pluripotency, we address its role through two different

approaches. First, both wild type and mutant cells were treated with FGF2 for 3 hours before gastrulation. Wild-type phenotype was partly recovered, as evidenced by the presence of contractile cardiomyocytes at day 15. Second, given that FGF2 is an important activator of RAS cascade that phosphorylates ERK1/2 (pERK), we incubated the cells with FGF2 for up to 5 hs in pluripotency media. Mutant cells exhibited an elevated pERK mark in ground conditions, and it was noticeable that the phosphorylation took place faster when they were treated. In summary these findings support a critical role of the C19MC microRNA cluster in early stages of primate differentiation.

301. (406) **THE HSA-MIR-216/217 CLUSTER IS DIFFERENTIALLY EXPRESSED DURING HUMAN EMBRYONIC STEM CELLS NEURONAL DIFFERENTIATION**

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Human embryonic stem cells (hESCs) are self-renewing cells that have the potential to differentiate into specialized cells. MicroRNAs (miRNAs) are small non-coding RNAs that play important roles in many key processes, such as cell differentiation. Large fraction of them are key regulators throughout neural development. Due to this, we aimed to identify and characterize miRNAs involved in hESCs neuronal differentiation. To this end, we derived and characterized neural stem cells (NSCs) from H9 hESCs and differentiated these cells into neurons (NEU). We next performed a high throughput small RNA sequencing of these cell populations followed by bioinformatics analysis. First, we select miRNAs with no less than 10 reads and visualized them as a heat map to represent the miRNA expression profile. Then, we carried out a differential expression analysis (FDR 0.5) and found 728, 270 and 798 differentially expressed miRNAs between hESCs-NSCs; NSCs-NEU and hESCs-NEU, respectively. RNA-seq results were validated analyzing, by RT-qPCR with specific stem loop primers, expression levels of known miRNAs involved in neuronal differentiation (hsa-miR-9-3p/9-5p/124-3p/125a-5p/125b-5p and 128-3p). From the list of differentially expressed miRNAs it caught our attention the hsa-miR-216a,b/217 cluster, which was only expressed in NSC and NEU. Moreover, this expression pattern was also validated by RT-qPCR. Furthermore, expression of this cluster was reported in published RNA-Seq bioinformatic datasets of other neural cell types but not along mesoderm and endoderm differentiations or in other species or types of pluripotent stem cells datasets. Besides, there is no bibliographic data of this family associated to neural differentiation or regulation of stemness. In a future, we aim to knock out the whole cluster using the CRISPR/Cas9 technology as a loss-of-function strategy. This knowledge will enable us to characterize the function and targets of this cluster in a neurogenic context.

302. (409) **3D CELL CULTURES IN HYDROGELS FOR BONE TISSUE ENFINEERING**

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Cells in living beings are disposed in three dimensions and, consequently, growing cells in 3D cultures are a more realistic, physiologically relevant than in a 2D environment, the last being the more extended due to their ease of use. For tissue engineering, 3D cultures can provide more representative information of the cell proliferation and differentiation capacity as well as a measure of the need of vascularization of the artificial tissue. Here, we explore the use of two hydrogels, alginate (ALG) and silk fibroin (SF) as scaffolds for 3D cultures of cells, making focus on soft gelation processes that avoid compromising the cell viability. Alginate gelation was performed by Ca2+ crosslinking, which was tailored by the addition of Ca2+ containing particles and pH control, while SF gelation was induced by sonication. Then, the rheological properties of the hydrogels containing ALG and/or SF was evaluated. Finally, the proliferation and differentiation capacity of MC3T3-E1 Subclone 4 cells were analyzed in both 2D and 3D environments. SF exhibited a more simple and customizable gelation procedure than ALG, although the latter showed a more stable gel than the former. 3D cultures, although more complicated to obtain, significantly changed the behavior of cells. Consequently, this type of culture is the more appropriate to determine the capacity of these hydrogels to perform as scaffolds for bone tissue engineering.

303. (411) **MESENCHYMAL STEM CELL-DERIVED EXOSOMES LOSE THEIR REGENERATIVE POTENTIAL UPON UV-C IRRADIATION**

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Exosomes are extracellular vesicles ranging from 30 nm to 150 nm that originate from the endosomal pathway. Their content will depend on the cell of origin and its physiological state, thus the message they convey might change in response to changes in cellular conditions. In particular, the DNA damage response (DDR) has been reported to modulate exosome secretion. This work aims to elucidate the effect that genomic damage induced by UV-C may have on the exosomal secretion of Mesenchymal Stem Cells derived from induced Pluripotent Stem cells (iPS-MSC).
To induce genomic stress, iPS-MSC were irradiated with three different UV-C intensities (0,001 J/cm2, 0,01 J/cm2 and 0,1 J/cm2) and it was assessed by immunofluorescence evidencing the expression of damage sensors such as S-15 phosphorylated p53 and H2AX-**γ**. Our group has previously demonstrated that although both the expression of genes involved in the exosomal pathway and the number of exosomes secreted by irradiated and non-irradiated iPS-MSC did not show a significant difference between conditions, a loss in pro-migratory properties was observed in irradiated iPS-MSC derived exosomes. For this reason, we hypothesized that a change in exosomal cargo could be responsible for such effects. To evaluate this, exosomes secreted by irradiated and not irradiated IPS-MSC were isolated from conditioned media using a Size Exclusion Chromatography column and proteomic analysis was performed by Tandem Mass Spectrometry. The results showed that a subset of cytoskeleton proteins and migration-inhibiting molecules were over-represented in exosomes from irradiated cells, such as Filamin A and Talin-1, known for negatively regulating cell motility. Altogether these results suggest that iPS-MSC irradiated exosomes carry a distinct and particular cargo that can explain the reverting of the pro-migratory capabilities of non-irradiated iPS-MSC exosomes.

304. (414) **EFFICIENCY OF POINT MUTATION BY CRISPR/CAS9 IN IPSCS DETERMINED BY NEXT GENERATION SEQUENCING**

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The arrhythmogenic cardiomyopathy (ACM) is a genetic disease characterized by the replacement of contractile myocardium by adipose tissue, causing ventricular arrhythmias and eventually sudden death in patients. One of the most commonly mutated genes is the Plakophilin-2 (PKP2) that codifies for a desmosomal protein. The aim of this work was to generate an induced pluripotent stem cell (iPSCs) line with a reported point mutation in PKP2 gene (C>T that generates a missense mutation p.S140F) by CRISPR/Cas9 for modeling the ACM in vitro. In order to generate this edition we designed two RNA guides (gRNA 1 and 2) targeting the Cas9 to the desired region of the PKP2 gene and a template DNA for each gRNA (ssODN 1 and 2) complementary to the sequence containing the point edition. We co-transfected the plasmid containing the CRISPR system with gRNA1 or

gRNA2 together with the ssODN1 or ssODN2, respectively, to 2x10^5 iPSCs in two different
concentrations (1 ug or 2.5 ug of each DNA construction): gRNA1-1, gRNA1-2.5, gRNA2-1, gRNA2-2.5 groups. After puromycin selection, genomic samples from the 4 groups were taken for amplicon sequencing analysis. PCR amplicons from the pool were sequenced using Miseq in CD-Genomics. These results were analysed with CRIS.py, a python-based program for multiple sequence analysis. The analysis revealed 24.7%, 27.6%, 0.2% and 5.5% of C>T edition, 9%, 10.9%, 3.2%, 25.7% of indel, and 67.8%, 62.9%, 91.2% and 47% of wild type sequences for gRNA1-1, gRNA1-2.5, gRNA2-1, gRNA2-2.5, respectively.
With these results, cells from gRNA1-1 were clonally expanded and 15 clonal cell lines were Sanger sequenced, obtaining 3 clonal cell lines with the desired edition (20%).
In conclusion, gRNA1 was more efficient than gRNA2 independently of the DNA concentration used. Our next steps are to characterize the phenotype of the C>T PKP2 clonal cell lines and to determine whether we can model the ACM in-vitro after differentiating these cell lines into cardiomyocytes.

305. (436) **WALKING BACK OSTEOSARCOMA STEPS: PROTEOMIC PROFILING OF BONE MARROW MESENCHYMAL STEM CELLS AND PRIMARY AND LUNG COLONIZING OSTEOSARCOMA HUMAN CELL LINES**

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Osteosarcoma (OS), the most common malignant bone tumor, has a 20% five-year survival rate for metastatic disease and treatment-resistant patients. Rapid lung dissemination and acquired chemotherapy resistance remain as major clinical challenges. Mesenchymal stem cells (MSC) may contribute directly or indirectly to OS origin and progression. To identify potential metastasis biomarkers, we made a proteomic screening of non-metastatic SAOS2, metastatic LM7 OS cells and BM-MSC using a shotgun approach by a tandem nanocapillary liquid chromatography-mass spectrometry system. We identified 1049 proteins for BM-MSC, 1567 for SAOS2, and 1424 for LM7. To obtain gene ontology terms of the identified proteins, an enrichment analysis of the gene groups was carried out. The three cell populations shared 661 proteins corresponding to protein metabolism, metabolism, and energy-related pathways (25.72%, 22.37%, and 22.37% respectively). Individually, SAOS2 and LM7 cells showed the same number of shared proteins with BM-MSC, but the 64-shared proteins were not the same. Most relevant differences were that VEGF and PDGF signaling pathways were 2.25 fold-increased in LM7-MSC vs. SAOS2-MSC shared proteins. Further, citric acid and electron transport pathways were upregulated in SAOS2-MSC shared proteins. A comparison between SAOS2 and LM7 also shows upregulation of VEGF/PDGF signaling and other metastatic-related pathways in LM7 cells. Our results on the comparison of both OS cells to MSC, suggest that MSC may have a relevant role in OS progression, dictating not only tumor initiation but also metastatic dissemination. Further, LM7 cells had higher expression levels of proteins related to a mesenchymal phenotype and stem-related genes, suggesting a closer relation with MSC. Lung disease remains a major mortality factor in OS. Identification of mechanisms and differentially expressed genes associated with metastasis would help in discovering promising markers and therapeutic targets.

306. (482) **STUDY OF NON-CO-LINEAR EVENTS IN HUMAN PLURIPOTENT STEM CELL**

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RNA sequences topologically inconsistent with the correspondent DNA sequence in the reference genome are known as “non-co-linear events” (NCLe). These events can be linear (trans-splicing) or circular (circRNA) and both are post-transcriptional events. In human pluripotent stem cells (iPSC), NCLe were described to contribute to the regulation of early lineage differentiation; in particular circRNAs formed by quaking protein (QKI) 5 were described as necessary for cardiac differentiation. Trans-splicing events are formed by separate pre-mRNA with inverted and repeated sequences (Alu) while circRNA originate from a backspliced junction in a pre-mRNA. The aim of this work was to characterize NCLe and the role of QKI 5/6/7 in circRNA formation in iPSC. RNAseq data from an iPSC line was analyzed with NCLscan pipeline, revealing 1109 NCLe, among which 3 occurred between different genes. To validate these intergenic junctional events, we amplified them by RTq-PCR with specific primers and sequenced the product, corroborating that these alternative junctional organizations were not informatic artifacts. PCR on purified DNA showed they are not genomic rearrangements. Furthermore, using magnetic oligo dT beads we also demonstrated that the 3 events are polyadenylated and they are sensitive to degradation with RNase R, thus linearly conformed. In parallel, we designed RNA guides to knock out QKI in FN2.1 and H9 using CRISPR/Cas9. PCR and immunofluorescence analysis revealed the absence of the target, indicating that the strategy was successful. In conclusion, we identified NCLe in an iPSC line and characterized 3 different trans-splicing. We were also successful in preparing knockout lines for QKI to assess its role in differentiation as well as the circRNAs dependent on its function. In the future we plan to assess these knockout lines by RNAseq and functionality of the trans-splicing with CRISPR/Cas13 and characterize using northern blot.

METABOLISMO Y NUTRICIÓN

307. (001) **EFFECTS OF METFORMIN AND LOSARTAN ON NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH MESENTERIC ADIPOSITY IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME IN THE RAT**

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Non-alcoholic fatty liver disease (NAFLD) has been described as a histological manifestation of metabolic syndrome (MS). Mesenteric fat that drains into the portal circulation is the largest contributor to visceral adiposity. There is great interest in the pleiotropic effects of metformin (M) and losartan (L) in the treatment of risk factors for MS. We studied the effects of M (500 mg/kg/day) and L (30 mg/kg/day) on NAFLD and its relationship with mesenteric vascular bed (MVB) adiposity, insulin resistance (IR) and systolic blood pressure (SBP) in an experimental model of MS for 9 weeks. Six groups of Sprague-Dawley rats were used: control (C, standard diet), high-fat plus fructose-overload (HFF, 50% w/w bovine fat plus 10% w/v fructose solution), M-treated (CM), L-treated (CL), M-treated HFF diet (HFFM) and L-treated HFF diet (HFFL). Adiposity index was calculated as MVB adipose tissue weight/body weight x 100. Homeostasis model of assessment of IR (HOMA-IR), SBP, hepatic steatosis and perivascular fibrosis (hematoxylin-eosin and Sirius Red techniques) were measured.
HFF diet produced significant ($p<0.001$) increments on MVB adiposity index (%, 1.75±0.07 vs C: 0.81±0.04), HOMA-IR (0.50±0.06 vs

C: 0.11±0.003), SBP (mmHg, 154±2 vs C: 120±2), hepatic steatosis (%, 81.5±2.5 vs C: 1.3±0.3) and perivascular fibrosis (%, 52.0±3.3 vs C: 12.3±1.1). Compared with HFF rats, M and L treatments (HFFM and HFFL respectively), significantly (p<0.001) ameliorated MVB adiposity index (%, 1.23±0.02 and 1.18±0.08), HOMA-IR (0.13±0.01 and 0.20±0.03), SBP (mmHg, 127±1 and 116±3), hepatic steatosis (%, 51.6±3.2 and 56.5±5.2) and perivascular fibrosis (%, 33.4±3.4 and 31.0±2.8). Moreover, we found that both steatosis and perivascular fibrosis positively correlated with MVB adiposity index, HOMA-IR and SBP.
Both M and L prevented MVB adiposity increase and consequently exhibited beneficial effects on the stages of NAFLD in a context of IR and hypertension.

308. (016) **TOTAL AND UNDERCARBOXYLATED OSTEOCALCIN (OCN) IN NON-DIABETIC WOMEN HAVING OR NOT METABOLIC SYNDROME (MS)**

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Bone controls glucose homeostasis and insulin resistance through OCN. We wanted to know if body mass index (BMI) and the presence of MS could influence total and/or undercarboxylated OCN (tOCN and ucOCN, respectively) levels.
We compared ucOCN and total tOCN levels in 95 non-diabetic normoglucemic women (52.7±13.2 years) having or not metabolic syndrome (MS and nMS, respecively) and different degree of obesity. ELISA was used except for 25OHD where an immune-competitive method was used. Different letters indicate statistical differences (one-way ANOVA) and (*) p<0.05: Ms vs. nMS (Student t´test).
Results (mean±SD): Overweight (OW), type I, II, III obesity (OB) in nMS and MS, respectively. tOCN(ng/mL): 32.0±14.5^{b}, 22.3±13.1^{b}, 36.5±3.0^{b}, 10.7±4.5^{a}; 28.5±12.5, 24.3±12.1, 21.2±14.9, 27.3±12.9* ucOCN(ng/mL): 2.2±1.8^{a}, 2.5±1.7^{a}, 3.1±2.1^{ab}, 4.5±0.3^{b}; 1.0±0.4^{a}, 3.7±2.4^{b}, 3.6±1.6^{b}, 3.9±1.1^{b} Insulin(μUI/L): 6.9±2.6, 8.6±3, 9.0±1.6, 7.9±3.6; 11.1±4.6*, 12.5±4.0*, 12.7±4.9*, 13.9±5.6* Leptin(ng/mL): 10.3±5.5^{a}, 17.9±11.9^{b}, 23.9±6.5^{b}, 38.8±17.6^{c}; 9.9±6.3^{a}, 12.8±2.1^{a}, 22.2±10.7^{b}, 27.5±6.9^{b}
CTX (ng/L): 433±203, 417±166, 562±13, 380±201; 355±177, 411±172, 361±197, 436±147 25OHD (ng/mL): 22.9±8.1, 21.4±7.9, 20.0±7.3, 18.2±6.5; 22.4±8.0, 22.2±10.1, 16.4±2.9, 17.5±6.0.
Levels of tOCN were similar, CTX decreased and ucOCN, insulin and leptin increased with in the degree of OB in MS group.
Instead, in nMS women tOCN decreased and ucOCN increased with OB degree; while OB type III presented the lowest CTX levels.
In both groups, leptin increased and 25OHD showed a tendency to decrease with the degree of OB.
Conclusion: ucOCN levels appear to be directly associated with the degree of OB in both MS and nMS women, while tOCN did not change with OB in MS women but showed a high reduction in nMS women associated to the decrease in CTX levels i.e bone turnover.
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309. (017) **EFFECT OF FEEDING A LOW LACTOSE YOGURT-BASE DIET HAVING GALACTOOLIGOSACCHARIDES (GOS) ON BONE HEALTH: PRECLINICAL MODEL OF NORMAL GROWTH**

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GOS are natural prebiotics of human milk that could have positive actions in calcium (Ca) absorption and bone health. They can be incorporated in fermented dairy products by enzymatic action on milk lactose, resulting in a low lactose-containing food.
We evaluated and compared the effect of feeding a low lactose-yogurt containing GOS (EY) in bone health during normal growth of weaning rats. Rats (n=10/group) fed 3 diets: control AIN'93-G (C); GOS-free yogurt (Y) or EY during 30 days.
We evaluated food consumption; body weight (BW); Ca absorption (% AbsCa) by balance methods; in cecum Lactobacilli growth (LB) by microbiological culture, pH by a pHmeter and short chain fatty acids (SCFA) by HPLC-IR; total skeleton (Et), lumbar spine (Ls) and proximal tibia (Pt) bone mineral density (BMD) and Et bone mineral content (BMC) by densitometry; bone volume (%BV) and intestinal crypt depth (ICD) (μm) by histology; maximal load, fracture strength and elastic modulus by biomechanical test. ANOVA and Bonferroni *post hoc* test were used to evaluate statistical significances.
Food consumption and BW were similar thought out the study. EY showed higher %AbsCa (p<0.05), LB colonies (p<0.05); SCFA concentration (p<0.001) and lower cecal pH (p<0.01) than Y and C. EY had the highest Ls and PtBMDs (p<0.05), %BV (p<0.01), ICD (p<0.0001) than YC and C without differences in EtBMC vs C. EY had higher biomechanical parameters than Y (p<0.01) without differences vs. C.
Conclusion: YE could be a useful tool to ensure an optimal bone growth in lactose intolerance conditions.
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310. (022) **PREBIOTICS GALACTOOLIGOSACCHARIDES/ FRUCTOOLIGOSACCHARIDES (GOS/FOS®) MIXTURE, CALCIUM (Ca) ABSORPTIOM AND RESORPTION; AND INSUFFICIENCY OF Ca AND VITAMIN D (VD)**

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Prebiotics favors Ca absorption (CaAbs) and retention in bone. VD positively affects both processes. We evaluated if the prebiotic mixture assayed here exert similar effects under conditions of VD insufficiency and low Ca intake, using a preclinical model of postmenopausal bone loss.
Adult ovariectomized rats fed a commercial diet during 15 days post-surgery. Then, for an additional 45-days period, 16 rats fed a VD-free (0 IU%) diet (-D groups) and 16 a normal VD diet (100 IU%) (+D groups). Both isocaloric diets content 0.3% of Ca (0.3%). At day-60, each group was subdivided into 2 groups which continuing feeding the same diet, having or not 0.25% of prebiotic mixture: +DPM and +D or -DPM and –D, respectively. We evaluated zoometric measurements, lactobacilli (LS) growth in feces by culture; activity of 4 fecal enzymes; cecal pH; CaAbs% by balance; femur Ca content (biochemically), bone volume fraction (BV/TV), epiphyseal cartilage total length (GPC.Th) and intestinal crypts depth (CD) by histology; total skeleton (TS) bone mineral content (TSBMC) and bone mineral density (TSBMD), lumbar spine (LS), proximal tibia (PrT) BMDs by densitometry. ANOVA and Bonferroni *post hoc* test were used to determine statistical significances.
No differences in cecal pH, lactobacillus colonies, β-glucuronidase, urease and tryptophanase and β-glucosidase; CD; LS and PrTBMDs were observed between +DPM and -DPM; CaAbs, femur Ca content, TSBMC, TSBMD, TF BMD, BV/TV and GPC.Th were significantly higher in +DPM vs. -DPM (p<0.05). Results showed that hypovitaminosis D negatively affected the prebiotic GOS/FOS® action on Ca Abs and bone retention.

Conclusion: Although prebiotics would a beneficial tool to improve Ca bioavailability, especially when requirements are not met, it is important to monitor VD nutritional status to avoid the least effect in Ca absorption and retention.
Grants of CONICET (PIP 11220100100004) and UBA (UBACyT 20020130100091BA).

311. (026) **EFFECTS OF CANNABIS OIL ON LIVER INJURE AND OXIDATIVE STRESS IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME**

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Liver damage and oxidative stress are closely related to the alterations present in Metabolic Syndrome (MS). Interest in the beneficial effects of Cannabis sativa L. has increased in recent years. Cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD) have been found to be potent antioxidants. The aim of this study was to evaluate the effects of cannabis oil on liver injury and oxidative stress in insulin-resistant dyslipidemic rats fed a sucrose-rich diet (SRD). Male Wistar rats were fed the following diets for 21 days: Reference Diet (RD): standard commercial laboratory diet, Sucrose rich diet (SRD) and SRD+Cannabis oil (SRD+Ca): the oral administration of 1 mg/kg of body weight of cannabis oil daily. The cannabis oil presented a ratio of total cannabinoids THC:CBD of 1:2. We analized: a) Serum: triglycerides, cholesterol, glucose, transaminases (AST and ALT), alkaline phosphatase (AP) and non-enzymatic antioxidant capacity (FRAP). b) liver: triglyceride content, transaminases AST and ALT, AP, reactive oxygen species (ROS), substances reactive to thiobarbituric acid (TBARS), FRAP and Catalase activity. Results: In the SRD+Ca group, serum triglyceride and cholesterol levels decreased significantly, reaching similar values to the RD group, without changes in glucose levels. In addition, AST, ALT, AP and TBARS levels were decreased ($P<0.05$), reaching reference values. FRAP was increased ($P<0.05$), reaching reference values. In liver tissue, triglyceride content and AST, ALT and AP were decreased ($P<0.05$). TBARS and ROS levels were reduced ($P<0.05$), reaching values similar to RD group. In addition, FRAP and the activity of the enzyme Catalase was increased ($P<0.05$), although the values of the latter were still lower than RD group. In summary, this work show that cannabis oil improves liver damage and oxidative stress in SRD-fed insulin-resistant dyslipidemic rats. Therefore, it would have a hepatoprotective effect on metabolic disorders included in MS.

312. (029) **EFFECT OF PROANTHOCYANIDINS-ENRICHED EXTRACT FROM *LIGARIA CUNEIFOLIA (Lc)* ON HEPATIC CHOLESTEROL METABOLIZATION AND EXCRETION IN WISTAR RATS FEEDING WITH HYPERLIPEMIC DIET**

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In folk medicine,*Ligaria cuneifolia(Lc*is used to increase blood fluidity by lowering plasma cholesterol(Cho).Previous results showed that a proanthocyanidin-enriched fraction(P*Lc*)led to decreased levels of plasmatic Cho and Triglycerides(TG)in rats fed with high fat diet(HFD).***Aim:*** We aimed to evaluate the effect of P*Lc* on hepatic metabolization and biliary excretion of Cho in Wistar rats fed with HFD(standard diet added with 40% of the first bovine juice) for 28 days and treated with P*Lc* for 3 days. ***Material and Methods:*** Animals were divided into HFD Group(vehicle,i.p.,n=12)or HFD-P*Lc*(group, Plc 30mg/kg b.w.,n=12). At fourth day, rats were anesthetized with ketamine/xylazine (100 mg/kg/3mg/kg,i.p.).A cannula was placed in the common bile duct and bile was collected every 15 minutes for 60 minutes to assess bile flow. Blood was obtained by cardiac puncture and the liver was removed.A liver sample was homogenized and the microsomal-enriched fraction was obtained by differential centrifugation. In blood we determined total Cho;LDL-Cho,HDL-Cho and TG by using commercial detection kits. In the microsomal-enriched liver fraction we determined the protein expression of the enzyme Cholesterol-7-alpha hydroxylase (Cyp7a1)by Western Blotting. ***Results:***Plasmatic levels of Cho(mg/dL)HFD: 97.5±4.7,HFD-P*Lc*:53.5±4.1*;LDL-Cho (mg/dL)HFD:24.1±1.2, HFD-P*Lc*:19.0±0.3*;HDL-Cho(mg/dL)HFD:25.0±0.9,HFD-P*Lc*:24.0±0.8;TG(mg/dL)HFD:164.6±29.5,HFD-P*Lc*: 83.3±6.6*.Bile Flow(ml/min/100gr liver weight)HFD:2.6±0.4,HFD-P*Lc*: 3.3±0.3*(mean±EE;*p<0.05 vs.HFD.Student's *t*-test for unpaired data).Also,P*Lc* treatment increased Cyp7a1 protein expression by 2,6-fold(p<0,05). ***Conclusion:*** We propose that P*Lc* treatment showed a lipid-lowering effect.Lower levels of plasma Cholesterol would be explained, in part, by the rice of Cyp7a1 expression that led to an increase in bile salts synthesis and biliary excretion improving the bile flow rate.

313. (036) **CHIA SEED MODULATES LIPOLYTIC ENZYMES AND pAKT LEVELS AND REDUCES COLLAGEN DEPOSITION IN ADIPOSE TISSUE OF AN EXPERIMENTAL MODEL OF VISCERAL ADIPOSITY AND INSULIN RESISTANCE**

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The seeds of *Salvia hispanica* **L.** (chia), rich in 18:3 n-3, have been shown to have beneficial effects on several metabolic disorders including visceral adiposity. Although many advances have been made in the study of this topic, the mechanisms involved are not completely understood. This study aimed to explore the effect of chia seed upon key enzymes of lipolysis, AKT levels and morphological changes of visceral adipose tissues in sucrose-rich diet- fed rats, a widely recognized model of visceral adiposity and insulin resistance. Male Wistar rats were fed a sucrose rich diet (SRD) for 3 months. Half of the animals continued with the SRD until month 6, the other half was fed a SRD in which the fat source, corn oil (CO), was replaced by whole chia seed from month 3 to 6 (SRD+CHIA). Another group consumed a reference diet all the time. We analyzed in epididymal (eAT) and retroperitoneal adipose tissues (rAT): a- morphological changes (adipocyte size, collagen deposition and inflammatory cells infiltration), b- lipolytic enzymes: HSL and ATGL levels; c- total and pAKT levels (basal and post insulin stimulation -euglycemic hyperinsulinemic clamp-). Besides, serum levels of triglycerides, free fatty acids, glucose, and insulin were determined. The replacement of CO by chia seed in the SRD: a- reduces adipocytes hypertrophy, lipid content and collagen deposition in both eAT and rAT ($p<0.05$), b- reduces the inflammatory cells infiltration in the eAT, c- decreases HSL and ATGL protein levels in eAT and HSL protein level in rAT ($p<0.05$); d- increases basal pAKT levels in eAT and improves the insulin response of pAKT in both tissues ($p<0.05$). No differences in total AKT were observed between the 3 dietary groups, e-serum levels of lipids and glucose were reduced. This work provides novel information about the mechanisms involved in the beneficial effect of chia seed in different deposits of adipose tissue.

314. (044) **EFFECT OF SUPPLEMENTATION WITH FATTY ACID OMEGA 3 IN DIET CONTAINING OMEGA 6 AS FAT SOURCE**

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Introduction: The importance of the diet in the maintaining of health statue is widely accepted. **Objective**: To study the effect of ω3 fatty acid supplementation of diet containing sunflower oil as fat source, on serum fatty acid profiles of growing rats. **Material and methods**: Weanling Wistar rats received during 10 days normocaloric diet and fat was provided by sunflower oil (S group). The others groups received the same diet supplemented with 24mg/day of fish oil (SF group) o chía oil (SCh). Control group (C) received AIN´93 diet. Serum fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA test. Results: (expressed as %Area) SERUM: OLEIC C:10.11±1.84, S:12.13±3.84, SCh:12.74±1.56, SF: 13.12±2.82; ARACHIDONIC C:13.40±4.39, S:17.61±4.09, SCh: 15.75±0.89, SF:15.41±1.76; LINOLEIC C:20.52±3.37, S: 19.80±3.36, SCh: 21.14±2.12, SF: 18.92±3.87; LINOLENIC (ALA) C:0.93±0.27a, S:0.19±0.06 b, SCh: 0.28±0.08b, SF:0.22±0.05b; EPA C:0.80±0.22, S:0.68±0.15, SCh: 0.74±0.18, SF: 0.67±0.14; DHA C:1.60±0.55a, S:1.14±0.35a, SCh:1.70±0.45a, SF:4.22±0.93b. Media that didn't present a letter (a,b) in common, were different (p<0.01). In sera, S, SF and SCh groups showed lower ALA levels compared to C. SF group presented high levels of DHA. Diet S was mainly a contributor to linoleic acid with a ratio ω6/ω3 = 250 (recommended value: 5-10). **Results**: ω6 family was exacerbated and ω3 family decreased. Chia supplement showed a tendency towards higher values of ω3 family but were significantly lower than C. Fish oil supplement increase significantly DHA values. Diet containing sunflower oil as fat source provoked changes in serum fatty acids profiles and the supplementation with ω3 fatty acid provided by chía or fish oil do not increase ALA values significantly. **Conclusion**: Diet influences the serum fatty acid profile, being not only important the percentage of lipids on it but also the different fatty acids pattern. UBACyT: 20020190100093BA.

315. (046) **CERAMIDES AND LIPOPROTEIN LIPASE IN EPICARDIAL ADIPOSE TISSUE: A NEW LINK FOR CARDIOVASCULAR DISEASE?**

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Together with the widely known cardiovascular disease (CVD) risk factors, scientific community still look for new ones. Epicardial adipose tissue (EAT) is a visceral adipose tissue surrounding myocardium and coronary arteries, proposed to be an independent risk factor for CVD. The lipidomic study of tissues reveals the role of many bioactive lipids such as ceramides (Cer) on their metabolism. In EAT, these lipids may contribute to CVD risk. We previously demonstrated that Lipoprotein Lipase (LPL) activity is increased in EAT from coronary patients (CAD), probably contributing to an increase in its volume and therefore in CVD. We assessed the lipidome of EAT from CAD as well, demonstrating an enrichment in Cer. In this opportunity, our aim was to evaluate links among EAT LPL, its inhibitor Angiopoietin-like protein 4 (ANGPTL4), its activator glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein-1 (GPIHBP1), and Cer content in the tissue.
Methods and results: in EAT and subcutaneous AT (SAT) from CAD undergoing coronary artery bypass graft (n=25) or valve replacement (No CAD,n=14), LPL activity, ANGPTL4 and GPIHBP1 levels were evaluated by a radiometric assay and western blot, respectively. Tissue lipidomes were evaluated by UHPLC-MS, in positive and negative ionization modes. For statistics, the MetaboAnalyst software was used. In EAT, we found an increase in LPL activity, directly associated with total Cer content (p= 0.002), being the strongest correlation with Cer d18:1/24:1 (p<0.0001). Furthermore, Cer content inversely correlated with ANGPTL4 (p=0.04), but not with GPIHBP1 (p=0.26), although its levels were higher. In EAT, LPL activity was also associated with short and long chain triglycerides, diglycerides and plasmalogens. No associations were observed in SAT.
Conclusion: as a first approach, we propose that the increase in Cer, in particular d18:1/24:1, might be one mediator in the increase of LPL activity, contributing to CVD risk.

316. (064) **MICROENCAPSULATED PEPTIDES FROM BREWER'S SPENT GRAIN HAVE BENEFICIAL EFFECTS UPON LIPID METABOLISM DISORDERS DEVELOPED IN AN EXPERIMENTAL MODEL OF DYSLIPIDEMIA AND INSULIN RESISTANCE**

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Recently, it was demonstrated that peptides obtained from Brewer's spent grain (BSG) - a byproduct of the brewing industry- can exert *in vitro* biofunctional activities including hypolipidemic and antidiabetogenic effects. However, it is unknown whether the bioactive properties remain *in vivo*. The aim of this work was to evaluate the effect of dietary intake of microcapsules containing BSG peptides upon biometric parameters and lipid metabolism disorders in an experimental model of dyslipidemia and insulin resistance. Male Wistar rats were fed from weaning (3-week old) and for 100 days with 1 of 3 randomly assigned experimental diets: a-Control group (C) received a standard commercial rodent diet, b- SRD group received a sucrose-rich diet (SRD), c- SRD+P group was fed with a SRD containing 700 mg/kg body weight/day of BSG protein hydrolysate microencapsulated. We analyzed: a- serum levels of triglycerides (TG), free fatty acids (FFA), cholesterol (Ch), glucose (G) and uric acid, b- biometric parameters-body weight (BW), body length (BL), thoracic (TC) and abdominal circumferences (AC), body mass index (BMI), Lee index- and energy intake, b- visceral adiposity index (VAI), c- In epidydimal adipose tissue (AT): fat cell size, number and lipid content, d-cholesterol esterase (CE) and pancreatic lipase (LP) enzyme activities in cecal contents, e- liver TG and Ch content. Compared with SRD-fed rats SRD+P group shown a significant reduction (P<0.05) of the increased serum lipid levels, hyperglycemia and hyperuricemia. BL, AC, BMI and Lee index were similar in the 3 dietary groups. BW, TC and VAI were increased (P<0.05) in SRD and SRD+P groups, however, a reduction in energy intake and AT adipocytes size and TG content were observed in SRD+P rats. These changes were accompanied by a decrease (P<0.05) of CE and LP enzyme activities and liver lipid content. The results show that BSG protein hydrolysates prevent/ attenuate lipid metabolism disorders developed in SRD-fed rats.

317. (072) **THE CONSUMPTION OF FRIED OIL DURING GROWTH ADVERSELY AFFECTS THE MANDIBULAR BONE FORMATION**

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Dietary lipids play an important role in bone health, which is relevant to develop effective interventions to prevent bone diseases or disorders, especially during the period of growth.The present study evaluated the effects of feeding growing rats with a diet containing fried sunflower oil, on morphometric properties and biomechanical competence of the mandible. **Methods**: Male Wistar rats (21±1 days old) (n=21) were assigned to one of three diet groups: those fed a control diet (C), and a high-fat diet containing either sunflower oil (SFO) or SFO which was repeatedly heated (SFOx). Total body weight and food intake were weekly recorded. After 8 weeks, rats were euthanized. Mandibular growth was estimated with digital calipers on removed bones by taking measurements between anatomical points. Structural properties (load at fracture(Wf), load at yield(Wy), and stiffness(Wydy)), were determined by using a three-point bending test (Instrom 4442). **Results** (mean±SD, ANOVA-SNK): final body weight was lower in SFOx rats as compared with C and SFO groups (310.0±14.7g<339.4±16.6g=336.4±23.0g, respectively; P<0.05). Mandibular weight and length (P=0.001) were negatively affected by high-fat diets rich-SFOx.The deleterious effect of SFOx on mandibular growth was more accentuated on the posterior part (C:11.4±0.3mm= SFO:11.1±0.2mm >SFOx:10.7±0.2mm; P=0.0005). The anterior/posterior ratio indicates that SFOx induced some deformation of the mandible. Structural properties, Wf, Wy and Wydy of the mandible were negatively affected in rats fed SFO either fresh or fried, as compared to the C group (P<0.01). Although Wf and Wy did not show significant differences between SFO and SFOx groups, fried oil intake induced a significant reduction in bone stiffness (Wy/dy). **Conclusion:** The potential adverse effects of consuming SFOx may affect mandibular mass, growth and development. Awarded by UBACyT: 20020170100138BA & 20020170200055BA.

318. (073) **INTAKE OF FRIED SUNFLOWER OIL AND RISK OF HEPATOESTEATOSIS**

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Previous studies by us in growing male rats indicated the prominent role of fried sunflower oil (SFOx) consumption in determining the risk for growth, cardiovascular and bone effects. In this study, we evaluated the effect of SFOx diet on body fat content, visceral fat, hepato-somatic index (HSI) and liver fatty acids (FA) profile of growing rats.

Male weaning Wistar rats (n=21) were randomly assigned to feedings of one of three different diets. The control rats were fed a commercial diet (C) and the experimental rats received either SFO or SFOx diets. SFO was repeatedly heated for a total of 40 hours (SFOx). SFO and SFOx were mixed with commercial rat chow. Rats were fed ad *libitum* throughout the experiment. Total body weight and food consumption were recorded every other day. At eight-week experimental period, body fat content and visceral fat were measured; HSI and liver FA profile (GC,Testo Equipment, Model 270 at 50°C) were determined. Results. SFOx vs. SFO and C showed that total body weight (323.1±9.1< 335.5±10.9=337.7±8.9 g; p=0.044), % body fat (13.7±0.8b vs.15.9±1.7cvs.10.2±1.3a; p=0.001); % visceral fat (3.55±0.36>3.08±0.38> 2.55±0.33; p=0.008) and HSI (4.17±0.34 vs. 3.64±0.27 =3.66±; p=0.016) were altered. In SFOx rats, serum T-Chol and nonHDL-Chol were the highest (p=0.025 and 0.029, respectively). SFO, SFOx and C groups attained similar serum concentrations of triglycerides and HDL-Chol (P=0.057 and P=0.265, respectively). There were significant higher liver profile of total saturated fatty acids; *trans* FA and unidentified minor compounds in SFOx group (p=0.001, p=0.001 and p=0.025; respectively); meanwhile, SFOx group presented significantly lower in total PUFA (p=0.001) and MUFA (p=0.001) than SFO and C groups. Conclusions. The potential adverse effects of SFOx denoted the risk for liver dysfunction. Dyslipidemia and increased visceral fat content contribute to the presence and/or progression of hepatosteatosis.

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319. (074) **SIMVASTATIN EXERTS A PLEIOTROPIC EFFECT ON BONE IN RATS UNDER A HYPERCHOLESTEROLEMIC DIET CONSUMPTION**

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There is evidence that lipid and bone metabolisms are mutually regulated. Previously, we had demonstrated the negative effect of high-cholesterol diet(HCD) on bone health. Simvastatin(SMV) blocks cholesterol(chol) biosynthesis in hepatic cells. However, the overall benefits observed with SMV appear to be greater than changes in lipid levels. Indeed, recent studies indicate that statins have beneficial extrahepatic effects. This study evaluated the effects of SMV administration on liver and bone under a hypercholesterolemic diet consumption. **Methods**: 40 rats were assigned to 1 of 4 groups: 1) control(C): fed pellets; 2) fed HCD; 3)C+SMV, fed pellets and SMV was given by gavage(5mg/day); and 4)HCD+SMV. After 5 weeks, rats were euthanized, blood was drawn and chol (mg/dL) and transaminase activities [AST,ALT(UI/l)] were determined. The liver was removed and weighed. The hepato-somatic index (HSI% (organ mass(g)/body mass(g)%)) and Oxidation of 2′,7′ dichlorofluorescein diacetate(DCFH-DA)levels(spectrofluorimetry,u.a./min. mg.prot) were calculated. Structural properties of the femur (load at fracture(Wf), load at yield(Wy), diaphyseal stiffness(Wydy)), were determined (Instrom 4442).

Results (mean±SD,ANOVA-SNK): SMV did not reduce chol (C:63.2±1.1 = C+SMV:67±16.8 < HCD+SMV:214.8±31.2 = HCD:209.3±39.3mg/dL, p<0.001) but improve transaminase activity (p<0.001). In liver, SMV decrease DCFH-DA levels (C 1.87±0.07=C+SMV2.03±0.04 < HCD+SMV3.49±0.26 < HCD4.03±0.1 u.a./min.mg prot, p<0.001). HSI% was higher in HCD groups (p<0.001).However, SMV could revert deleterious effects of HCD on the structural properties of the femoral diaphysis, Wf, Wy and Wy/dy, (p<0.01). Moreover, C+SMV revealed more benefits than expected.

Conclusion: in this study, SMV failed to manage liver and plasma chol concentration, but exhibit “pleiotropic” properties on bone beyond lipid effects. Further clinical evidence is required to evaluate SVM role in the treatment of bone diseases.

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320. (080) **EFFECT OF SOCIAL ISOLATION DUE TO COVID-19 ON BMI IN SCHOOL CHILDREN**

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Introduction: Preventive and compulsory social isolation (ASPO)

and the resulting closure of schools and suspension of sports practice reduced physical activity; this could affect children causing changes in lifestyle and consequently body mass index (BMI).
Objective: to evaluate the effect of ASPO (March-October 2020) on the changes in BMI and lifestyle in children aged 6 to 9 years old in La Plata, Buenos Aires.
Methods: Children who underwent their health checks at La Plata Children Hospital-IDIP Health Observatory in the 4 months prior to the ASPO were summoned in November 2020 to assess anthropometric variables (weight, height, BMI expressed as z-score) and lifestyle (diet, physical activity and sleep). A historical control group (GH) was established with anthropometric data from older children who did not experience ASPO between 6 and 9 years old.
Results: 140 children were evaluated. Body weight increased during the ASPO (DzBMI/age: 0.47 vs GH=0.04; p<0.001). Children reported less physical activity (52.3%), increased screen time (75%) and sleeping more hours (46.6%) than before the ASPO. Children with pre-existing overweight/obesity gained more weight (6.25 vs 3.0 kg in normal weight children; p<0.001), associated with reduced physical activity (2 h/day vs 3 h/day in normal weight children; p<0.001) and not to changes in diet.
Conclusion: ASPO affected lifestyle and weight gain in children; those with pre-existing overweight/obesity were more affected. Our findings can guide efforts to preserve and promote child well-being during lockdown, helping governments to decide the confinement rules to apply to children, especially regarding school closing.

321. (090) **CELIAC DISEASE: DAILY ANTIOXIDANT INTAKE FROM FOOD**

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Celiac disease (CD) occurs in 1 out of 167 adults in our country. The immune response due to the ingestion of gluten produces injury to the small intestine with an increase of oxidative stress markers. The only treatment is a gluten-free diet (GFD). Many studies propose that antioxidants contained in some foods contribute to prevention and improvement of some diseases. **Objective**: to determine the contribution of antioxidants from the diet in celiac and non-celiac patients. **Materials and Methods**: 68 people were surveyed using Google Forms as a tool where the frequency and quantity of food were recorded. The estimations of the total antioxidant capacity (TAC) were carried out with the intake data of these foods and the tabulated TAC values (umol TE / g of food) in previous publications. **Results**: It was found that 31% of the participants did not consume nuts or almonds. Within the group of vegetables, the most consumed were potatoes (71%), onions (64%) and tomatoes (59%). Tangerine, banana, almond, potato and tomato showed statistically significant differences between the average consumed by celiac people and controls (p <0.05, 95% confidence), finding the highest intake in the celiac group. 81% of the total have a consumption greater than 10,000 trolox (umol TE / day, recommended intake according to bibliography). It was observed that 25% out of the 8 patients who consumed less than the recommended amount of antioxidants were celiac patients (12.5% did not adhere to the GFD). Mean mate ingestion contributed to 35.8% and 28.6% of the total antioxidants intake from the foods surveyed in celiac patients and controls, respectively. There were no statistically significant differences in the contribution (%) of mate's TAC between the two groups (p = 0.3308, > 0.05). **Conclusions**: most of the surveyed people showed an adequate antioxidant ingestion. Mate was identified as the main source of TAC. Other biomarker studies are necessary to evaluate and confirm these results.

322. (136) **ALTERATION OF KEY METABOLIC COMPONENTS IN A FOLLICULAR PERSISTENCE MODEL IN DAIRY COWS**

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Several local metabolic factors are involved in the ovarian follicular persistence associated with anovulation similar to the ovaries from cows with cystic ovarian disease. Therefore, we aimed to study the protein expression of AdipoR1, AdipoR2, AMPK, CPT1 and ACOX1 in ovarian follicular structures during the development of follicular persistence in cows. Non-lactating Holstein cows (n=25) with regular estrous cycles without previous reproductive disease were studied. The persistence model was performed with an intravaginal progesterone device to get subluteal concentrations of progesterone, obtaining dominant follicles by ovariectomy at the expected day of ovulation (n=5; P0) and follicles that persist for 5 (n=5; P5), 10 (n=5; P10) or 15 days (n=5; P15) relative to the expected time of ovulation. Controls cows, without progesterone treatment, were ovariectomized in proestrus (n=5; C). Expression of target protein was assessed by indirect immunohistochemistry in ovarian tissue sections. In theca cells, AdipoR1 expression was higher in persistent follicles of P15 than persistent/dominant follicles of other groups (p<0.05). AdipoR2 expression in theca cells was higher in persistent follicles of P15 than follicles of groups C and P0 (p<0.05). AMPK expression tends to increase (p=0.078) in the granulosa cells of the persistent follicles of the P15 group with respect to persistent/dominant follicles of the other groups. In granulosa cells, the expression of CPT1 was higher in persistent follicles of P5, P10 and P15 groups than C and P0 groups (p<0,05). In theca cells, the expression was higher in persistent follicles of P15 than C, P0 and P5 groups (p<0.05). ACOX1 expression showed no differences (p>0.05) in follicular structures analyzed. These results evidence a local alteration in some metabolic sensors from the initial stages of persistence, possibly related to alterations in the oxidation of fatty acids at intraovarian level.

323. (148) **HIGH TRIGLYCERIDES/HDL-CHOLESTEROL INDEX AND REMNANTS LIPOPROTEINS ARE ASSOCIATED WITH COVID-19 SEVERITY**

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Coronavirus disease 2019 (COVID-19) patients with severe complications present comorbidities like cardiovascular-disease, hypertension and type 2 diabetes mellitus (DM). These clinical disorders share metabolic alterations like insulin resistance (IR) and dyslipidemia. Scarce data are reported about lipid parameters and COVID-19 severity. Our aim was to evaluate the association between different components of the lipid profile, particularly the TG/HDL-Cholesterol index as surrogate marker of IR and Remnants lipoproteins-cholesterol (RLP-C), in patients with COVID-19 at hospitalization. Methods and results: 193 patients (68 (29-96) years; 49.7% male) hospitalized for COVID-19 and 200 negative COVID-19 patients (46 (18-79) years; 52.5% male) consecutively attended at Hospital de Clínicas during the same period, were included. Lipoprotein profile and glucose were assessed in patients and controls. Procalcitonin (PCT), as a surrogate marker of COVID-19 severity, was measured in COVID-19 patients. COVID-19 patients were older (p<0.001), without differences in DM frequency (21% in both) neither

in gender between groups. COVID-19 patients presented higher glucose and TG, but lower total, LDL, HDL and no-HDL-Cholesterol levels (p<0.001) than controls. RLP-C and TG/HDL-Cholesterol were increased in COVID-19 (p<0.001). Regarding PCT, the population was divided by terciles: 0.010-0.070 ng/ml (n=46), 0.071-0.390 ng/ml (n=60) and higher than 0.391 ng/ml (n=54). Using Kruskal Wallis test, no differences in age, gender and DM were observed. As the PCT values rises, a decrease in total, LDL and HDL Cholesterol (p<0.03) was observed with no differences in TG, meanwhile levels of RLP-C and TG/HDL-Cholesterol increased (p<0.001). Conclusion: Lower levels of all the Cholesterol fractions were related with the presence and severity of COVID-19. The increase in RLP-C and TG/HDL-Cholesterol index would indicate in COVID-19 patients a lipid metabolic disorder characteristic of IR states.

324. (172) **ALTERATIONS IN INTERMEDIATE METABOLISM AND AORTA MORPHOLOGY INDUCED BY ZINC DEFICIENCY AND/OR HIGH FAT DIET DURING GROWTH**

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Introduction: Zinc deficiency can coexist with overweight and obesity during growth predisposing to metabolic disorders in adult life.
Objective: To evaluate, in adult male rats, alterations in intermediate metabolism and aorta morphology induced by fetal and postnatal zinc deficiency and/or high fat diet during post weaning life.
Methodology: Female Wistar rats received low (L:8ppm) or control (C:30ppm) zinc diets from pregnancy to offspring weaning. C male offspring continued with C(C) or HFD (60% of total calories) (CH) diets. L offspring were fed L(L) or L and HFD(LH) diets. At day 81, we determined blood oxidative stress, glucose tolerance test (GTT), lipid profile, plasmatic adiponectin levels and thoracic aorta morphology. Two way ANOVA, Bonferroni post-test, mean±SEM, *p<0.01 vs C, †p<0.01 vs L, ‡p<0.01 vs CH. N=8 per group. FFyB-UBA-CICUAL approval Exp 0061021/18.Res 4370.
Results: CH and LH showed higher bodyweight (C:418±13; CH:505±9*; L:401±10; LH:444±5‡†g). LH and CH showed an increase of GTT curve area (C:27797±504; CH:30827±971*; L:27826±809; LH:34851±1344‡† min.mg/d). L, LH and CH showed higher plasma glucose levels after 3 hours of glucose overload. Zinc deficiency exacerbated alterations induced by HDF. HFD and zinc deficiency increased triglycerides concentration (C:85±4;CH:168±15*;L:112±6*;LH:127±4†‡mg/dL), plasmatic TBARS(C:1,7±0.2; CH:2,8±0.2*; L:2,9±0.2*; LH:2,6±0.3*pmol. MDA/mg.prot) and adiponectin (C:8,3±0,6; CH:6,4±0.4*; L:8,6±0.9; LH:6,4±0.1† µg/ml) levels. No changes were observed in cholesterol levels. LH and CH showed higher thoracic aorta media lumen ratio (C:0,22±0,02; CH: 0,35±0,01*; L: 0,234±0,004; LH: 0,31±0,01†).
Conclusion: Zinc deficiency and/or HFD during growth increases systemic oxidative stress, reduces glucose tolerance, alters lipid metabolism and induces aorta remodeling in adult life. Zinc deficiency during fetal and postnatal life exacerbates some of the alterations induced by HFD.

325. (175) **INHIBITION OF MICROSOMAL TRIACYLGLYCERIDE TRANSFER PROTEIN (MTP) BY LOMITAPIDE FAVORS TUMOR GROWTH IN MICE**

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Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as very low-density lipoproteins (VLDL). The MTP inhibitor lomitapide binds directly to MTP inhibiting the synthesis of VLDL in the liver. The objective of this work was to study the effect of MTP inhibition on tumor growth. Adult male Balb/c nude mice were subjected to a xenograft model where Huh7 cells ($5x10^6$/mouse) were injected subcutaneously into the right flank of mice. After 4 days, mice were divided into 2 groups. Control group received vehicle (methylcellulose, gavage) and another group received 5 mg/kg bw/day lomitapide (gavage) for 15 days. Tumors were monitored using a caliper and volumes were estimated based on the formula "1/2 x l x w x h". At the end, mice were sacrificed, and tumors excised and weighed. Lomitapide-treated mice showed higher tumor volume and weight (2-fold) than control mice. Plasma levels of triacylglycerol and cholesterol were decreased (-30%, and -40%, respectively) in lomitapide-treated mice compared to control mice. Tumor histology showed no differences between groups on tissue architecture; however, lomitapide-treated mice presented with accumulation of cytosolic lipid droplets. Analysis of proliferation by immunoblotting in total tumor homogenates showed that lomitapide-treated mice presented with increased expression of proliferation cell nuclear antigen (PCNA) (+58%). In line, positive Ki-67-stained nuclei were increased in tumor sections from lomitapide-treated mice. Conclusion: these studies demonstrate that MTP inhibition, blocking lipid secretion from the liver, could lead to increased tumor growth, and represent the first steps in the evaluation of the role of MTP in cancer development.

326. (179) **FUNCTIONAL PROPERTIES OF A BREAD MADE WITH MALTED RYE FLOUR IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RATS**

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Rye is the second most important winter forage cereal in Argentina. The whole grain can be malted and the flour obtained from malting rye increases the amount of soluble fibers that have functional properties. Bread made with this flour, can be a product that could help to maintain a healthy diet.
The aim of this study was to evaluate the intake of a bread made with malted flour rye (MRB) on biomechanical bone parameters and volume stool during 60 days, in a rat model.
A total of 16 male Wistar rats recently weaned (8/group) were fed with a control diet prepared according to the American Institute of Nutrition Diet (C) and a semisynthetic diet prepared with MRB.
During the experience, feces were collected every 30 days to determine the moisture content. At the end of the study rats were anesthetized and the cecum from each animal was excised, split open, and the pH of the cecal content was measured. Right femur was also excised and Limit elastic load (Wy), Diaphyseal stiffness (Wy Dy) and Maximum fracture load (Wf max) were measured.
MRB group presented a higher moisture content of the feces than C group at 30 and 60 days respectively (day 30: 22.0±1.2 vs 13.8±1.3, *p <0.0001* ; day 60: 21.1±2.7 vs 11.5±2.2, *p <0.0001*). The cecal content of MRB presented a lower pH than C (6.67±0.05 vs 7.13±0.14; *p< 0.0001*).
The MRB group presented significantly higher biomechanical bone properties than group C: Wy (127.4±7.5 vs 102.8±15.4; *p=*

0.0025), Wydy (227.0 ±11.5 vs 189.9 ±26.9; *p< 0.01*) and Wfmax (150.2±17.9 vs 124.3±12.3; *p< 0.0087*).
Conclusions: the high fiber content of MRB would be correlated with water retention and increased stool. On the other hand the intake of MRB, showed a prebiotic effect improving bone quality. These intestinal and bone benefits led us to affirm that MRB could be considered as a functional food., to carry out a diet with healthy characteristics. Financed by UBACyT Nº 20020170100148BA and PIO 80020190300027UM.

327. (181) **EFFECT OF HIGH FAT DIET AND CHRONIC STRESS EXPOSURE ON LIPIDIC METABOLISM AND ADIPOKINES mRNA EXPRESION IN ADIPOSE TISSUE**

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Overweight and obesity are defined as abnormal or excessive fat accumulation caused by inadequate dietary habits and stress. Adipose tissue is a metabolically active organ that secrete adipokines involved in the maintenance of metabolic homeostasis. We have previously shown that mice fed with high fat diet (HFD) showed overweight and glucose intolerance. Chronic mild stress exposure (CMS) induced a decrease in body weight related to a decrease in caloric efficiency and worsening in glucose intolerance. The aim of this work was to study the participation of lipidic metabolism and adipokines expression in subcutaneous (SCAT) and visceral –abdominal- adipose tissue (VAT) in glucose intolerance induced by HFD and-or CMS exposed C57Bl/6J male mice. HFD fed mice presented an increment in total cholesterol (p<0.0001) and free fatty acids (p=0.00089) without significant changes in triglycerides levels measured by commercial kits. CMS exposure did not produce any alteration in these parameters. Adipokines mRNA expression was determinate by q-PCR real time. Results indicate that HFD induced an increase in VAT resistin expression (p=0.0002) and a diminution in SCAT (p=0.0005). Respect to adiponectin, a decrease was observed in both tissues (VAT p=0.0769, SCAT p=0.0003). In relation to leptin, no significant changes were observed. CMS exposure didn't produce significant changes in resistin and adiponectin expression but a non-significant increment in VAT leptin expression was observed (p=0.095).
These findings indicate that HFD feeding alters lipid metabolism and modify the pattern of adipokines expression in different way in SCAT and VAT that in turn could influence the metabolic consequences of HFD feeding and stress exposure.

328. (201) **EFFECTS OF LIRAGLUTIDE ON VASCULARIZATION AND MMP-2 ACTIVITY IN EXPANDED ADIPOSE TISSUE**

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Visceral adipose tissue (VAT) expansion occurs in obesity and is related to cardiometabolic risk. Metalloproteinases are endopeptidases involved in adipose tissue remodeling. Liraglutide, a GLP-1 agonist, has been recently approved for obesity treatment; however its direct effects on VAT are still unknown. Aims: to evaluate the effects of liraglutide on VAT remodeling and gelatinolytic activity in an animal model of diet-induced obesity. Methods: Male C57/BL6 mice (8 weeks old) were divided into 2 groups: Control (C, n= 9) fed with standard diet, and high fat diet group (HFD, n=8) fed a diet with 40% of total calories from fat during 15 weeks. Then, both groups were subdivided according to the subcutaneous administration of liraglutide (L, 200ug/kg/day) or vehicle (equivalent volume) for 5 weeks. Body weight, food and water consumption were registered weekly. The study was approved by the Ethic Committee of BIOMED. Serum glucose, triglycerides and HDL-cholesterol were measured post- L administration. Epididymal AT, as representative of VAT, was removed and weighed. Histological characteristics (adipocyte area and adipocyte and vascular density) were evaluated, and metalloproteinase 2 (MMP-2) activity was measured by gelatinolitic zymography. Results: as expected, body weight and VAT mass was higher in HFD compared to Control group (p<0.05 and p= 0.014 respectively). In HFD+L group, a significant decrease in body weight (p<0.01), VAT mass (p<0.01) and glucose (p=0.001) levels compared to HFD was observed. Moreover, HFD+L VAT presented higher adipocyte and vascular density than HFD (p<0.05). MMP-2 activity was increased in HFD+L compared to HFD (p<0.05) and it was directly associated with vascular density (r= 0.821, p=0.045). Conclusions: In obesity, liraglutide would improve adipose tissue functionality by favoring tissue vascularization in association with an increase in gelatinolytic activity.

329. (207) **THE SELECTIVITY OF COPPER-INDUCED PROTEIN AGGREGATES IN SERUM**

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Copper (Cu) reactivity is largely based on its ability to participate in redox chemistry and nature has employed it in the active site of different enzymes. Cu-induced damage has also been related to redox reactions. However, no antioxidant therapy has been approved in Cu-related pathologies and Cu-mediated non-redox reactions remain extensively unexplored in such diseases. Previously, we have shown the relevance of Cu-induced protein aggregation over oxidative damage and found that Cu aggregates albumin (Alb) at high concentrations. We speculated that the heterogeneous group of proteins in serum should comprise proteins with higher propensity to aggregation. Objective: To assess the selectivity of Cu-induced protein aggregation in serum. Methods: Optic density (OD), SDS-PAGE and proteinogram in cellulose acetate. Results: OD kinetic analysis of albumin (Alb), serum or plasma exposed to Cu was performed. Protein aggregation from healthy rat serum occurred at lower Cu concentrations (less than 100 μM) than Alb. No significant differences were observed between serum and plasma. The aggregation of serum proteins is dependent of Cu but independent of protein concentration. The aggregation curve of serum is flatter than Alb, likely due to the heterogeneous group of proteins present in serum. With SDS-page we determined that serum aggregates exposed to low concentrations of Cu are enriched in high molecular weight proteins and some low molecular weight proteins while very poor in Alb. The proteinogram of serum pellet obtained after Cu-induced aggregation showed enrichment in gamma-globulin fraction (p<0.01). Conclusion: The formation of protein aggregates is a pathologic hallmark of many diseases. Here, we show Cu can induce aggregate formation of specific proteins in serum. Likely, additional adverse conditions such as pH, fever, inflammation, etc. may also contribute to aggregate formation. The biological responses elicited by these aggregates require further research.

330. (220) **SPEXIN IMPROVES METABOLIC DELETERIOUS**

EFECTS CAUSED BY FRUCTOSE RICH DIET INTAKE

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Spexin (SPX) is a novel hormone distributed in numerous tissues, including white adipose tissue (AT). SPX regulates lipid and carbohydrate metabolism, caloric intake and body weight loss. Here, we evaluate the ability of SPX to improve metabolic profile and visceral AT function in fructose rich diet (FRD) obese mice. Four male mice (C57BL/6J) groups were studied: CTR mice, FRD mice (10 weeks of 20% w/v fructose in drinking water), and two similar groups that were treated or not with SPX for ten days prior to the end of the protocol (ip. 29 μg/kg/day; CTR-SPX and FRD-SPX). Body weight and caloric intake were recorded every day. Glucose Tolerance Test (GTT) was performed. Plasma was collected for triglycerides (TG) and glucose (GLU) levels quantification. AT depots (Inguinal (IAT), retroperitoneal (RPAT) and Epididymal (EAT)) were dissected, weighted and EAT was used for quantification of Ob, Adiponectin, PPARγ2 and GALR2 by qPCR. Two-way ANOVA was used to determine variable (SPX and FRD) and interaction (FRDxSPX) effects. SPX caused weight loss (p<0.01), regardless the diet, and a positive correlation was observed between the initial body weight and the body weight loss after the 10 days SPX treatment ($P_{CTR\text{-}SPX}$=0.0153 and $P_{FRD\text{-}SPX}$= 0.0161). FRD increased total caloric intake (P<0.001), while SPX decreased it (P<0.05). TG and GLU showed no changes. However, GLU intolerance in FRD mice (P<0.001) was reverted by SPX treatment (P<0.05). FRD increased all AT depots masses (P<0.05), but SPX only generated a more marked decrease in IAT mass from FRD mice (SPXxFRD=0.05). SPX treatment generated a beneficial lowering in mRNA expression of EAT markers, which was greater in FRD obese mice (FRDxSPX P<0.05). In conclusion, SPX caused weight loss; improved GLU metabolism and visceral AT function in FRD mice. Overall, our results support the beneficial role of SPX treatment, especially for metabolic alterations associated to obesity. PICT2017-2038, PICT2019-1851, PICT2019-2787.

331. (227) **THE CHLORIDE ANION IS NECCESARY FOR THE DEVELOPMENT OF ARTERIAL HYPERTENSION AND RENAL DAMAGE INDUCED BY SALT**

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The excessive consumption of sodium chloride is a risk factor for arterial hypertension (AH) and induces a renal inflammatory and oxidative response. The contribution of the chloride anion (Cl^-) to these deleterious effects is unknown.

The objective was to evaluate whether Cl^-, independent of sodium (Na^+), would be involved in the renal and oxidative inflammatory response and in the development of AH.

Male Wistar rats were divided in four groups (n=8/group) and fed with different diets (3 weeks): control (C); high sodium chloride (NaCl 8%); high Na^+ without Cl^- ($Na_3C_6H_5O_7$ 11.8%); high Cl^- without Na^+ ($CaCl_2$ 3.80%; KCl 3.06%; $MgCl_2$ 1.30%). Systolic blood pressure (SBP), renal function and oxidative parameters in renal cortex were determined: activity and expression (by WB) of enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). We also determine chloride channels expression (CLCN5 and CLCNKa) as key transporters to elicit this process.

Results: SBP increased in NaCl and Cl^- groups (p<0.05). TBARS production increased in all three diets, without changes in the activity and expression of SOD and CAT, while the activity of GPx increased only in Cl^- group (p<0.05). Additionally, compared with C group, NFkB expression in the kidney was increased in NaCl and Cl^- groups (p<0.05), while CLCNKa expression increased in Cl^- group and CLCN5 decreased in NaCl group.

Conclusion: Cl^- contributes, at least in part, in the development of AH induced by NaCl overload, and diets with a high chloride content would be associated with a higher prooxidant state in kidney than only sodium salt diets. The role of chloride transporters as mediators of oxidative damage or for the development of AH remains to be elucidated.

332. (228) **VALIDATION OF A SHORT SELF-ADMINISTERED QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE TO ASSESS HABITUAL VITAMIN D INTAKE AMONG ARGENTINIAN CAREGIVERS DURING COVID-19 PANDEMIC**

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Objectives: The objectives of the presented study were to develop and evaluate the validity and reproducibility of the ARVID-FFQ (ARgentine VItamin D–Food Frequency Questionnaire) in a group of caregivers during the COVID-19 pandemic in Argentina, where there is no vitamin D data in food composition tables.

Materials and Methods:

The self-administrated ARVID-FFQ was validated against the average of four 24-h dietary recalls (DRs) in a sample of 178 nurses, technicians and physicians. Reproducibility of the FFQ (n=84) was assessed by ARVID-FFQ1 and the same questionnaire, ARVID-FFQ2, 1 year after. The assessment of validity and reproducibility was conducted by verifying standard errors of estimation, kappa coefficient, and percentages of individuals classified into quartiles, correlations and Bland-Altman plots.

Results: Vitamin D intake for over 93% surveyed participants are characterised by intake values lower than 5.0 μg per day and over 99% by intake values lower than 10.0 μg per day. The following vitamin D intakes were observed in the studied group: 2.58 μg (0.05–12.2 μg) for 4x24-hDRs, 3.57 μg (1.1–10.6 μg) for ARVID-FFQ1, 3.61 μg (0.1–10.4 μg) for ARVID-FFQ2. The Bland-Altman indexes in assessment of validity and reproducibility were 5% and 1.1%, respectively, with mean differences of -0.15 μg and 0.02 μg, as well as limits of agreement -0.65–0.34 μg and -0.07–0.12 μg. The kappa coefficient indicated a fair agreement for validity (0.35) and moderate for reproducibility (0.48), while correlations were significant ($p < 0.0001$, $r = 0.54$ for validity; $p < 0.0001$, $r = 0.61$ for reproducibility).

Conclusion: Vitamin D intake in most cases was inadequate for this particular population at high risk for COVID-19. The VIDEO-FFQ can be considered a practical tool with satisfactory validity and reproducibility, making it convenient for estimating vitamin D intake in the Argentine population.

333. (253) **DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR DETERMINATION OF FREE 3-NT IN URINE AND LIVER SAMPLES OF LACTATING DAIRY CATTLE**

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During the transition from pregnancy to lactation, cows experience higher energy demand and oxygen requirements leading to oxidative stress. The 3-nitrotyrosine (3-NT) has been described as a specific biomarker of oxidative damage mediated by peroxynitrite. This study aimed to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of 3-NT in urine and liver tissue samples from dairy cows and described the 3-NT concentration in plasma, urine, and liver during the transition period in the spring (SG) and winter (WG) seasons. The developed method was validated following the Food and Drug Administration guideline. The matrix effect was negligible for urine and there was a medium matrix effect for liver tissue. The calibration curve of 3-NT was fitted with a $1/x^2$ weighted linear regression over the concentration range of 0.24-19.31 mg/L, for urine, and 2.27-102.04 mg/L, for liver tissue. Regarding the dilution study, the factor was 1/5 for urine and 1/10 for liver tissue. The precision was < 15 % and the accuracy was in the range of 85–115 %. 3-NT was stable in the different matrices after short-term storage for 30 days at -80 °C, after long-term storage for six months at -80° C, and after three freeze and thaw cycles. In plasma, urine, and liver samples, 3-NT concentration was higher in cows of the SG than in those of the WG ($p < 0.05$). A high correlation was observed between 3-NT concentration in urine and liver ($r = 0.74$; $p < 0.01$) and a moderate correlation between plasma and urine ($r = 0.61$; $P < 0.01$) and between that in plasma and liver ($r = 0.55$; $p < 0.01$). This study describes the development and validation of an LC-MS/MS method for the quantification of an important oxidative stress biomarker in fluids and tissue from groups of animals with different heat stress, showing that it could be a very useful parameter to evaluate the comfort of dairy cows, especially during the transition period.

334. (261) **GLAUCOMA INDUCES REDOX IMBALANCE IN THE CORNEA**

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Glaucoma is a neurodegenerative disease that affects eye structures and brain areas related to the visual system. Oxidative stress plays a key role in the development and progression of the disease. The aim was to evaluate the redox balance in the cornea in a glaucoma model.

3-month Wistar rats were operated by cauterizing two of the episcleral veins in the left eye: glaucoma group (G n=4); the control group (C n=4) received a sham procedure. Seven days after surgery rats were euthanized, eyes were enucleated and right (RC) and left (LC) corneas were isolated (CICUAL FFyB n° 3314). Damage to macromolecules, antioxidant enzymes activities and NOX and iNOS expression were evaluated.

When compared to CG, GG-LE showed an increase of 100% in protein oxidation (*p<0.01*) and 38% in nitrotyrosine expression (*p<0.05*). Both NOX4 and iNOS expression were 76% (*p<0.01*) and 160 % (*p<0.001*) higher in G-LC, respectively. A 65% increase in SOD activity (*p<0.05*) was measured in both corneas in G. However, GPx activity and CAT levels increased 46% (*p<0.001*) and 80% (*p<0.001*) in G-RC, respectively, without any changes in LC. Finally, there was a 50% decrease in GR activity in G-LC.

These results suggest that glaucoma induces damage to the cornea, such as oxidative modifications to macromolecules, due to an enhancement in oxidative species production from NOX and iNOS. In this context, the RE cornea presents an adaptive response increasing the antioxidant enzymes, while SOD is the only enzyme increased in LC. In addition, glutathione levels could be impaired since its recycling is decreased due to the decay of GR activity in LC. The understanding of corneal impairment in this pathology is important since it could lead to the development of novel therapeutic approaches.

335. (264) **MATERNAL FRUCTOSE CONSUMPTION IMPACTS ON THE DEVELOPMENTAL OUTCOME OF ITS PROGENY**

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Recent epidemiological evidence suggests that exposure to maternal obesity increases the risk of neurodevelopmental disorders in offspring. Given the rise in the prevalence of metabolic syndrome (MetS), a clinical condition closely related to obesity, it is important to understand the molecular mechanisms by which maternal MetS might impact offspring behavior and brain function.

Ten, two-months-old female Wistar rats from our colony were separated in two groups of five rats each, control (C) and fructose 20% (F). The former drank tap water while the later drank fructose 20 % (w/v) *ad libitum* during 10 weeks. Both F- and C dams were mated to chow-fed male rats on the 7th week of treatment and maintained on their respective diets throughout pregnancy and sacrificed on day 4 postpartum. At postnatal day (PN) 1, the progeny from both groups were separated from their mothers and continued lactating from control nurse dams. From PN3-PN21 neurodevelopmental reflexes were evaluated. At PN22 all pups were weaned and behavioral tests (open field, marble test, elevated plus maze, novel object recognition, social reciprocal interaction test, tail flick test, rotarod, Kondziela's inverted screen test) were performed on 4- to 12-week-old female and male rats. Results were considered statistically different between the C and the F group when a *p value* of 5% or lower ($p< 0.05$) was obtained when applying the *t-student* test.

Our findings strongly associate maternal fructose consumption with the induction of MetS and infertility. In addition, offspring from the F group presented alterations in the developmental milestones and social behavior; decreased grip strength and increased anxiety. Furthermore, long term memory also showed a tendency to be reduced. No modifications were noted in compulsive like behaviors, locomotion nor in nociception. All in all, maternal fructose consumption impacts on the developmental outcome of its progeny.

336. (310) **OFFPRING FROM MALNOURISHED PARENTS ARE PREDISPOSED TO DEVELOPING METABOLIC DISEASES**

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Growth restriction in utero is associated with the development of obesity and diabetes. The current understanding is that intrauterine deprivation programs the individual for a deprived environment, and that such programming is maladaptive in a no deprived environment. The liver plays an essential role in metabolism regulation.

The objective of this work was to analyze the liver function of the offspring of malnourished parents. Two types of experiments were carried out: 1) the parents (F1) were chronically malnourished from their gestation. They were studied at 120 days of age; 2) other group of malnourished rats at 120 days were mated and their offspring were analyzed at weaning. On day 120 (F1) and day 24 (F2) blood was obteined and the liver was dissected out. Body weight and biochemical parameters were measured. Chronicle protein malnutrition induced increased serum glucose and insulin ($p<0.05$) in F1. Secondly, serum glucose, triglycerides and cholesterol were significantly higher in the malnourished (F2M) group with respect to the control (F2C) and liver proteins and glycogen contein were lower ($p<0.05$). In F2, the values of insulin were higher with respect to the control ($p<0.05$) while the values of leptin and adiponectin were significantly lower in the F2M group. Oxidative stress markers (ROS, lipid peroxidation, and protein carbonylation) showed higher values with respect to the control. These changes were associated with increased pro-inflammatory cytokines production. Serum IL-6, TNF-α and TGF-β were significantly higher in F2M group with respect to

the control (p<0.05).These results suggest that protein malnutrition during the development predisposes to the occurrence of diabetes and the increment of liver inflammation and oxidative stress markers in their offspring.

337. (314) **EFFECT OF DIETARY SUPPLEMENTATION WITH RESVERATROL, ALPHA-TOCOPHEROL AND PIPERINE ON OXIDATIVE STRESS AND INFLAMMATION IN OLDER ADULTS WITH RISK FACTORS FOR METABOLIC SYNDROME**

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Oxidative stress, hypertension, blood glucose level and lipid profile are risk factors for metabolic syndrome (MS) and cardiac disease in older adults. The aim of this study is evaluate the protective role of resveratrol supplementation on chronic inflammation and oxidative stress associated to MS. Voluntary patients with a diagnosis of MS (n=92) based on the diagnostic criteria of the National Cholesterol Education Program, Adult treatment Panel III, 2002 received a dietary supplement (RTP: 50 mg resveratrol, 25 mg alpha-tocopherol and 5 mg piperine) along with their usual treatment for a period of 3 months. Piperine increases resveratrol and alpha-tocopherol absorption. Control was the patient himself in baseline conditions, avoiding interindividual variables and bioavailability of active principles. Venous blood was collected from 23 patients (68 ± 5 years), and biochemical markers were assessed in plasma: protein oxidation (measured as carbonyl protein, CO), and interleukin 6 (IL-6); and in red blood cells (RBC): catalase activity. Patients were classified into 3 groups according to the amount of risk factors (blood glucose, HDL cholesterol, triglycerides, waist circumference and blood pressure) for MS: 3/5 (n=7), 4/5 (n=5) and 5/5 (n=11) risk factors. Preliminary results showed increase in catalase activity (22-32%, p<0.05) in the 3 groups of patients after RTP treatment, without differences between groups. When blood glucose, HDL-cholesterol and Triglycerides risk factors were evaluated separately, it was observed that they all impact on catalase activity in the same way after RTP treatment. No significant differences were observed in IL-6 and CO after RTP neither among groups. RTP treatment improves the enzymatic antioxidant response by protecting cells from oxidative damage by hydrogen peroxide generated in inflammatory processes, regardless of the risk factors of the MS patient.

338. (326) **EFFECTS OF FLAXSEED OIL RICH IN LIGNANS ON LIPID ALTERATIONS INDUCED BY CAFETERIA DIET IN RATS**

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Cafeteria (CAF) diet, composed by palatable foods high in saturated fat and refined carbohydrates, leads to a deregulation of lipid metabolism with hepatic triacylglycerol (TAG) accumulation which can progress to non-alcoholic fatty liver disease (NAFLD). Flaxseed oil (FO) can be used as a functional food ingredient due to its potential health benefits and excellent nutrient profile. The principal component of virgin FO is α-linolenic acid, which can be enriched with lignans (LGN). This preliminary study aimed to investigate the effects of virgin FO rich in LGN on the alterations induced by the cafeteria diet. Wistar rats (310g) were fed during 60 days with the diets: control (SO): soybean oil 4%, CAF (C): lipids 30% and CAF+FO (CF): lipids 26%+4% FO rich in LGN. Food intake, body weight gain, relative epididymal (EAT) and retroperitoneal (RPAT) adipose tissues and liver weights; and serum TAG, cholesterol (CHO) and glucose (GLU) levels, were measured. Liver fatty acids profile was determinate by gas chromatography. Data were analyzed by One-Way ANOVA followed by Tukey´s test (p<0.05). The results were: food intake: SO(108,2^{a}±6,5), C(140,1^{b}±6,5) and CF(125,9^{b}±2,6), body weight gain: SO(115^{a}±3,6), C(170,6^{b}±4,1), CF(155,8^{b}±7,7); relative tissues weights: EAT: SO(2,44^{a}±0,12), C(3,09^{b}±0,07) and CF(2,53^{a}±0,11); RPAT: SO(3,95^{a}±0,14), C(4,60^{b}±0,11) and CF(3,81^{a}±0,24) and liver: SO(2,24^{a}±0,04), C(2,52^{b}±0,07) and CF(2,52^{b}±0,03); serum TAG: SO(2,31^{a}±0,14), C(3,10^{b}±0,12) and CF(2,33^{a}±0,26), CHO: SO(0,98^{a}±0,02), C(0,80^{b}±0,04) and CF(0,81^{b}±0,04) and GLU: SO(1,53^{a}±0,07), C(2,17^{b}±0,20), CF(1,56^{a}±0,05). In liver n-3 polyunsaturated fatty acids (PUFA) were increased (200%) and n-6/n-3 ratio was diminished (70%) by CF vs C. In conclusion, these preliminary results showed that virgin FO rich in LGN attenuates some negative effects on lipid alterations induced by cafeteria diet, diminishing adipose tissues weights and serum TAG levels, as well as, improving liver fatty acids profile.

339. (341) **EFFECTS OF MINERAL AND VITAMIN SUPPLEMENTATION IN THE OXIDATIVE STRESS BIOMARKER 3-NITROTYROSINE IN PLASMA, URINE AND LIVER TISSUE OF DAIRY CATTLE DURING THE TRANSITION PERIOD**

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The intensification of milk production has generated a great metabolic demand in the animals causing an increase in the oxidative stress, particularly during the transition period of dairy cattle. Therefore, we aimed to study the concentration of free 3-nitrotyrosine (3-NT) in plasma, urine and liver tissue, as a marker of the oxidative stress, and other metabolic biomarkers in plasma of cows supplemented with vitamins and minerals during the transition period. The supplemented group (SG; n = 11) received subcutaneously a dose of 5 ml of the vitamin supplement ADAPTADOR® Vit and 5 ml of the mineral supplement ADAPTADOR® Min (Biogenesis Bagó, Bs. As.; vitamin A palmitate 3.5% and vitamin E acetate 5%, copper edetate 1%, zinc edetate 4%, manganese edetate 1% and sodium selenite 0.5%) on -60, -30 and 7 days relative to calving. The control group (CG; n = 11) received two injections of 5 ml of 0.9 % sodium chloride. Blood, urine and liver biopsies were sampled at -21, 7 and 21 days relative to calving and to evaluate 3-NT. Also, plasma concentrations of non-esterified fatty acids, beta-hydroxybutyrate, glucose, albumin, cholesterol, bilirubin, aspartate aminotransferase, gamma-glutamyl transferase and liver triacylglycerol content were spectrophotometrically measured. In cows of the SG, 3-NT concentration was greater in plasma ($p < 0.05$) and lesser in the liver tissue ($p < 0.05$). In addition, a lesser liver triacylglycerol content and greater glucose concentration was detected in cows of the SG ($p < 0.05$). Regarding albumin, plasma concentration was greater in cows of the SG ($p < 0.05$), with a supplementation x time effect ($p < 0.05$), being particularly greater on day 21 pre-calving and day 4 post-calving. No statistical differences were evidenced in the other studied parameters. These results suggest that mineral and vitamin supplementation could ameliorate the oxidative stress in the liver and the transition of dairy cows.

340. (399) **COMPARISON OF INTESTINAL AND HEPATIC MTTP IN THE STATE OF DYSBIOSIS ASSOCIATED WITH INSULIN RESISTANCE**

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Microsomal triglyceride transfer protein (MTTP) is essential for the assembly and secretion of chylomicron particles (CM) in the intestine and very low-density lipoprotein (VLDL) in the liver. Intestinal dysbiosis, related to insulin-resistance, may alter the expression of MTTP in different manners in intestine and liver, which could affect the triglyceride-rich lipoproteins contributing to atherogenic dyslipemia. Aim: to evaluate intestinal (I) and hepatic (H) MTTP expression and their association with triglyceride-rich lipoprotein characteristics in a diet-induced dysbiosis animal model. Methods: 12 male Wistar rats (180-200g) were fed with standard diet (Control, n=6) or standard diet plus 40% fat+15% sucrose (HFSD, n=6) during 14 weeks. Glucose, free fatty acids (FFA), lipoprotein profile and lipopolysaccharides (LPS) as altered gut microbiota marker, were measured in sera. CM and VLDL were isolated by ultracentrifugation and lipid composition was measured. In I and H MTTP expression were evaluated by Western Blot. Results: Compared to Control: HFSD showed higher LPS ($p<0.01$), TG ($p<0.001$), nonHDL-chol ($p=0.01$), FFA ($p<0.05$) and TG/HDL-chol as surrogate of insulin-resistance ($p<0.001$). CM and VLDL composition showed higher TG ($p<0.001$) and phospholipids ($p<0.05$) proportion. H-MTTP was increased ($p<0.001$) although no differences were found in I-MTTP ($p=0.145$). I-MTTP was associated with CM-TG ($r=0.72;p<0.01$), TG/HDL-chol ($r=0.58;p<0.05$) and inversely with LPS ($r=-0.79;p=0.03$). H-MTTP correlated with VLDL-TG ($r=0.50;p<0.05$), TG/HDL-chol ($r=0.65;p<0.01$), and LPS ($r=0.68;p<0.05$). Conclusions: This is the first time that I- and H- MTTP are reported in a diet-induced dysbiosis model. MTTP promotes TG over-enriched CM and VLDL, that probably to the atherogenic profile. The difference in I- and H- MTTP levels and the opposite correlation that maintain with LPS, suggest an earlier response of the liver to intestinal dysbiosis, in the studied period.

The study was approved by the Comité Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL)-FFYB-UBA (REDEC-2020-2292-E-UBA-DCT FFYB & REDEC-2021-592-E-UBA-DCT FFYB).

341. (430) **TIGHT JUNCTION (TJ) PROTEINS IN KIDNEY OF HIGH-FAT FED MICE: EFFECT OF (-)-EPICATECHIN**

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(–)-Epicatechin (EC) is a flavanol which presence in human diets is associated with health benefits. High-fat (HF) fed mice develop obesity, insulin-resistance, and dyslipidemia, all of which are conditions that also contribute to chronic kidney disease. Previously, we showed that EC supplementation mitigated dysmetabolism developed by HF-fed mice related to an attenuation in TLR-4 mediated inflammation in kidney. In this work, we studied, in the same experimental model, modifications in proteins involved in TJs in kidney responsible for the permeability barrier, i.e. zonula occludens-1 (ZO1) and claudin-2 (cldn2). C57BL/6J male mice were divided into 4 groups: control (C), control + 20 mg EC/kg body weight (CE), HF diet (60% fat from lard), and HF diet + EC (HFE). At the end of a 14-d treatment, mice were euthanized, and blood and kidney samples were obtained. Serum creatinine levels were 41 % higher in HF respect to the other groups ($p<0.05$, ANOVA, Tukey's test) showing a protection of EC against renal damage; this protection was also observed in parameters of inflammation and fibrosis. ZO1 and cldn2 abundance were evaluated by histochemistry. ZO1, expressed as positive area in %, was C: 45±3, CE: 52±1, HF: 13±1*, and HFE: 13±1* (*$p<0.05$ vs C and CE, Kruskal–Wallis, Dunn's test), would imply a loss of integrity in the permeability barrier that was not attenuated by EC supplementation. Changes in cldn2 detection showed a 28% of increase in HF respect to C and CE, and a decrease of 49% in HFE respect to HF. These results are indicative that the HF-diet in the present conditions affects the two proteins differentially. The action of EC restoring HF triggered high levels of clnd2 to below control values, is relating clnd2 to the observed renal function protection, including inflammation and fibrosis. PIP-CONICET 11220170100585CO, PICT 2018-03052, and UBACyT 20020170100586BA (MG); UBACyT 20020190100157BA (CF).

342. (431) **VLDL-R EXPRESSION IN PATIENTS WITH NAFLD WOULD CONTRIBUTE TO HEPATIC STEATOSIS**

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Non-Alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in adults worldwide, highly associated with obesity, type 2 diabetes and cardiovascular disease. Liver steatosis depends on triglycerides (TG) accumulation from the novo fatty acids (FA) and TG synthesis, the influx of free FA (FFA) and the metabolism of remnant lipoproteins (RLP). In physiological states, the contribution of VLDLReceptor (VLDL-R) in the latter pathway has not been reported. Besides, the release of liver VLDL, depends on its synthesis throughout Microsomal TG transfer protein (MTTP). Our aim was to evaluate the expression of VLDL-R and MTTP in liver biopsies from NAFLD patients and their possible association with the steatosis grade (SG).

Methods: We studied 17 patients with NAFLD of both sexes (56±12 years, women: 82%). In liver biopsies, protein levels of VLDLR and MTTP were evaluated by Western blot. In serum samples we assessed lipoprotein and hepatic profile; platelets were measured in blood. We calculated the fibrosis scores APRI, FIB-4 and NFS.

Results: Studied patients were mostly obese, with normal hepatic function. Glycemic control was altered, and an atherogenic dyslipidemia was verified, with increased insulin-resistance markers. VLDL-R was expressed in liver biopsies, tending to increase in those patients with higher SG (0.44±0.20 RU in patients with <33% SG *vs* 0.64±0.13 RU in patients with >33%, p=ns). VLDL-R showed a tendency to positively correlate with APRI score and negatively with RLP-Cholesterol (C) despite not being significant (p=0.42 and 0.39 respectively). MTTP expression tended to associate positively with nonHDL-C (p=0.06) and LDL-C (p=0.07).

Conclusion: The expression of VLDL-R in NAFLD patients is a challenging finding that would contribute to the influx of FFA to the liver, worsening the TG accumulation in the hepatocytes. Further studies in a larger number of patients would allow to elucidate the role of this receptor in NAFLD.

343. (433) **PERIVASCULAR ADIPOSE TISSUE IN HIGH-FRUCTOSE FED RATS: EFFECT OF (-)-EPICATECHIN**

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It was demonstrated that the flavanol(-)-epicatechin (EC), highly present in edible plants, was able to avoid perivascular adipose tissue (PVAT) dysfunction in high-fat fed mice. The aim of this study was to investigate whether in high-fructose fed rats, EC treatment induces changes in hemodynamics and thoracic aorta PVAT (taPVAT) characteristics in terms of thermogenic capacity and NLRP3 inflammasome activation.Male Sprague-Dawley rats were divided into 4 groups: C: control diet and tap water; CEC: EC (20 mg/kg BW/d) in the diet and tap water; F: control diet and 10% (w/v) fructose in the water, and FEC: EC in the diet and fructose in the water. After 8 w, BP was determined, animals were euthanized and blood (plasma), aorta, and taPVAT were obtained for biochemical and histological determinations.As was expected, EC supplementation attenuated BP increase induced by high-fructose diet. Aorta morphometry did not show differences among the experimental groups. taPVAT area/aorta lumen showed higher value in F respect to C (16%, $p<0.05$), that was attenuated by EC supplementation. Adipocyte size was not significantly affected by the treatments. Levels of uncoupling-protein 1 (UCP-1) and the mitochondrial marker VDAC, were measured by western blot. UCP-1 expression was higher only in CEC respect to C (64%, $p< 0.05$). NLRP3 inflammasome pathways was evaluated in taPVAT, but NLRP3, caspase-1 and IL-1β did not show significant differences among the experimental groups. In conclusion:i) EC-induced alterations in taPVAT could be linked to changes in BP, ii) EC showed a browning effect in control animals that was not present in high-fructose fed animals, iii) no changes were observed in the inflammasome NLRP3 pathway. Further research is necessary to understand the relevance of taPVAT modifications in hemodynamics changes in this model. UBACyT 20020170100087BA (AMB), 20020170100586BA (MG) and 20020190100157BA (CF), PIP-CONICET 11220170100585CO, PICT 2018-03052.

344. (435) **HEMIN IMPROVES LIPID METABOLISM IN A RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE**

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The liver plays a major role in controlling systemic lipid metabolism and an imbalance between lipids intake and secretion can lead to insulin resistance and Non-Alcoholic fatty liver disease (NAFLD), the most prevalent chronic liver disorder that is associated with cardiovascular disease. Previous results from our laboratory showed an increase in serum triglyceride (TAG) levels in rats fed a sucrose rich diet (SRD) for 12 weeks. The administration of hemin (15mg/kg ip, every 48hs) during the last two weeks of SRD reduced TAG levels, while no changes were detected in systemic insulin resistance and hepatic triglyceride levels. Based on these results the aim of the present study was to analyze the effects of hemin on hepatic lipid metabolism. Male Wistar rats were randomly distributed into control (C) and SRD groups (30% sucrose in the drinking water). Hemin was administered as described (SRD+H). Livers were harvested and total RNA and proteins were extracted. Our results showed an increased gene expression of the transcription factor ChREBP, and the lipid synthesis enzymes FAS and ACC1 (0,001 vs. C group), as well as FAS protein expression (0,001 vs. C) in SRD treated rats. Although hemin administration had no effect on the gene expression of ChREBP or ACC1, it caused a significant increase in SREBP1c, FAS, and DGAT1/2 levels (0,05 vs. SRD or C). Interestingly, when we analyze the genes involved in the liver capacity to oxidize lipids we found that SRD+H treated animals showed an increase in the gene expression of PPARα, CPT1α and ACOX1α (0,01, 0,05, and 0.001 vs. SRD respectively). Furthermore, protein levels of p-AMPK and nuclear protein levels of PPARα were also increased (0,05 and 0,01 vs. C and SRD, respectively). In summary our results led us to hypothesize that hemin administration associated with a reduction of triglyceridemia is due to an increase in the oxidation of lipids within the liver, possibly through the stimulation of the AMPK/PPARα pathway.

345. (444) **HO-1 AS A THERAPEUTIC TARGET IN A RAT MODEL OF MAFLD: KEY ROLE OF KUPFFER CELLS**

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Currently, metabolic dysfunction associated with fatty liver affects a quarter of the world population, but no pharmacological treatment has been recommended yet. We have previously shown that depletion of Kupffer cells (KC) in rats fed a sucrose-rich diet (SRD) for 12 weeks attenuates tissue injury and prevents liver inflammation, without changing the degree of steatosis. The aim of this study was to evaluate the effects of hemin treatment (an HO-1 inducer) on liver damage induced by SRD and identify the underlying mechanisms.
SRD-treated rats are presented with IR, hepatic steatosis, and high serum levels of NEFAS, glycemia, and triacylglycerides (TAG). Administration of hemin for the last two weeks of the dietary intervention (15 mg/kg/48h, SRD+H) did not modify these parameters, except for the observed reduction in serum TAG levels. A lower degree of ballooning (histological change compatible with injury) as well as a decrease in oxidative stress parameters (TBARS and 3-nitrotyrosine levels, SOD and catalase activities), UPR (expression of XBP1s, ATF4 and GRP78) and apoptosis (TUNEL and cleaved caspase-3 expression) were also detected in SRD-treated rats. The induction of HO-1 expression in KC by hemin was associated with lower tissue levels of IL1β, TNFα and pP65 compared to the SRD group. Induction of PEPCK as well as the response to pyruvate were blocked by hemin, that also restored the ratio pAkt/Akt altered by SRD. Finally, animals in the SRD+H group showed an increase in the expression of PPARα, CPT1α and ACOX1α (proteins involved in lipid oxidation), and an increase in pAMPK (vs. SRD). In summary, our results lead us to hypothesize that administration of hemin attenuates liver injury induced by sucrose diet by reducing the pro-inflammatory tone of the KC associated with the induction of HO-1. Moreover, hemin treatment is also able to decrease TAG serum levels by increasing lipid oxidation through the stimulation of the AMPK/PPARα pathway in the liver.

346. (466) **FAT MASS ESTIMATION BY PREDICTION EQUATIONS AND DETERMINATION BY ISOTOPIC DILUTION TECHNIQUE AS REFERENCE METHOD IN SCHOOL CHILDREN**

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Obesity is defined by WHO as excessive fat accumulation that presents a risk to health. Therefore, it is relevant to study fat mass (FM) modifications from childhood as a potential evaluation tool in health programs. Different techniques are applied to determine body com-

position (BC), particularly FM. Simple methods as anthropometry (A) and bioelectrical impedance analysis (BIA) use prediction equations to estimate BC meanwhile isotopic dilution technique (ID) is used as reference method. The aim was to estimate the FM of school children by prediction equations using anthropometric measurements (Aeq) and bioelectrical impedance analysis (BIAeq) and to compare them to that determined by ID as reference method. 250 school children aged 6-12 years from JV. González School of the University of La Plata and from neighborhood clubs of La Plata, Olmos and Villa Elisa were studied. Body weight (kg), height (cm) and skinfolds (mm) were evaluated to estimate %FM by five anthropometric equations and resistance values (ohms) were used to estimate fat free mass (%FFM) through eight BIA equations. Deuterium oxide dose (0.5g/kg body weight) was given orally, and a saliva sample was collected. Deuterium concentration was measured by FTIR, %FFM was determined and %FM was obtained as 100-%FFM. %FM estimated by Aeq and BIAeq were between 23.3±4.6-27.1±8.3 and 18.4±7.2-28.2±7.9, respectively, while %FM determined by ID was 29.3±7.2. All values were statistically lower regarding ID ($p<0.0001$). When Bland-Altman test was applied to analyze the agreement between methods, high variability and even bias were observed. FM estimated through different prediction equations was different than the one obtained using a reference method, such as ID. This fact strengthens the need to have our own equations, developed and validated in local populations. In this way, local equations could be applied depending on the availability of anthropometric or BIA instruments.

347. (468) **TLR4 KO MICE ARE LESS SENSITIVE TO THE EFFECTS OF A HIGH-FAT DIET INTAKE THAN C57BL/6J MICE BUT MORE SUSCEPTIBLE TO STRESS**

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Previously, we and others described that male C57BL/6J (WT) mice are sensitive to diet-induced obesity, leading to an increased inflammatory state. Mice under chronic stress (CS) gained less body weight (BW). TLR4 receptor knockout mice (KO) do not show inflammation under high-fat diet (HFD) intake, although their stress response is unknown. Here we studied the metabolic and behavioral effects of HFD intake and CS exposure in both strains.
At 4 weeks of age, mice received a standard diet (SD) or a HFD. After 12 weeks, they were exposed (or not) to CS for 8 weeks. Resulting in 4 groups for each strain: 1) SD, 2) SD+CS, 3) HFD and 4) HFD+CS. We recorded BW, blood glucose (GLU), lipid metabolism and behavioral tests.
After 12 weeks of HFD, WT showed higher BW gain ($p<0.01$) and GLU ($p<0.01$) vs WT SD mice. However, KO showed a lower BW gain ($p<0.001$ vs KO SD) without changes in GLU. After 8 weeks of CS, WT CS mice showed a lower BW gain (SD: $p<0.01$ and HFD: $p<0.001$), whereas KO HFD+CS had a higher BW gain than HFD ($p<0.05$). GLU levels at the end of the experiment were higher in KO under CS than non-CS mice, while HFD induced an increase in GLU in WT mice. Although, we have not observed any changes in the lipid metabolism estimated by total cholesterol and triglycerides.
In relation to behavioral performance, CS+HFD increased locomotor activity in WT ($p<0.05$) but not in KO. In contrast, KO mice showed a lower habituation capacity than WT. Moreover, the spatial object recognition test was altered in WT by HFD and CS, whereas in KO only CS exposure produced a decrease in the discrimination rate in this test ($p<0.05$). Also, spontaneous alternation was unaffected in WT mice, although we observed a reduction induced by CS in KO for both diets ($p<0.05$).
These findings suggest that KO mice have not responded to HFD but are more sensitive to stress exposure. Further experiments are necessary to explore the role of Toll Receptors in the enhanced stress sensitivity observed.

348. (511) **DETERMINATION OF ZINC LEVELS IN COMMERCIAL NOODLES AND BIOACCESSIBILITY**

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The present work aims to evaluate the bioaccessibility of Zn in commercial noodles (common, fortified with zinc, wholemeal and gluten-free) and to study the effect of cooking time and added salt on the loss of Zn and solids from said noodles.
For the bioaccessibility analysis, the samples were digested following the steps of gastrointestinal digestion "in vitro". The bioaccessibility of zinc was estimated through the dialysability of the mineral (% DZn) expressed as the bioaccessible percentage of the total content in the sample, using the following formula: % DZn = (mg Zn dialyzed / mg Zn sample) x 100. For On the other hand, the potential contribution (APZn) was calculated considering a ration of 80 g of crude product (portion established in the product label), through the following formula: APZn = Zn concentration x% DZn x ration (g). On the other hand, the loss of solids and zinc (Zn) was evaluated at two cooking times (optimal time indicated by the manufacturer) and an overcooking (10 minutes), without adding salt to the cooking water and with the addition of salt: 2g / 200mL (1%) and 10g / 200mL (5%). Zinc quantification was performed by inductively coupled plasma mass spectrometry.
The %DZn was less than 10% in all the analyzed noodle variants, without significant differences between them. The APZn in all cases was less than 168 μg / 80g (standard portion of noodles). The results showed significant losses of zinc during cooking ($p<0.05$), increasing according to the treatment time and registering lower losses with the addition of salt to the water. On the other hand, significant weight losses were observed when the cooking time was prolonged ($p<0.05$). They were lower at a higher concentration of added sodium chloride ($p<0.05$).
Considering its bioaccessibility and cooking losses, this type of food hardly contributes to meeting the requirements of the mineral in question.

349. (518) **EXTRACTION AND CHARACTERIZATION OF CAIMAN OIL FOR USED AS A FOOD SUPPLEMENT**

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In Argentina, the Proyecto Yacaré aims to conserve the *Caiman latirostris* (CL) through its sustainable use as a renewable resource. Its leather is appreciated in the fashion industry and meat is valued as a source of high quality animal protein because it contains essential fatty acids (FA) important for human nutrition. Fat is generally discarded because it has no commercial value, increasing environmental pollution and eliminating a potential source of valuable natural oils. The objective was to extract oil from the fat of CL and perform the chemical and microbiological characterization for its use as a dietary supplement. In addition, the acceptability of the oil was carried out in a group of 100 consumers and it was expressed on a hedonic scale from 1 -9, with 9 being the most accepted. Atherogenicity index (AI), thrombogenicity index (TI) and the presence of aerobic mesophilic and coliforms bacteria, *Escherichia coli* and *Salmonella* were determined. Four methods of oil extraction based on solvent extraction and melting were evaluated and data analysis

was performed by using ANOVA. The selected extraction method (melting at 80ºC) presented an excellent yield (89% w/w) and by means of gas chromatography it was determined that the FA content does not change after heating. The oil obtained contains FA of high nutritional quality such as oleic acid (34%), linoleic acid (30%) and α-linolenic acid (2%). The AI was 0.29 and TI was 0.47. The oil contained no organic solvents and no microbial load. Due to its excellent oxidative stability, it can be produced and stored at 25ºC for 4 months maintaining its physicochemical properties and nutritional quality. This may be related to the presence of reducing substances and free radical scavengers that increase its antioxidant capacity. The acceptability was 8,1 ± 1,3, which indicates that it was highly accepted by consumers. Due to its excellent nutritional quality, the oil obtained from caiman fat could be used for nutrition.

350. (527) **METABOLISM REWIRING IN CRC: INSIGHT FROM BIOINFORMATIC FREE TOOL ANALYSIS**

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It has been suggested that colorectal cancer (CRC) cells show a distinctive metabolic organization at different development stages as in response to stress. We determined the value of metabolism reprogramming in CRC patients against controls and at different stages (I-IV) by means of bioinformatic tools. We employed 49 CRC samples from E-GEOD50421 microarray (569.593 probes specifics to human genes) and 10 controls. The informatics free access tools were: Mev4 to heat map (1.5, 2 & 2.5 fold differences); DAVID and Reactome. The study of gene expression in CRC patients versus controls revealed that this disease alters the levels of expression of genes associated with cell adhesion and proliferation that promote the cell growth and mobility. Other overexpressed genes in CRC samples correspond to prognosis, tumor diagnosis or genes closely associated to cell metabolism. Gene related to carbohydrate and lipid metabolism differed by 2 fold in CRC patients. At stages I and II our results showed a significant increase in oncogenic markers and genes of glutaminolysis, while at stage III, the significant 2 fold expression was on fatty acids pathway genes, including acyl-CoA and phosphatidylserine. At stage IV, we evidenced an increase in the expression of tetraspanin 5, CRNEP and MMP7, genes associated to cancer progress in advanced stages and metastasis. In conclusions, during the development of the disease, the metabolic pathways are reprogrammed, increasing glycolysis, glutaminolysis and fatty acid synthesis. Our results showed that CRC cells exhibit the “Warburg Effect” at early stages (I-II) while at stage III cancer cells increase the uptake of extracellular lipids and lipoproteins, and up regulate de novo lipid biosynthesis and synthesis of cholesterol, which produce lipid metabolites for cell membranes at stage III and IV of CRC, suggesting that patients would have higher circulating levels of fatty acids than patients at early stages.

351. (529) **PHYSICAL ACTIVITY, ENERGY EXPENDITURE AND BODY COMPOSITION IN OLDER SUBJECTS FROM ARGENTINA: PRELIMINARY STUDY**

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Energy expenditure (EE) is mainly composed by the basal metabolic rate and the physical activity (PA). The aim of this work was to study physical activity, energy expenditure and body composition in a group of older subjects from Argentina. A descriptive study was conducted in 64 women (74.9±9.9y) and 23 men (75.1±6.7y) who attended community centers or nursing homes previous signature of informed consent. The protocol was approved by the Ethics and Research Committee of the HIGA San Roque. Body weight (BW,kg) and height (H,m) were determined to calculate Body Mass Index (BMI=BW/H^2,kg/m^2). Fat-free mass (FFM) was determined by isotope dilution technique after an orally dose of deuterated water and fat mass (FM) was obtained as FM= BW-FFM and expressed as percentage. Physical activity was measured in 19 subjects (8 men,11 women) by accelerometry (ActivPal) for 5-7 days and the number of steps/day (S), the stepping time (ST, hs/day), the energy expenditure ($EE_{ActivPal}$, expressed in total Mets and kcal/day) and the physical activity level (PAL as total Mets/total hours) were recorded. PAL was used to estimate EE (Kcal/day) by FAO/WHO/UNU/2001 (FAOeq), DRI (DRIeq), Harris-Benedict (HBeq) and Mifflin (Meq) equations; statistical analysis was performed by ANOVA. Regarding BMI, 39% were overweight and 32% were obese. %FM was 32.6±4.2 in men and 41.8±5.5 in women ($p<0.0001$), being 74% and 81% higher than the suggested values. S and ST were 6235±2784 and 1.4±0.6, respectively, and tended to be lower as %FM increased. PAL determined by ActivPal was 1.38±0.08. No statistically difference was observed between $EE_{ActivPal}$ and EEFAOeq. This preliminary study showed high obesity prevalence as well as low physical activity in this elderly group. Moreover, PAL obtained in this study would be suitable for estimating daily energy requirements in elderly when prediction FAO equation is used to assess energy adequacy in future studies.

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352. (542) **PORPHYRIAS DURING SARS-CoV-2 PANDEMIC. A RETROSPECTIVE ANALYSIS**

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Porphyrias are a group of metabolic diseases produced by partial and specific deffect in one of the enzymes of heme biosynthesis. There are eight different porphyrias, classified in cutaneous: Porphyria Cutanea Tarda (PCT), Erythropoietic Protoporphyria (EPP), Congenital Erythropoietic Porphyria (CEP) and Hepatoerythropoietic Porphyria (HEP); acute: Acute Intermittent Porphyria (AIP) and New Acute Porphyria (NPA) or mixed: Variegate Porphyria (VP) and Hereditary Coproporphyria (HCP), according to symptoms. Some papers in 2020 indicated that SARS-CoV-2 attacked β-hemoglobin chain releasing heme and producing protoporphyrin IX and Fe, favoring virus infectivity by an interaction with the porphyrin. It was suggested that hydroxychloroquine, used to mobilize and eliminate porphyrins, could be a suitable treatment. This hypothesis made us think about how COVID-19 could affect porphyric patients who usually have high content of porphyrins. Pandemic affected the amount of diagnosis during 2020, being diagnosed only 6 cases: 4 PCT, 1EPP, 1 CEP. Until August 2021 we diagnosed: 22 PCT, 7 PAI, 2 VP and 1 EPP. A review of last 10 years showed that AIP cases in 2021, were about the median value (4.5±2.2) while PCT were significantly low (64±9.6). Moreover, 29 patients had COVID-19: 20 AIP and 3 PCT in spite of PCT is 10 times more frequent (PCT 1:20000, PAI 1:215000). Two AIP patients were hospitalized and only one needed respiratory assistance. Another AIP patient suffered an acute attack two months after COVID-19 positive. Heme parameters values were similar to that usually determined in these patients. This retrospective analysis allows us to conclude that COVID-19 does not have a different expression than that of general population, no worsening related to the porphyric condition was observed. We consider that an update of COVID-19 cases in porphyric patients would allow us to have more evidence about possible differences between acute and cutaneous porphyrias.

353. (571) **EFFECT OF THE CO-ADMINISTRATION OF DOCOSAHEXAENOIC ACID AND EXTRA VIRGIN OLIVE OIL ON LONG CHAIN PUFA DEPLETION IN LIVER AND EXTRAHEPATIC TISSUES OF MICE FED A HIGH-FAT DIET**

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Consumption of high-fat diet (HDF) can negatively affect health. The metabolic effects of HFDs are closely related to the reduction of n-3 long-chain polyunsaturated fatty acids (LCPUFA) in tissues. Docosahexaenoic acid (C22:6n-3, DHA) has been associated with cardiovascular and brain protection. In addition, extra virgin olive oil (EVOO) with high content of polyphenols and tocopherols has shown anti-inflammatory, antioxidant and preventive properties against metabolic alterations. Moreover, previously we showed that DHA+EVOO co-administration exhibits synergistic beneficial effects. Accordingly, the aim of this work was to evaluate the effect of DHA+EVOO co-administration on the fatty acids (FA) profile in the liver and extrahepatic tissues in a mouse model of HFD-induced obesity. Male C57BL/6J mice received: control diet (CD) (10% fat), DC+DHA, DC+EVOO, DC+DHA+EVOO, high fat diet (HFD) (60% fat), HFD+DHA, HFD+EVOO, HFD+DHA+EVOO for 12 weeks conforming 8 experimental groups (Doses DHA and EVOO: 50 mg/kg/day). The total FA composition was analyzed by gas-liquid chromatography in: liver, brain, heart, kidney, intestine, testicle, plasma and erythrocytes. Statistical analysis was performed by two-way ANOVA and Tukey's test, $p<0.05$ was considered to be significant. The HFD administered for 12 weeks caused increase in saturated FA, mainly C16:0 palmitic, reduction of n-3 LCPUFA and increased n-6/n-3 LCPUFA ratio in all studied tissues. In HFD-fed mice that received DHA+EVOO a normalization in n-3 LCPUFA content was achieved in all tissues. Interestingly, the reduction in eicosapentaenoic acid caused by HFD was attenuated by 60% in this group. The results of the present work suggest that the combined administration of DHA+EVOO could be considered as a preventive nutritional strategy to address metabolic diseases. New studies are necessary in order to evaluate the effects and mechanisms involved in extrahepatic tissues.

354. (593) **CHARACTERIZATION OF A GENETIC MURINE MODEL OF ACUTE INTERMITTENT PORPHYRIA. AN OVER TIME STUDY**

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Acute intermittent porphyria (AIP) is an inherited disease due to Porphobilinogen deaminase (PBG-D) deficiency. Mouse models of human Porphyrias are useful to investigate disease pathogenesis and to develop new therapies. AIP model is a knockout mouse with targeted disruption of PBG-D that exhibits the typical biochemical/neurological characteristics of human AIP. The aim was to evaluate heme metabolism, hepatic damage and oxidative parameters in a genetic AIP model compared to wild type strain (*C57BL/6*). The study was performed in liver and brain using three groups (males and females): Wild type, T1 (PBG-D activity 55% reduced) and AIP (PBG- D activity 70% reduced. T1 and AIP PBG-D activity was according to the model in liver; in brain it was also reduced (40-50%, p<0.01) without differences between both genotypes. As was expected, 5-Aminolevulinic acid synthetase activity, heme regulatory enzyme, was elevated in liver (T1: 140%, p<0.01; AIP: 45%, p<0.05) and brain (T1: 257%, AIP: 95%, p<0.05) in both genotypes. Heme oxygenase (HO), involved in heme catabolism, was 100% (p<0.05) higher than wild type in brain in both sexes and genotypes, being hepatic HO only induced in females (50-100%, p<0.05). HO alteration, GSH variation and Catalase reduction (140%, p<0.05) would indicate stress oxidative instauration. Glutathione S-Transferase, hepatic damage marker, varied depending on the genotype. Tryptophan pyrrolase activity, pool regulatory heme marker, was elevated in liver and brain (87-140%, p<0.05) of AIP female. Considering that aging is a significant risk factor for impaired tissue functions and chronic diseases, alterations in the measured parameters were evaluated throughout life, using animals 12-15 months old; no major variations were observed. In conclusion, the present study has demonstrated that the differences among wild type and genetic models were more striking in AIP genotype respect to T1 and age did not affect significantly the metabolisms analyzed.

NANOMEDICINA

355. (221) **AMORPHOUS SILICA NANOPARTICLES: NEW THERAPEUTIC APPROACH FOR DRUG DELIVERY IN TRIPLE NEGATIVE BREAST CANCER**

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Triple Negative Breast Cancer(TNBC) is a heterogeneous group of tumors with difficult clinical management. Nanotechnology represents a strategy to improve current therapies. The aim of this work is to develop amorphous silica nanoparticles(SiNPs) as carriers for drugs involved in TNBC. Synthesis, physicochemical characterization and evaluation on the viability of TNBC cell lines of two SiNPs formulations were performed: amino-functionalized SiNPs(Si@NH_2) and SiNPs modified with folic acid(FA) (Si@FA). Modified Stöber process was applied to obtain Si@NH_2. FA was covalently linked (Si@FA). Characterization was performed by FTIR and DLS to determine hydrodynamic diameter(Hd). Viability assays by crystal violet staining were performed in human MDA-MB-231, murine 4T1 TNBC cell lines and in non-malignant murine breast HC11 cells (10 - 500 µg/mL SiNPs, 24 h). Reactive oxygen species (ROS) generation was determined by DCDCDHF assay in MDA-MB-231 cells (500 µg/mL SiNPs). A pilot in vivo assay was conducted in mice to evaluate SiNPs acute toxicity: 30 mg/kg of Si@NH_2, Si@FA or control were administered weekly for 1 month. FTIR spectra confirmed functionalization with NH_2 and FA; Hd resulted as 643.7 nm and 600.0 nm respectively. Si@FA displayed a significant reduction of the viability of MDA-MB-231 and 4T1 TNBC cells. Si@NH_2 decreased 4T1 cell viability (p<0.001) although no effect was found for MDA-MB-231 cells at any of the concentrations tested. Both NPs increased ROS production with respect to control (Si@FA: p<0.001; Si@NH_2: p<0.01) and between them (p<0.001). Regarding to HC11 cells, NPs had no effect on viability. The observation of the internal organs of the animals showed no macroscopic alterations; no changes in hematocrit, behavior and body weight were observed. Altogether, these results suggest that Si@FA could be a promising nanotechnology for TNBC treatment. Further studies are in course to evaluate their effects in combination with conventional drugs.

356. (225) **LIPOSOMAL ASCORBIC ACID - PHYSICOCHEMICAL CHARACTERIZATION**

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Objective: Physicochemical characterization of pure sunflower lecithin liposomes and sunflower lecithin liposomes combined with cholesterol, and ascorbic acid, by means of Z-potential, particle size, conductivity, density and ultrasound velocity measurements dependence with temperature. This work is meant as a preliminary screening for the development of improved delivery and bioavailability of vitamin C systems.

Materials and methods: Phosphatidylcholine from sunflower (H100), Cholesterol (Chol) and ascorbic acid (AA). H100, H100-Chol, H100-AA and H100-Chol-AA liposomes were prepared. *Zeta potential (ZP), size and conductivity* measurement of liposomes was determined with a Zetasizer Nano ZS90 equipment. Anton-Paar DSA 5000 was used to get continuous and automatically, densities (ρ) and sound velocities (u).

Results: ZP curves as a function of temperature showed that the most negative surface charge was that of the H100 Chol system, with positive surface charges for the others. H100 and H100-Chol conductivities were the lowest measured and were found to be very close to each other. H100 AA y H100 Chol AA systems had the highest conductivities. Particle size was 150 nm for H100 Chol AA liposomes, 200 nm for H100 AA liposomes and 250 nm for H100 and H100 Chol and remained constant. The lower specific volume of H100-Chol-AA liposomes in comparison with H100 membranes may be indicative of a more compact lipid bilayer structure for the former. Specific compressibility values for H100 Chol AA and H100 were similar.
Conclusions: Ascorbic acid encapsulated in sunflower phosphatidylcholine liposomes shows well-organized morphology, uniform particle size, which might lead to an improved bioavailability. It could result to be a good alternative to protect and transport vitamin C in the body.

357. (243) **CHARACTERIZATION AND IN VITRO EFFECTS OF GERANIOL-LOADED PECTIN MODIFIED NANOSTRUCTURED LIPID CARRIERS ON LUNG CANCER CELLS**

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Lung cancer is the first cause of cancer-related deaths in the world. Many of the current therapies are still inefficient and/or present highly toxic undesirable side effects. The aim of the present work was the design of biocompatible and non-toxic hybrid pectin (P) nanostructured lipid carriers (NLC) containing geraniol (GOH), a monoterpene with antitumor activity, as a novel platform for the bioactive delivery of anticancer drugs.
Nanoparticles (NPs) were prepared by hot homogenization/ultrasonication method. Different pectin formulations were prepared by modifying their amount (0.1 and 0.5%, w/v) in the formulation and/or metoxilation degree -Low (LMP, 33%) and high (HMP, 74%). Ten different formulations were prepared: NLC/GOH, NLC/LMP0.1/GOH, NLC/LMP0.5/GOH, NLC/HMP0.1/GOH, NLC/HMP0.5/GOH, and their respective counterparts without GOH. NPs were characterized by TEM microscopy and DLS: particle size, z-potential (z-pot), and polydispersity index (PI). The encapsulation efficiency (EE) was determined by UV-Vis spectroscopy. Cell viability (MTT) and mitochondrial membrane potential (MMP, fluorescence spectroscopy) in human lung adenocarcinoma A549 cells were evaluated. NPs showed spherical shape, sizes in the range of 110-180 nm with narrow distribution (PI< 0.3), and negative z-pot ranging from
-10 to -19 mV. The EE of GOH was higher than 89% in five formulations. GOH-loaded NLC inhibited A549 cell viability in a dose (0.25-2.00 mM) and time (24 and 72 h) dependent manner. GOH-loaded NLC decreased cell viability up to 10-fold compared to free GOH (GOH 1.5 mM, 24 h, p< 0.001). In addition, GOH-loaded NLC strongly decreased (p< 0.001) MMP (50 to 94%) in comparison with free GOH (37%) in A549 cells. These results suggest that hybrid NLC/P nanoparticles containing GOH are promising bioactive and innovative systems for targeted delivery of antineoplastic drugs to treat lung cancer.

358. (252) **HISTAMINE COMBINED STRATEGIES TO IMPROVE THE THERAPEUTIC EFFICACY OF PACLITAXEL IN TRIPLE NEGATIVE BREAST CANCER**

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Breast cancer is the most frequently diagnosed neoplasia and a leading cause of cancer related death in women worldwide. Triple negative breast cancer (TNBC) is an aggressive subtype, associated with poor prognosis. The standard treatment regimen for TNBC is based on the administration of highly toxic chemotherapeutic drugs (anthracyclines/taxanes). Paclitaxel (PTX) is a first-line therapy for TNBC and has low water solubility, poor permeability, and produces severe adverse effects, which limit its clinical use. The aim of this work was to improve the therapeutic index of PTX through the design of combined strategies. For that purpose, we developed nanomicellar polymeric formulations of Soluplus® (S) and Soluplus® surface decorated with glucose residues (SG) co-loaded with histamine (HA, 5 mg/mL) and PTX (4 mg/mL). The micellar size, evaluated by dynamic light scattering, showed a hydrodynamic diameter between 80 and 100 nm for loaded nanoformulations with a unimodal size distribution. Cytotoxicity and apoptosis assays were performed to assess their efficacy *in vitro* in human MDA-MB-231 and murine 4T1 TNBC cells. Results showed that histamine improved the antitumoral activity of free PTX and Genexol® (commercial micellar-based PTX- nanoformulation) at low concentrations (0.01-0.1 uM) in both cell lines (P<0.05). HA-PTX co- loaded SG micelles exhibited enhanced cytotoxic and pro-apoptotic effects compared to PTX loaded SG micelles, Genexol®, and free PTX (P<0.05).
99mTc-radiolabelled S and SG micelles' distribution was analyzed using *gamma* camera imaging in the TNBC model developed in BALB/c mice with 4T1 cells, showing tumor uptake. Importantly, the *in vivo* studies showed that histamine, both in combination with free PTX and in the HA-PTX loaded SG micellar system, reduced cardiotoxicity associated with PTX administration. We conclude that histamine enhances the efficacy of nanotechnology based PTX therapy, representing a promising approach for TNBC treatment.

359. (272) **CURCUMIN NANOVESICLES FOR INHALATORY PHOTODYNAMIC THERAPY**

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Curcumin (CUR) is a polyphenol obtained from the roots of *Curcuma longa* that produces singlet oxygen and other reactive oxygen species (ROS) when irradiated with blue light. In a previous work we developed an inhalatory formulation of CUR encapsulated in archaeolipid nanovesicles, called nATC, and a conventional formulation of liposomal CUR, nLTC. While nATC had excellent stability against nebulization and long-term storage, nLTC released CUR after 1 month of storage and partially lost its CUR content during nebulization. Therefore, the aim of this work was to evaluate nATC as a photosensitizing agent to be used in an inhalatory photodynamic therapy (PDT) against lung cancer. We studied cytotoxic effects of nATC, nLTC and free CUR on A549 cells -a human lung adenocarcinoma cell line-, after irradiation at 420 nm with a fluence of 9 J/cm^2. First, we determined cell proliferation by MTT 24 h after irradiation and obtained an IC50 of 3.7 ± 0.5 μM, 4.9 ± 0.8 μM and 8.5 ± 1.6 μM for nATC, nLTC and free CUR, respectively. Cells were labeled with Annexin V/Propidium Iodide 6 h after irradiation and it was determined that only nATC induced apoptosis (12.5 ± 3% of cells analyzed by flow cytometry), while free CUR only induced necrosis (18 ± 2%). 24 h after irradiation, all formulations induced LDH release and complete depolarization of mitochondrial membrane potential. Furthermore, nATC and CUR completely inhibited cell migration 96 h post irradiation, whereas nLTC did not. Finally, only nATC was able to completely inhibit the activity of matrix metalloproteinases. These

differential effects of nATC could be explained by 1) the higher cytoplasmatic CUR content within the cells and 2) the higher concentration of ROS and nitric oxide detected after nATC irradiation. We concluded that nATC outperformed liposomal CUR and free CUR making it a good candidate for inhalatory PDT that deserves further study to determine its anti-angiogenic activity and immunomodulatory effects.

360. (275) **HEMOCOMPATIBILITY STUDY OF AMORPHOUS SILICA NANOPARTICLES**

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The study of potential toxicity of amorphous silica nanoparticles (SiNPs) has been assessed in human blood to better understand their usefulness as drug carriers, in special for hematological disorders. Size represents a crucial parameter in terms of internalization and disruption of blood cells. In this work, SiNPs were synthesized by Stöber method with modifications, varying the amounts of water and ethanol; temperature and time of reaction as variables determinant of size. Besides, SiNPs were modified by treatment with NaOH to generate pores in the surface for future pharmacological prospects. The SiNPs obtained were characterized by FTIR spectroscopy to evaluate qualitative composition; by DLS to determine hydrodynamic diameter (Hd) and zeta potential to infer surface charge. Hemolytic activity, LDH levels and oxidative stress were studied. Exposure to SiNPs was conducted by mixing NPs dispersed in isotonic saline solution of NaCl 0.9% at concentrations of 1000, 500, 250, 100 and 50 μg/mL with healthy human blood, during 1h. Positive controls were processed. Free hemoglobin was determined by UV spectroscopy. LDH levels were studied employing a commercial kit. Evaluation of oxidative stress was conducted by TBARS determination. FTIR spectroscopy confirmed SiO_2 as main component of NPs. All formulations obtained resulted monodisperse with Hd near 200nm with negative surface charge.
Results have not evidenced changes in erythrocytes lysis in comparison to control ($p<0.001$). No changes were observed in cytology. LDH levels were registered as normal for all formulations at the concentrations tested. TBARS assay demonstrated the induction of oxidative stress at concentration of 500 μg/mL, in special for pore induced SiNPs formulation.
200nm sized SiNPs obtained did not induce significant alterations in the parameters observed for the study of hemocompatibility. However, the induction of oxidative stress was observable as dose dependent for concentration of 500 μg/mL.

361. (289) **INHIBITORY ACTIVITY OF MAGNETIC MESOPOROUS SILICA NANOPARTICLES LOADED WITH NORFLOXACIN**

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The effect of raw magnetic mesoporous silica nanoparticles ($MMSiO_2$) and 4.97% Norfloxacin-loaded $MMSiO_2$ (NFX-$MMSiO_2$) was tested on an *E.coli* bacterial growth to evaluate inhibitory activity. $MMSiO_2$, with a core (Fe_3O_4)-shell type structure, were prepared by a modification of the Stöber method. The average size, surface area and isoelectric point was 125 nm, 471 m^2g^{-1} and 2.4, respectively. A suitable range of concentrations of NFX-$MMSiO_2$ [16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL] was seeking to the minimum inhibitory concentrations known by NFX. Microdilution inhibitory assay was conducted for of $MMSiO_2$, NFX-$MMSiO_2$ and NFX. The samples were incubated for 18h at physiologic temperature of 37°C and absorbances were measured in a Multiskan GO VWR Co Microplate Spectrophotometer, Thermo Scientific. The results were standardized subtracting the absorbances of the vehicle and data were statistically analyzed.
The evidence suggested differences in the inhibitory activities depending on NFX-$MMSiO_2$ concentrations when compared to NFX. At low doses, the major prevalence of growth decrease was observed for NFX-$MMSiO_2$ in comparison to the same doses of the antibiotic, whereas at higher concentrations of NFX-$MMSiO_2$ the effect decreased in comparison to free NFX. There was exposed a decline in the inhibitory growth activity of NFX according to its minimum inhibitory concentration (MIC) in contrast to $MMSiO_2$ which activity remained constant. It was also observed that $MMSiO_2$ did not present a direct effect on the bacteria. However, these NPs could have enhanced the antibiotic effect at lower doses. This fact may be due to the outcome of iron from the NPs and to changes in the ionic force of growing medium.
Results demonstrate that loading NFX into $MMSiO_2$ would be a strategy to target the drug to specific sites in the organism under the influence of an external magnetic field, at low concentration to ensure a better performance in comparison to free NFX.

362. (297) **VALIDATION OF A SCALABLE METHOD USING CHROMATOGRAPHY TO ISOLATE ENGINEERED EXTRACELLULAR VESICLES CARRING IGF1 DERIVED FROM HUMAN UMBILICAL CORD PERIVASCULAR CELLS FOR THE TREATMENT OF EXPERIMENTAL LIVER FIBROSIS**

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Introduction: Extracellular vesicles (EVs) derived from human umbilical cord perivascular cells (HUCPVC) over-expressing IGF1 mediates therapeutic effect on liver fibrosis in mice. We aimed to validate these results using EVs isolated by affinity chromatography, a scalable method.
Methods: HUCPVC were infected with adenoviruses codifying for human IGF1 (AdIGF1) or green fluorescence protein (AdGFP). Viability was determined by MTT assay. IGF1, TNF-α, and CD63 levels were determined by ELISA. EVs were isolated from HUCPVC supernatants by anion exchange chromatography and characterized by electron microscopy. Expression of pro-fibrogenic genes (Col1A2 and αSMA) on hepatic stellate cells (LX2) were determined by qPCR. Antifibrotic effect of EVs was determined in BALB/c mice with liver fibrosis (thioacetamide for 8 weeks). The treatments were administered on week 6 (Groups: Saline, EVs-AdIGFI or EVs-AdGFP, 3 doses, 15 μg/dose/mice every 5 days).
Results: First, we found that HUCPVC infected with 2.5 to 30 MOI showed over-expression of IGF1, keep cell viability and exert an anti-inflammatory capacity on J774 macrophages revealed by decreased TNF-α expression ($p<0.0001$ vs LPS). HUCPVC-derived EVs isolated by chromatography showed typically size, shape, and CD63 expression. Increased IGF1 levels were observed on lysed-dialyzed EVs-AdIGFI indicating its loading on EVs ($p<0.001$). Pro-fibrogenic genes expression was reduced in LX2 cells after treatment with IGF1-loaded EVs ($p<0,01$ vs. DMEM). *In vivo* treatment with EVs-AdIGF1 resulted in a further amelioration of liver fibrosis when compared to saline group ($p<0,001$).
Conclusion: Our results showed that EVs-AdIGF1 isolated by a chromatographic scalable method carry IGF1 and keeps anti-fibrotic activity. This data provides a novel approach of nanomedicine to generate therapeutic tools for the treatment of iver fibrosis.

363. (332) **DEVELOPMENT AND CHARACTERIZATION OF SOLUPLUS® NANOMICELLES ASSOCIATED TO SPECIFIC IgG AS AN INNOVATIVE STRATEGY FOR THE DETECTION AND NEUTRALIZATION OF SHIGA TOXIN TYPE 2**

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Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin producing *Escherichia coli* and is responsible for triggering Hemolytic Uremic Syndrome (HUS). We aimed to develop and characterize polymeric nanomicelles (PN) with the amphiphilic polymer Soluplus® coupled to anti-Stx2 IgG in order to introduce innovative proposals for the detection of Stx2 and treatment of HUS. PN of Soluplus® were formulated in PBS and coupled with IgG anti Stx2 from hyperimmune (PN-IgG-Stx2) or control bovine colostrum (PN-IgG-Ctrl). The hydrodynamic size of PN, PN-IgG-Stx2 and PN-IgG-Ctrl was evaluated by Dynamic Light Scattering. Morphology of PN or PN-IgG-Stx2 was analyzed by Transmission Electron Microscopy (TEM). The PN toxicity was evaluated on both Vero and Human Glomerular Endothelial cells (HGEC) and cell viability was determined by neutral red uptake. After coupling PN with IgG, Stx2 neutralization capacity of PN-IgG-Stx2 or PN-IgG-Ctrl was evaluated on Vero and HGEC cells and the percentage of cell viability was analyzed. The hydrodynamic size of the PN of Soluplus® and IgG-Stx2 showed an average diameter of 70.2 ± 1.5 nm and 40.9 ± 2 nm, respectively. When both components were coupled, a single peak with a similar hydrodynamic size of the PN was observed (70.4 ± 0.2 nm). TEM analysis revealed circular particles with a diameter corresponding to 100 nm either in PN and PN-IgG-Stx2 particles. PN-IgG-Stx2 were able to neutralize Stx2 on Vero and HGEC cells in a dose dependent manner. When comparing the neutralization capacity of Stx2 by IgG-Stx2 *vs* PN-IgG-Stx2 a significant improvement in the cell viability of Vero and HGEC was observed with the PN-IgG-Stx2 ($p<0.001$). The association between anti-Stx2 IgG from bovine colostrum and Soluplus® PN was optimized and characterized. Encouraging results of antibody functionality coupled with PN were registered. These results may open the perspective of the design of new nanoplatforms for neutralization and/or detection of Stx2.

364. (358) **ANTIOXIDANT NATURAL COMPOUNDS ADSORBED ON SILICA NANOPARTICLES**

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Therapeutic nanotechnology involves the use of nanoparticles (NPs) as vectorization, protection and controlled release systems for molecules of therapeutic interest like antioxidants. These vectors make possible to improve the biodistribution of fragile or complex molecules and favor their interaction with specific tissues. Antioxidant compounds are of great interest since uncontrolled production of free radicals has been related to several diseases. The aim of this work was to obtain fungal enriched protein extracts with antioxidant activity and to adsorb them on silica NPs in order to evaluate their antioxidant potential. To achieve this goal, *P. ostreatus* and *A. bisporus* fruiting bodies were homogenized, centrifuged and the supernatants were treated with ice cold ethanol to precipitate soluble proteins. The precipitates were resuspended in buffer and enriched proteins extracts were obtained. Silica nanoparticles (SiNPs) were synthesized by the Stöber method and a portion of these were grafted with APTES to add amino groups to their surface, obtaining modified SiNPs ($SiNPsNH_2$). Both variants of NPs were characterized by TEM, DLS and Z potential. These NPs of spherical form were a homogeneous population of diameter 110 ± 10 nm. Fungal extracts were incubated with NPs at 40 rpm and 4°C overnight to allow the proteins adsorption on the NPs. Antioxidant activity was evaluated using ABTS radicals generated by the reaction of ABTS and potassium persulfate overnight at room temperature in the dark. For the assay, samples were incubated with the radical ABTS for 30 min in the dark and the absorbance was measured at 734 nm. The results showed that a high amount of the proteins of *A. bisporus* extracts were adsorbed on both SiNPs and $SiNPsNH_2$, while *P. ostreatus* proteins were not adsorbed. But only *A. bisporus* proteins-SiNPs complex presented significant antioxidant activity. Therefore, this complex is of interest to be studied as a possible therapeutical tool.

365. (372) **TITLE: ANTI-INFLAMMATORY EFFECT OF ARCHAEOLIPID NANOPARTICLES ON HUMAN ENDOTHELIAL CELLS**

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Background: New generation of liposomes called archaeosomes (ARC) are nanoparticles (NPs) made of archaeolipids employed as drug delivery systems that exhibit high structural stability. So far there are no reports on the effects of ARC on human vascular endothelium, critical data for their eventual clinical use.
Aim: The present study investigated the effects of archaeosomes on human umbilical vein endothelial cells (HUVECs) in physiological and under inflammatory conditions and compared them with conventional liposomes.
Methods: HUVECs were non-stimulated or stimulated with LPS, Pam3CSK4, *E. coli,* TNF-α, or IL-1 β in the absence or presence of nanoparticles. We analyzed cell viability and apoptosis (acridine orange/ethidium bromide), cell proliferation (EdU assay), expression of ICAM-1 and E-selectin (cytometry), secretion of IL-6 and von Willebrand Factor (vWF) (ELISA), mRNA expression of ICAM-1 and vWF (RT-qPCR), uptake of NPs (cytometry and confocal microscopy) and signaling pathways (cytometry and western blot). Results were analyzed by ANOVA followed by Bonferroni test ($p<0.05$, n=4-6).
Results: None of the NPs affected the viability, cell proliferation, and expression of ICAM-1 and E-selectin under basal conditions, but ARC reduced the expression of both molecules and the secretion of IL-6 induced by LPS, Pam3CSK4 or *E.coli*, an effect not observed with TNF-α or IL-1 beta. Interestingly, ARC significantly decreased basal vWF levels and the increase induced by all stimuli. None of these parameters were modified by the liposomes. Only archaeosomes were endocytosed by HUVECs and reduced mRNA expression of ICAM-1 and vWF via NF-kB and ERK1/2 in LPS-stimulated HUVECs.
Conclusions: Our data show that archaeosomes exert an anti-inflammatory effect on endothelial cells. Loaded with anti-inflammatory drugs, they could magnify their activity on inflamed endothelium, and therefore their research in vasculopathies is of special interest.

366. (434) **IMPACT OF PHOTOTHERMAL THERAPY USING GLUCOSE-FUNCTIONALIZED GOLD NANOPARTICLES IN HUMAN LUNG CANCER CELLS**

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Despite the recent data on cancer incidence showing that molecular targeted treatments have accelerated a progress against lung cancer, it remains the principal cause of cancer mortality worldwide.

Additionally, most patients are diagnosed in advanced stages, restricting their therapeutic options. Nanoparticles-based photothermal therapy has been studied as a low toxic and feasible treatment for solid tumors in the recent years. In this work, we synthetized gold nanoparticles (AuNPs) in the forms of rods, spheres and stars and analyzed the in vitro toxicity of AuNPs, polyethylene glycol-(PEG-AuNPs) and glucose-functionalized AuNPs (GlucAuNPs) on human lung carcinoma cells. Also, we evaluated the ability of cancer cells to uptake each type of GlucAuNPs and assessed the capability of irradiated N-GlucAuNPs to induce thermal damage. From our results, we detected that AuNPs toxicity significantly decreases when they were functionalized with PEG, in comparison with as prepared AuNPs ($p<0.001$). Importantly, the viability of A549 cells was completely preserved when AuNPs were functionalized using glucose (GlucAuNPs). Quantification of cellular uptake by inductively coupled plasma mass spectrometry (ICP-MS) shown that GlucAuNPs were more incorporated by A549 cells than PEG-AuNPs. In order to evaluate in vitro, the efficiency of GlucAuNPs for photothermal therapy, we irradiated A549 with a 980 diode laser at 1 W and identified that GlucAuNPs plus laser had an antiproliferative effect and inhibited tumor cell viability compared to laser or GlucAuNPs without irradiation ($p<0.001$). In addition, similar studies on normal fibroblast incubated with GlucAuNPs did not shown photothermal damage. Our findings exhibit that glucose functionalization of AuNPs improves the uptake of nanoparticles by cancer cells and selectively induces loss of viability and cellular death. We suggest that our approach can be used to develop new tools for advanced lung cancer.

367. (477) **IMPACT OF OUTER MEMBRANE VESICLES IN ANTIMICROBIAL RESISTANCE**

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The outer membrane vesicles (OMVs) are able to harbor genes associated with antimicrobial resistance (RAM). In addition, OMVs possess both defensive and protective functions and thus participate in community related functions. Our goal was to characterize and look for the presence of genes associated with RAM in the OMVs from three relevant resistant strains, *E. coli* SM5 that belongs to pandemic lineage ST131, *E. coli* M19736 that harbors *mcr-1*, and *P. aeruginosa* PAE981 that belongs to epidemic clone ST2867. In addition, we evaluated if these OMVs could protect other bacteria against a membrane-active antibiotic, such as colistin. To characterize OMVs 1) total quantification of OMVs was performed using the Micro BCA Protein Assay Kit, 2) the images of OMVs was obtain by transmission electron microscopy (TEM), and 3) we estimated biophysical characteristics using nanoparticle tracking analysis (NTA). Finally, by PCR assay we looked for genes associated with RAM. NTA results showed a size between 100–300 nm in diameter. PCR assays allowed us to detect in the OMVs from SM5 the $bla_{\text{CTX-M-15}}$ gene, from M19736 the *mcr-1* gene, and from PAE981 the $bla_{\text{VIM-2}}$ gene. Chemical, electro and natural transformation was performed for SM5 using the OMVs from M19736. The colonies obtained were evaluated by PCR for the presence of *mcr-1*. The three transformation assays with a strain that was previously susceptible to colistin, showed colonies which then were able to survive to this antibiotic without acquiring *mcr-1*. Therefore, we propose that the OMVs isolated from M19736 could protect SM5 from colistin. The present study shows an efficient isolation method in different bacterial genera that allows the detection of genes associated with RAM by PCR in OMVs. Also, the results obtained indicate that OMVs have a dual impact on the generation of antimicrobial resistance, both through horizontal genetic transfer and through a protective physical effect in clones with epidemic behavior.

368. (536) **SYNTHESIS AND EVALUATION OF THE ANTITUMORAL ACTIVITY OF RIBOFLAVIN-TARGETED 8-HYDROXYQUINOLINE PLATINUM(II)-LOADED NANOSTRUCTURED LIPID CARRIERS**

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Platinum-based drugs have been used as chemotherapeutic agents for decades, although they show a high level of toxicity, chemoresistance, low solubility, and consequently low bioavailability. Nanostructured lipid carriers (NLC) are the second generation of lipid nanoparticles that were developed to overcome these drawbacks. It has been reported that riboflavin (RFV) receptors show up-regulation in many tumor cells, so RFV could be an efficient ligand for tumor-specific drug delivery. RFV, also known as vitamin B2, was functionalized in the NLCs to study actively target tumor cells. The drug 8-hydroxyquinoline platinum(II) (8HQ-Pt) loaded, RFV-targeted NLC of myristyl myristate (MM) (RFV-8HQ-Pt-NLC) were synthesized by ultrasonication. Cytotoxicity, cell uptake, and apoptosis assays against the human colon adenocarcinoma cell line HT-29 were studied. The NLCs with spherical shape and narrow size distribution in the range of 136-162 nm mean particle diameter and 75% encapsulation efficiency were shown. RFV-8HQ-Pt-NLC exhibited better cytotoxicity than the untargeted-NLC (8HQ-Pt-NLC) and the free 8HQ-Pt (2.8-fold and 3.8-fold, respectively at 5 μM). RFV-8HQ-Pt-NLC 3.2-fold more efficiently incorporated into HT-29 cancer cells at 2.5 μM in comparison to 8HQ-Pt-NLC. Also, RFV-8HQ-Pt-NLC showed higher levels of apoptosis than the free 8HQ-Pt at 2.5 and 5 μM. As a conclusion, we showed that RFV targeting improved the antitumoral activity of NLC. Ligand-targeted nanosystems offer promising results in the future of chemotherapy.

369. (550) **DEVELOPMENT OF TRIAMCINOLONE ACETONIDE NANOCRYSTALS FOR OCULAR ADMINISTRATION**

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Objectives: Triamcinolone acetonide (TA) is a powerful anti-inflammatory drug, used in the treatment of inflammatory ocular disorders. Poor drug solubility and ocular anatomical barriers hinder an optimum therapy. The principal problems related to the use of injectable approved formulations of TA include particle size (>1μm), which can hinder the administration, and also the presence of excipients as preservatives, which can generate cell damage. The purpose of this work was to obtain a formulation based on triamcinolone acetonide nanocrystals (TA-NCs) to improve ocular corticosteroid therapy.
Material and Methods: The microsphere assisted nanomilling (MAN) followed by spray-drying (SPA) techniques were used to obtain dried TA-NCs, which were characterized by studies of dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), thermal analysis, X-ray diffraction (XRD), and Electron microscopy (SEM). Preliminary studies of anti-inflammatory efficacy of a suspension of dispersable TA-NCs via subconjunctival route (n=5) were carried out in an endotoxin-induced uveitis (EIU) rabbit model and compared with a control group (normal saline solution).
Results: Dispersable TA-NCs presented an average particle size of (266±77) nm; a narrow size distribution and zeta potential of (-27±1) mV, which remained stable during 30 days under storage conditions at 4 °C. TA-NCs showed uniform and spherical morphology (SEM). FTIR and XRD spectra showed no apparent chemical and crystallinity changes, while no apparent thermal changes were revealed by thermal analysis. The subconjunctival administration of TA-NCs in

albino male white rabbits did not show clinical signs of ocular damage. *In vivo* preliminary studies showed that dispersable TA-NCs treatment alleviated the inflammatory response in the anterior chamber and iris in EUI rabbit eyes.
Conclusion: Dispersable TA-NCs are a promising approach to obtain a novel nanometric TA formulation for ocular disorders.

370. (556) **EFFICACY OF TOPICAL MILTEFOSINE FORMULATIONS IN AN EXPERIMENTAL MODEL OF CUTANEOUS LEISHMANIASIS**

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Objectives: Cutaneous leishmaniasis (CL) is a neglected tropical disease endemic in ~ 90 countries, with an increasing incidence. Presently available pharmacotherapy implies the systemic administration of moderately/very toxic drugs. A topical treatment would have the great advantage of minimising the systemic circulation of the drug, preventing side effects. We set out to design and characterize a topical treatment based on miltefosine (Milt).
Methods: We prepared dispersions containing Milt and liposomes of different compositions to enhance/modulate trans-epidermal penetration and evaluated *in vitro* and *in vivo* efficacy and toxicity, in vitro release rate of the drug and particles size stability with time. Treatments were topically administered to BALB/c mice infected with *Leishmania (Leishmania) amazonensis*.
Results: The dispersions containing 0.5% Milt eliminated 99% of the parasites and cured the lesions with a complete re-epithelisation, no visible scar and re-growth of hair. Fluid liposomes decreased the time to heal the lesion and the time needed to eliminate viable amastigotes from the lesion site. Relapse of the infection was not found 1 month after treatment in any case. Ultraflexible liposomes on the other hand had no significant *in vitro* effect but decreased *in vivo* efficacy.
Conclusion: A topical Milt formulation including fluid liposomes seems a promising treatment against CL.

371. (557) **EFFICACY OF TOPICAL MILTEFOSINE FORMULATIONS IN AN EXPERIMENTAL MODEL OF CUTANEOUS LEISHMANIASIS**

Ma. Florencia Peralta[1], Nadina Usseglio[1,2], Ma. Elena Bracamonte[3], Ma. Laura Guzmán[2], Ma. Eugenia Olivera[2], J. Diego Marco[3], Paola A. Barroso[3], Dolores C. Carrer[1]
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Objectives: Cutaneous leishmaniasis (CL) is a neglected tropical disease endemic in ~ 90 countries, with an increasing incidence. Presently available pharmacotherapy implies the systemic administration of moderately/very toxic drugs. A topical treatment would have the great advantage of minimising the systemic circulation of the drug, preventing side effects. We set out to design and characterize a topical treatment based on miltefosine (Milt).
Methods: We prepared dispersions containing Milt and liposomes of different compositions to enhance/modulate trans-epidermal penetration and evaluated *in vitro* and *in vivo* efficacy and toxicity, in vitro release rate of the drug and particles size stability with time. Treatments were topically administered to BALB/c mice infected with *Leishmania (Leishmania) amazonensis*.
Results: The dispersions containing 0.5% Milt eliminated 99% of the parasites and cured the lesions with a complete re-epithelisation, no visible scar and re-growth of hair. Fluid liposomes decreased the time to heal the lesion and the time needed to eliminate viable amastigotes from the lesion site. Relapse of the infection was not found 1 month after treatment in any case. Ultraflexible liposomes on the other hand had no significant *in vitro* effect but decreased *in vivo* efficacy.
Conclusion: A topical Milt formulation including fluid liposomes seems a promising treatment against CL.

372. (562) **RADIOSENSITIZATION OF HUMAN MELANOMA CELLS BY SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES**

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Melanoma is the deadliest form of skin cancer, highly metastatic and resistant to therapies. Superparamagnetic iron oxide nanoparticles (SPIONs) have shown great potential for diagnosis and therapy. The aim of this study was to evaluate the radiosensitizing effect of SPIONs in A375 human melanoma cells.
SPIONs were synthesized and stabilized by methyl-poly(ethylene glycol). After that, it was physicochemically characterized by transmission electron microscopy, X-ray diffraction, magnetometry and tested in vitro. Superparamagnetic behavior and low dispersion in shape and sizes (8-17 nm) were obtained. No cytotoxicity was found in A375 cells exposed up to 250 μg/ml for 24 h. Dichloro-dihydro-fluorescein diacetate assay revealed higher reactive oxygen species levels in treated cells ($p<0.05$). Survival curves obtained by combined treatments of SPIONs and gamma irradiation (^{137}Cs) (SPIONs-IR) and fitted to the linear-quadratic model, demonstrated a significant increase in radiation effect in SPIONs-IR treated cells ($p<0.05$), with surviving fraction at 2 Gy of 0.51 and 0.28 for IR and SPIONs-IR treated cells, respectively. Increased DNA damage by SPIONs-IR vs IR was observed by the detection of γH2AX foci at 30 minutes post-irradiation and a decreased repair capacity was found at 24 h post-irradiation by analyzing the persistence and size increase of γH2AX foci in SPIONs-IR compared with IR treated cells ($p<0.05$). In conclusion, SPIONs proved to be effective radiosensitizers of melanoma cells.

373. (569) **CARRIER IN CARRIER: DNA-DOXORUBICIN COMPLEX SELF-ASSEMBLED WITH AMPHIPHILIC CYCLODEXTRINS AS NANOSYSTEMS FOR CANCER THERAPY**

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The goal of the present work was to characterize the interaction between DNA–Doxorubicin (DNA-Dox) complex and catanionic vesicles based on amphiphilic cyclodextrins (ModCBHD) for their application as drug delivery systems. The systems were characterized by dynamic light scattering (DLS), Zeta potential (ζ), circular dichroism (CD), Emission spectroscopy, atomic force microscope (AFM), transmission electron microscopy (TEM) and drug release in two-compartment Franz cells. Fluorescence spectra, CD profile and ζ showed multifaceted interaction pathways between DNA and Dox, with ionic and intercalation interactions, trought of the amino sugar residue and the tetracene ring system of the drug, to form DNA-

Dox complexes with a negative surface charge. DNA behaves as a reservoir of Dox, that is slowly released from the complex triggered the presence of ions the medium. ModCBHD-DNA-Dox complexes were formed by self-assembling in aqueous solution without introducing any subsequent steps, and exhibited around 160 nm particle size, monodisperse size distribution (PDI 0.250) and spherical shape, which could be an advantage for the enhanced permeability and retention (EPR) effect in cancer therapy. All these results demonstrated that ModCBHD can load the DNA-Dox complex and indicated the potential of the ModCBHD-DNA-Dox systems as nanocarriers to be evaluated in both in vitro cancer cell lines and in vivo tumor models.

374. (572) **A^{BR}: PROTECTING BR STABILITY, ANTIOXIDANT, ANTI-INFLAMMATORY ACTIVITY WITH ARCHAEOLIPIDS**
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In recent years, oxidative stress induced by reactive oxygen species (ROS), has been implicated in the cause and progression of various diseases such as cancer and atherosclerosis through the oxidation of DNA, proteins and lipids. Antioxidant compounds decrease the rate of mutagenesis and carcinogenesis by inhibiting oxidative damage to cells.
Halophilic archaea produce polar (PA) and neutral (NA) archeolipids with unique and differential properties. PAs are composed of saturated isoprenoid chains linked by ether bonds to the glycerol carbons in the *sn* 2,3 position and are highly resistant to hydrolytic, oxidative and enzymatic attack. NA are mainly composed of bacterioruberin (BR), a carotenoid with high antioxidant activity, able to reduce effects caused by ROS as it inhibits oxidative damage to cells. However, free BR is hydrophobic and susceptible to oxidation. In this work we encapsulated BR in PA archaeosomes (A^{BR}) and studied its antioxidant and anti-inflammatory activity compared with the one of nanovesicles made of soybean phosphatidylcholine and BR (L^{BR}).
A^{BR} (135 ± 48 nm, -50 ± 18 mV Z-potential and 9,6 μg/mg phospholipids/BR rate) protected BR from light and high temperatures effect as antioxidant activity remained unchanged after incubation at 80º and room temperature without protection. This activity was lost in L^{BR} (154 ± 35 nm, -13 ± 3 mV Z-potential and 6,9 μg/mg phospholipids/BR rate). In murine macrophages cells we found that even though no high antioxidant activity was found in studied conditions, there is a remarkable anti-inflammatory activity as A^{BR} reduced the release of pro-inflammatory cytokine TNF- α induced by LPS (compared with the one of A without BR).
In conclusion, A was able to protect BR, even in hostile conditions, allowing future application and enabling its antioxidant and anti-inflammatory activity.

375. (577) **NANO-MATCHA: NOVEL NANOVESICLES CARRYING EGCG FOR DRY EYE DISEASE**
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Dry eye disease (DED) is an ocular surface disease that exhibits inflammation and oxidative stress. Hyperosmolarity at the level of the tear film surface, uncontrolled levels of reactive oxygen species (ROS) and pro-inflammatory stress lead to apoptosis of epithelial cornea cells. Antiinflammatory agents (steroids and cyclosporine A), hormonal therapy, antibiotics, nerve growth factors, essential fatty acids are used as treatment options of DED. Current therapies attempt to reduce the ocular discomfort by producing lubrication and stimulating gland/nerve(s) associated with tear production, without providing a cure for dry eye. Besides, eyes drops are associated with poor drug bioavailability due to transient contact time and rapid washout by tearing. Thus, it is necessary to overcome these drawbacks.
The objective would be to develop nanovesicles made from *Halorubrum tebenquichense total* archaeolipids, which contain PGP-Me a ligand for scavenger receptors class A and bacterioruberin the high antioxidant carotein with the incorporation of green matcha tea extract (nano-matcha). Thus, nano-matcha would achieve and maintain remission of acute inflammatory episodes and reduce ROS at baseline levels for topical administration. EGCG, one of the main catechins extracts of green tea, provides an inhibitory effect on the inflammation and ROS some studies demonstrated that green tea extract reduces the expression of IL-1, IL-6 and TNF-α through inhibition of NF-kB activation, and suggested that green tea may be useful to prevent or ameliorate diseases associated with inflammatory cytokines overexpression and stress oxidative on corneal epithelial cells1,2 Firstly, catechins of green matcha tea (GMT) were extracted using ethyl acetate followed by washing citric acid to decaffeine3. The extraction yield obtained was 73,2 mg ± 4,9 g catechins/GMT. We showed the presence of EGCG, a highly fluorescent molecule at 353 nm4 in the extract using a spectrofluorometer. Lightless thin-layer chromatography (TLC) method using Chloroform:Methanol:Distilled Water (65:35:10, v/v/v) as the mobile phase was developed and revealed using UV-light and Iode fumes. RF values for EGCG and catechin standards were similar to the extract. Besides, HPLC method was developed using a C18 column and mobile phase composed of acetonitrile, acetic acid and water (6:1:193 v/v/v) to determine and quantify EGCG in the extract of catechins5. EGCG was the main component of the extract. Nanosized, monodispersed and negative z potential in EGCG nano-matcha were obtained. A lower inhibitory concentration providing 50% reduction of the DPPH radical (IC50) in nano-matcha (the activity enhanced by co-encapsulation) than EGCG was obtained. Onwards, anti-inflammatory and antioxidant activity of nano-matcha on corneal and macrophage cell lines and a 3D ocular model will be tested.
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376. (581) **NEBULIZABLE NANOSTRUCTURED ARCHAEOLIPID CARRIERS DELIVERING TOBRAMYCIN AND *THYMUS VULGARIS* ESSENTIAL OIL: ANTI-BIOFILM, ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY**
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Lung infections caused by *Pseudomonas aeruginosa* (Pa) in cystic fibrosis patients are persistent due to their ability to grow in biofilms, that protect bacteria from host defenses and chemotherapy. Pa infections, together with chronic airway inflammation, diminish the quality of life of patients who often require aggressive treatments that frequently fail to eradicate the infection. Thus, development of novel therapeutic strategies is urgent. We developed NAC eoT-TB, a formulation that combines the broad spectrum antibiotic tobramycin (TB) and *Thymus vulgaris* essential oil (eoT) -which has anti-inflammatory and antioxidant properties- delivered by nanostructured archaeolipid carriers (NAC). NAC eoT-TB (195 ± 15 nm, -39 ± 4 mV), were structurally stable after nebulization, an important feature for an efficient delivery to the lungs by inhalation route.
The use of essential oil as pharmaceutical ingredient can be limited by its high volatility. However, the incorporation of eoT into NAC eoT-TB decreased its evaporation rate 1.7 times after 24 h at room temperature. On the other hand, while the viability of bacteria in established Pa biofilm was reduced in a similar degree by NAC eoT-TB and free TB, the former significantly decreased the biomass of the biofilm (86 ± 11% vs 39 ± 59%, respectively, at 18 μg/ml of TB). At these concentrations, NAC eoT-TB did not significantly reduce the

viability of human alveolar basal epithelial cells (A549), neither human macrophages (THP1). Also, A549 cells stimulated with 1 μg/ml LPS showed an improved antioxidant activity at 182 μg/ml eoT delivered by NAC eoT-TB which reduced 96 ± 7% of ROS generation, whereas free eoT reduced only 61 ± 51%. In addition, the incubation of THP1 with NAC eoT-TB significantly diminished the release of proinflammatory cytokine TNF-α. In summary, these results indicate NAC eoT-TB has improved antibiofilm, antioxidant and anti-inflammatory activity compared to free eoT and TB, and therefore it may be a successful approach to combat Pa infections.

377. (591) **NANOENCAPSULATION OF BETANIN PURIFIED FROM RED BEETROOT EXTRACTS IN CHITOSAN MATRIX BY IONIC GELATION METHOD**

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Betanin (BET: betanidin 5-*O*-β-glucoside) is a pigment with powerful antioxidant properties but labile to harsh environmental conditions. The radical scavenging activity of BET has been associated with several health benefits including anticancer properties. BET is also the most abundant component of the processed beetroot (*Beta vulgaris*) juice. Chitosan (CS) is a food grade, biocompatible and non-toxic biopolymer used as encapsulation material by virtue of its reactive -NH_2 and -OH groups along the molecule. In this work we have purified BET from beetroot juice by means of size exclusion chromatography (sephadex LH-20) and encapsulated into CS by ionic gelation method. The purified BET fractions were analysed by TLC (Thin Layer Chromatography) and UV-visible spectroscopy and compared with standard commercial BET. Spectroscopy analysis showed that BET has a prominent presence in the purified fractions. From 51,5 ± 2,6 mL of juice/100 g of beetroot we obtained 54 ± 13 mg of purified BET/mL of juice. Purified BET was lyophilized and incorporated into CS solutions (0,7 % w/w, pH 5.5) by using tripolyphosphate (TPP; 0,6 % w/w, pH 9.0) as crosslinking agent. A maximum encapsulation efficiency (42 ± 6 %) was reached when using the 3:1 CS/TPP relation (w/w). The mean size of nanoparticles (NP) was 239 ± 17 nm assessed by DLS (Dynamic light Scattering), with a low polydispersity index (0,322 ± 0,004) and was dependent on a previous ultrasonication step to prevent aggregation. The stability in water (pH 5.0) of NP-loaded BET was observed throughout several days. On the contrary, a fast liberation of BET occurred in the PBS buffer (pH 7.0), showing differential retention properties depending on vehicle and pH conditions. These preliminary results show that BET-CS-TPP NP could be suitable nanocarriers for BET protection and delivery. The maintenance of BET antioxidant properties after incorporation into NP and their biomedical applications should be determined further.

378. (594) **EVALUATION OF DOXYCYCLINE-FUNCTIONALIZED AUNPS AS A MITOCHONDRIA-TARGETED THERAPY FOR MELANOMA RESISTANT CELLS**

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Melanoma, the most lethal form of skin cancer, is a highly aggressive tumor whose incidence is increasing. Cancer stem cells (CSCs) are responsible not only for the tumor initiation, but also for its progression and recurrence. CSCs are quiescent cells that overexpress mitochondrial related proteins, thus disruption of mitochondrial activity may represent a new approach for the elimination of CSCs. Nanotechnology improves cancer therapy and reduces its adverse effects by specifically delivering drugs to tumor tissues.

In this work we present a nanotechnology strategy for targeting CSCs, using gold nanoparticles (AuNPs) conjugated with doxycycline (DOXY), an inhibitor of mitochondrial biogenesis.

Initially, the in vitro effects of free DOXY were studied using radiosensitive (A375) and radioresistant (A375-G10 and Mel-J) melanoma cell lines. All cell lines displayed a significant reduction on cell viability and metabolic activity at concentrations above 25 μM of DOXY, being the radioresistant cells more affected. Also, radiosensitization was evaluated by clonogenic assays in A375- G10 cells irradiated with a gamma rays source (137Cs). At 1 Gy survival fraction was reduced from 60% on control to 28% on those pre-treated with DOXY.

We propose different strategies to produce the nanoconjugates. Firstly, DOXY- AuNPs were synthesized using DOXY as a reducer and stabilizer agent in alkaline conditions. Secondly, AuNPs were obtained by a traditional Turkevich method, and a polymer-DOXY conjugate was attached. Hydrodynamic diameter measured by DLS was 15 nm for DOXY-AuNPs and 36 nm for traditional synthesized AuNPs, with a strong absorption band by UV-Visible spectroscopy at 520 nm and 525 nm, respectively.

We are currently working on determining the effects of these nanosystems on proliferative activity and clonogenic capacity of the cell lines. These preliminary results suggest the possibility of controlling melanoma resistant cells using a mitochondria targeted therapy.

379. (595) **T908 POLYMERIC MICELLES IMPROVED THE UPTAKE OF SGC8C-ALEXA IN MICE BEARING LYMPHOMA TUMOR INDUCED USING A20-CELLS THAT OVEREXPRESS PTK7 RECEPTOR**

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Aptamers are oligonucleotides that have the characteristic of recognizing a target with high affinity and specificity. The greatest challenges include to overcome the degradation by endo- and exo-nucleases and to increase its blood circulation. Sgc8c is an aptamer that recognizes the PTK7 receptor, described as a tumor target that has been previously studied by our research group, as a molecular imaging probe. In this work, we studied the optimization of Sgc8c delivery, as well as its stability, using linear and branched polymeric micelles (PMs), based of PEO-PPO-PEO copolymers: poloxamer F127® and poloxamines T1307® and T908®. For it, Sgc8c-(CH2)6-NH2 was conjugated to Alexa647 fluorophore (Sgc8c-ALEXA, probe) and its co-association with different PMs was exhaustively analyzed. The majority size-populations of Sgc8c-ALEXA-PMs were between 11 and 32 nm at 25ºC by *Dynamic Light Scattering* (DLS). Zeta-potentials were moderately negative in all cases. TEM and AFM data fitted well with DLS and showed nanometric sizes with marked different morphology between free- and co-associated probe-PMs. Finally, the biodistribution and pharmacokinetic studies in BALB-c mice bearing lymphoma tumor induced using A20-cells, revealed an enhanced circulation time and marked uptake into tumor for Sgc8c-ALEXA-T908 PMs. All data obtained from this work, suggested that PEO- PPO-PEO based PMs and, more specifically those PMs made of high molecular weight copolymer (~25 kDa), such as T908-based ones, are good candidates to improve the phar-

macokinetics and the tumor uptake of Sgc8c-ALEXA in models of mice bearing tumor.

NEFROLOGÍA

380. (054) **THE HORMONAL CONTEXT DETERMINES THE RESPONSE OF THE IMMUNE SYSTEM TO HIGH SODIUM DIET IN A RAT MODEL OF SALT-SENSITIVE HYPERTENSION**

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We work with an animal model of salt sensitive hypertension (HSS) in which the absence of sex hormones due to ovariectomy (oVx) in adult Wistar rats produces failures in renal regulatory mechanisms that leads to the development of HSS when they receive a high sodium diet. Innate and adaptive immunity have been linked to HSS. Emerging evidence supports the concept that immune cells are activated promoting inflammation and exacerbating disease.
Aim: to study in this HSS model if the increase in pressure is accompanied by the immune system (IS) cells infiltration in renal tissue, and to evaluate the expression of the CD4 + and CD8 + in peripheral blood lymphocytes (PBL) of intact female (IF) and oVx rats with a normal (NS) or high sodium (HS) diet.
Methods: At 60 days of life, half of the rats were ovariectomized, and at 145 days IF and oVx rats were divided into NS (0.24% NaCl) or HS (1% NaCl in drinking water) subgroups. At day 150 systolic blood pressure (SBP, tail-cuff method) was recorded and the animals were sacrificed. Previously, blood samples were taken for PBL separation. Expression of CD4 and CD8 was analyzed by Western Blot. Kidney histology was analyzed in hematoxylin-eosin stained sections, and common leukocyte antigen (CD45) was analyzed by immunohistochemistry.
Results: SBP (mmHg), IF NS: 126 ± 3.1, IF HS: 127 ± 7.1, oVx NS: 127 ± 3.0, oVx HS: 142 ± 7.3*.
* p <0.05 oVx HS vs all other groups. CD4 expression of PBL in IF HS is lower respect to IF NS (p <0.001), while in oVx HS it is higher respect to oVx NS (p <0.01). CD8 increased in only oVx HS respect to the other groups (p <0.05). The presence of CD45 was found only in the renal cortex of oVx HS rats.
The increase in CD4 and CD8 could indicate an activation and proliferation of IS in HSS promoting the infiltration of cells in key tissues. The response of the IS to the HS diet is different according to the hormonal context, which is a key point to understand the progression of the disease.

381. (230) **ROLE OF PURINERGIC SIGNALINGS IN MURINE MODELS OF DIABETIC NEPHROPATHY**

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We have previously reported that during an inflammatory or infectious stimulus, cells sense the hypoxic environment and release to the extracellular milieu large amounts of ATP, which functions as a pro-inflammatory metabolite driving the nature of the immune response. The purinergic ectoenzymes CD39 and CD73, metabolize extracellular ATP into adenosine, an anti-inflammatory mediator. These purinergic pathways have been studied in the context of metabolic diseases related to kidney damage, but their role in the type II diabetes mellitus (T2DM) nephropathy have not been completely established.
The objective of the present work was to study the role of CD73 in the development of diabetic nephropathy employing mice devoid of CD73 activity (CD73KO) and controls B6 (WT). To this aim, we developed two murine models of T2DM, feeding the mice with medium fat diet (17%) and water 20% fructose (model A) and high fat diet (60%) (model B) for 22 weeks, combined with a single dose of streptozotocin (65-100mg/kg).
In both models, at the end of treatment, blood glucose levels were higher than 190mg/dl; but, in model A KO animals gained significantly less weight compared to the WT (p=0,0043). In model A, both mouse strains showed comparable renal damage parameters, such as a decrease in diuresis and a loss of more than 50% of glomerular filtration rate as well as significantly higher values of microalbuminuria (p_{WT}=0,0327, p_{KO}=0,0500), and plasma creatinine (p_{WT}=0,0159, p_{KO}=0,0159). In contrast, in model B, diabetic KO mice show signs of improvement with a significant correction of diuresis (p=0.0346), albuminuria (p=0.0303) and proteinuria (p=0.0043) compared to diabetic WT. Furthermore, plasma and urinary creatinine from diabetic KO mice did not show significant differences respect to healthy KO (p=0.556, p=0.9667). In conclusion, the results suggest that CD73 activity could have a protective role in the development of diabetic nephropathy in a diet dependent manner.

382. (401) **EFFECTS OF A SUBLETHAL DOSE OF SHIGA TOXIN 2 ON RENAL FUNCTION IN ADULT FEMALE RATS**

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Shiga toxin-producing *Escherichia coli* causes acute renal failure and Hemolytic Uremic Syndrome. We have previously shown that an intraperitoneal (i.p.) injection of a sublethal dose of Shiga toxin 2 (Stx2) produced polyuria, increase in serum creatinine, renal necrosis and induced tubular cell proliferation in female rats with water *ad libitum*. Vimentin, is expressed in metanephric mesenchyme but not in tubules of mature kidneys, and it is reexpressed in renal epithelial cells during recovery from acute injury. The aim of our work was to study the effects of a sublethal dose of Stx2 on renal function, urine output, and tubular epithelial regeneration in female Sprague-Dawley rats. Rats (250g) were i.p. inoculated with 0.5ng Stx2/g body weight (bw) (St) or diluent (Ct). At 4 days post-injection (dpi), rats were placed in metabolic cages during 16 h under water deprivation. Blood and urine samples were collected to determine urinary flow (U_v), serum creatinine (S_{Cr}) and serum urea (S_{urea}). Rats were then euthanized and kidneys were removed to evaluate vimentin and Ki67 (proliferation marker) expressions by immunofluorescence. St rats showed a significant increase in U_v (9 ± 0.8 μl/min x 100g bw), S_{Cr} (8.9 ± 0.68 mg/l) and S_{urea} (54.5± 2.84 mg/dl), compared with Ct rats (U_v: 3 ± 0.3 μl/min x 100g bw; S_{Cr}: 6.5 ± 0.69 mg/L; S_{urea}: 36.5 ± 2,64 mg/dl) (p<0.01). A significant increase in Ki67 and vimentin expression was observed in renal medulla tubular cells of St rats with respect to Ct rats (p<0.01). About 18% of vimentin positive tubular epithelial cells colocalized with Ki67. In conclusion, Stx2 sublethal dose produced alterations on renal function and polyuria, similar to what we have previously observed in rats with water *ad libitum* at 4 dpi. On the other hand, vimentin and Ki67 expression suggest the reparation of the tubular epithelia after moderate damage caused by Stx2.

NEUROCIENCIAS

383. (033) **VBM ANALYSIS OF IPSILATERAL ANC CONTRALATERAL BRAIN STRUCTURES INVOLVED IN THE EP-**

ILEPTOGENIC ZONE AND CLINICAL CORRELATION WITH IMPULSIVITY AND DEPRESSION IN PATIENTS WITH DRUG-RESISTANT EPILEPSY

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Objective: The aim of this work was to analyze the epileptogenic zone (EZ) and their correlation with brain anatomical structures, and impulsivity and depression in patients with drug-resistant epilepsy.
Methods: Patients with drug-resistant temporal (TLE) and frontal (FLE) epilepsies were included. EZ and laterality were confirmed by VEEG. Psychiatric evaluation (SCID-I and -II, BDI-II, BIS-11, GAF) was performed. 3T MRI (T2 coronal slices and 3-mm FLAIR) images were obtained. SPM12 and CAT12 were used for volume calculation and image preprocess. For every patient and brain region, a z-score was calculated based on the grey matter volume relative (GMRV) to total intracranial volume. Mann Whitney and Spearman correlation were performed.
Results: 36 TLE and 7 FLE patients were included. Ipsilateral EZ showed lower GMRV values comparing contralateral structures in TLE and FLE patients ($p<0.05$). In TLE, impulsivity positively correlated with GMRV in ipsilateral entorhinal cortex (EC) ($p<0.05$). Higher BDI-II scores positively correlated with GMRV in EC, fusiform gyrus ($p<0.05$).
In ELF, impulsivity correlated positively with GMRV in both amygdalas and contralateral transverse temporal gyrus and rectus gyrus ($p<0.05$). Higher BDI-II scores were associated with higher GMRV in ipsilateral anterior insula and contralateral EC, gyrus rectus and superior frontal gyrus ($p<0.05$).
Conclusions: These findings could indicate cortical changes which could be involved in both the pathophysiology of behavioral symptoms and drug-resistant epilepsy.

384. (045) **PARKINSON'S DISEASE (PD) IS CHARACTERIZED BY THE PROGRESSIVE LOSS OF DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA (SN) AND A DECREASE IN DOPAMINE (DA) IN THE STRIATUM (CPU) WHICH MANIFESTS IN MOTOR BEHAVIOUR. PD IS PREDOMINANTLY MALE, WHICH IS WHY IT IS PROPOSED THAT FEMALE HORMONES HAVE AN IMPORTANT NEUROPROTECTIVE ROLE**

On postnatal day 60 (D0) rats were injected with 6-hydroxydopamine (6-OHDA) or vehicle (O) in left CPu. From D7-D17, they received a 10-day treatment with 17β-estradiol (E=0.1 µg/kg/day s.c) or V. Groups: HP (6-OHDA+V;n=18); HP+E (6-OHDA+E;n=18); E (O+E;n=15); C (O+V; n=15).
Motor behavioural activity was evaluated for RT and OFT on D27 after Amph and on D57 after Apo; on D60 all animals were euthanized for TH IHQ and DA/DOPAC evaluation by HPLC.
For OFT+Amph, HP decreased total distance travelled and average speed, also increased freezing and time in the corners, $p<0.05$. HP+E significantly improved movement parameters, $p<0.05$.
On OFT+Apo groups C, E and HP+E move more than HP, $p<0.05$. E and HP+E remained less time in the corners than the HP, $p<0.05$. HP increased freezing episodes and diminished ambulatory activity while the other experimental groups exhibited random movements.
In RT, compared to the other groups, HP increased ipsilateral turns ($p<0.05$) after Amph and increased contralateral rotations after Apo, $p<0.01$.
While 6-OHDA administration diminishes DA neurons, E attenuates dopaminergic loss. TH neurons were less for HP in comparison to the other groups, $p<0.0001$. E improves DA arborization and staining intensity and distribution.
In left CPu, the DOPAC/DA ratio for HP showed a drop in the functionality of the dopaminergic system. This detriment was statistically significant compared to the other groups, $p<0,0001$.
After 6-OHDA injury, E could restore ambulatory activity, physiological properties and morphology of neurons, enhance synapses and DA release.

385. (050) **APOPTOSIS SIGNALING PATHWAYS DUE TO AFTER-EFFECTS OF ACUTE ETHANOL EXPOSURE IN BRAIN CORTEX**

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Binge-drinking is the most common alcohol-related disorder whose direct consequence is known as alcohol hangover (AH). This one is defined as a combination of mental and physical symptoms experienced the day after a single episode of heavy drinking, starting when blood alcohol concentration (BAC) approaches zero. We demonstrated that AH induced strong oxidative stress, mitochondrial dysfunction and alterations in nitric oxide (NO) metabolism through the impairment of NMDAR/PSD-95/nNOS pathway in brain cortex synapses.
The aim of the present work was to study apoptotic signaling pathways in brain cortex at the onset of AH. Swiss male mice received an i.p. injection of ethanol (3.8 g/kg body weight, AH group) or saline (control group) and were sacrificed 6h afterwards (BAC=0). Determinations were conducted in mitochondria and lysates from brain cortex.
Impairment of calcium handling and MPT induction were observed in AH mitochondria ($p<0.05$) together with a 21% increase and 18% decrease in Bax and Bcl-2 protein expression ($p<0.05$) respectively. Moreover, a 4-fold decrease in cytochrome *c* mitochondria/cytosol ratio was found due to AH ($p<0.01$). In addition, citrate synthase, a mitochondrial enzyme marker, was 40% decreased in AH mitochondria ($p<0.05$). Interestingly, AIF protein expression was unchanged by AH. Caspase 3 and 9 activity and expression were increased by approximately 30% and 18-19% respectively ($p<0.05$) in brain cortex lysates from AH. In addition, although non-significant, p53 was found 14% increased in AH brain cortex ($p=0.057$). Lastly, SIRT-1, a histone deacetylase which promotes mitochondrial biogenesis was 63% decreased ($p<0.01$).
In conclusion, alcohol after-effects could result in the activation of intrinsic apoptotic pathways due to mitochondrial dysfunction and oxidative stress. Also, non-mitochondrial apoptotic processes can be triggered during AH due to the blockage of calcium entry at synapses after disruption of NMDAR/PSD-95/nNOS pathway.

386. (051) **EFECTO DEL ESTRÉS PRENATAL Y CRÓNICO: ¿PODRÍA EL ENRIQUECIMIENTO AMBIENTAL MEJORAR LAS ALTERACIONES INDUCIDAS POR UN MODELO DE ESTRÉS DE DOS IMPACTOS?**

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Existe evidencias de que el estrés en las primeras etapas del desarrollo puede causar alteraciones que resultan en disfunciones y/o vulnerabilidad a enfermedades psiquiátricas o fisiológicas en la edad adulta. El estrés prenatal (PS) se ha asociado con alteraciones conductuales a largo plazo en la descendencia, mientras que se ha demostrado que el estrés crónico (CS) tiene efectos deletéreos en la edad adulta. Este trabajo tiene como objetivo determinar, en un solo modelo experimental, las consecuencias del estrés pre y posnatal sobre la conducta y si el ambiente enriquecido (EE) podría revertir los efectos inducidos por la exposición a PS y CS en la edad adulta.

Se utilizaron como modelo de PS ratones hembra BALB / C preñados sometidos a restricción de movimiento durante 2 horas al día, desde el día 14 de preñez hasta el parto. Como modelo CS, las hembras de ratones de 2 meses nacidas de madres se sometieron a restricción de movimiento 2 horas al día durante 3 semanas. Los animales estuvieron expuestos a EE desde el día postnatal (DP) 21 a DP 90. Nuestros resultados mostraron que los grupos PS, CS, PS + CS presentan modificaciones en la memoria contextual y conductas relacionadas con la ansiedad que dependen del género. La exposición a EE durante la adolescencia y la edad adulta revirtió los cambios observados. Sin embargo, la combinación de PS + CS presenta un efecto sinérgico que evita que EE revierta los cambios. Sería necesario estudiar la existencia de nuevos tratamientos para los casos en los que se presenten ambos tipos de estrés

387. (052) **LONG-LASTING EFFECTS OF PRENATAL AND CHRONIC STRESS: COULD ENVIRONMENTAL ENRICHMENT IMPROVE ALTERATIONS INDUCED BY A TWO-HIT STRESS MODEL?**

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There is considerable evidence that stress in the early stages of development can cause disturbances that result in dysfunctions and/or vulnerability to psychiatric or physiological illnesses in adulthood. Prenatal stress (PS) has been associated with long-term behavioral alterations in the offspring, while chronic stress (CS) has been shown to have deleterious effects in adulthood. This work aims to determine, in a single experimental model, the consequences of pre and postnatal stress on behavior and whether the enriched environment (EE) could reverse the effects induced by exposure to PS and CS in adulthood.
Pregnant female BALB / C mice subjected to movement restriction for 2 hours a day, from day 14 of pregnancy to delivery, were used as a model of PS. As a CS model, 2-month-old female mice born to mothers were subjected to movement restriction 2 hours a day for 3 weeks. Animals were exposed to EE from postnatal day (PD) 21 to PD 90. Our results showed that the PS, CS, PS + CS groups present modifications in contextual memory and anxiety-related behaviors that depend on gender. Exposure to an EE during adolescence and adulthood reversed the observed changes. However, the combination of PS + CS presents a synergistic effect which prevents EE from reversing the changes. It would be necessary to study the existence of new treatments for cases in which both types of stress are present

388. (056) **MODULATION OF INFLAMMATORY MEDIATORS OF MATERNAL IMMUNE ACTIVATION BY RESVERATROL**

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Maternal immune activation (MIA) produces structural, metabolic and epigenetic changes in the fetus associated with increased risk for various neurodevelopmental and neurodegenerative disorders. The hallmark of brain neuroinflammation is the activation of microglia, one of the main effectors of the innate immune response in the CNS. Excessive microglial activation leads to CNS damage due to excessive production of pro-inflammatory mediators, like interleukins, MMP, PGE2, NO and ROS.
It has been demonstrated that resveratrol has anti-inflammatory, antioxidant and epigenetics properties that translate into neuroprotective effects in adults.
Objectives: To study the possible neuroprotective effects of resveratrol in the fetal CNS in a model of MIA induced by bacterial lipopolysaccharide (LPS) and to determine the pro-inflammatory mediators involved in the modulation of the response.
Resveratrol was administered to Balb/c females on gestational day 15, which were then exposed or not to LPS. After LPS administration, amniotic liquid was collected to evaluate activity of MMP2 and MMP9. Additionally the brain of the offspring were collected to evaluate the expression of two inducible enzymatic pathways (COX-2 and iNOS) that produce mediators (prostaglandins and nitric oxide) known to cause inflammation and to evaluate the acetylation of H3 and H4 since DNA acetylation is associated with the regulation of inflammatory gene expression.
Results:
Preliminary results show that the LPS-triggered MIA induces COX2 expression in the fetal brains ($p < 0.05$ respectively) while resveratrol prevents this effect. Regardless to the activation of iNOS and the acetylation of H3 and H4 we couldn't see any significant variation between the different treatments.
On the other hand, LPS we observed that LPS decreases MMP2 while Resveratrol induces MMP9 in the amniotic fluid.
Conclusion: Maternal immune activation increases COX2 expression in fetal brains.

389. (082) **GENISTEIN AMELIORATES NEUROINFLAMMATION IN THE HIPPOCAMPUS OF MALE SPONTANEOUSLY HYPERTENSIVE RAT (SHR)**

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Hippocampal neuropathology is a recognized feature of the spontaneously hypertensive rat (SHR). Previous studies have found abnormalities in the hippocampus of SHR consisting of decreased neurogenesis and increased astroglial and microglial reactivity. These abnormalities are reversed by exogenous administration of estradiol, an active neuroprotective and hypotensive factor. Also, these hippocampal alterations are associated with cognitive impairment. Genistein (GEN) is a phytoestrogen which binds to ERβ and GPER and it is known to have neuroprotective actions. We have recently demonstrated that GEN exerts neuroprotective actions, increases neurogenesis, decreases astrogliosis and also improves hippocampal dependent memory.
To further investigate if this phytoestrogen exerts neuroprotective effects in hypertensive encephalopathy, we focused on neuroinflammation. We treated 5 month old SHR (BP>180 mm Hg) during 2 weeks with 10mg/kg daily s.c injections of GEN. We measured the expression of IBA1+ microglia in the CA1 region and hilus of the hippocampus by immunocytochemistry and classified microglia according to its morphology. Furthermore, we evaluated the expression of pro-inflammatory (COX-2) and anti-inflammatory (TGFβ) factors by real-time PCR. We found that while ramified microglia predominated in normotensive rats, SHR presented an increased proportion of the hypertrophied phenotype. GEN produced a shift in microglial phenotypes towards a ramified type. Furthermore, GEN treatment decreased expression of the pro-inflammatory factor COX-2 and increased the expression of the anti-inflammatory factor TGFβ
Our results indicate that GEN was able to exert direct neuroprotective actions ameliorating neuroinflammation in the hippocampus of SHR. These data, along with previous data of GEN improvements on neurogenesis, astrogliosis and memory deficits, opens an interesting possibility for proposing this phytoestrogen as an alternative therapy in hypertensive encephalopathy.

390. (088) **NEUROGENESIS IS REGULATED THROUGH THE CLASSICAL PROGESTERONE RECEPTOR DURING AGEING.**

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A new role has emerged for progesterone after discovering its potent actions away from reproduction in both the central and the peripheral nervous system. Progesterone effects beyond reproduction include myelin formation, neuroprotection, anti-inflammatory actions and neurogenesis regulation. Despite the fact that the precise mechanism of Progesterone actions is controversial, most studies support a role of the progesterone classical receptor (PR) in those actions. Given that during ageing hippocampal neurogenesis decreases, oligondendrocytes die and neuroinflammation is enhanced due to reactive gliosis, we decided to study whether progesterone can regulate these parameters in the aged PR knock out mouse (PRKO). This strain is depleted of both PR isoforms (PRA and PRB), as shown by postnatal genotyping. Five month, one and two year old PRKO and wild type mice (WT) were analysed by immunohistochemistry. Neurogenesis was assayed by identifying doublecortin (DCX) positive immature neurons in the subgranular zone of the dentate gyrus of the hippocampus using stereologic methods. In the course of the time analysed, neurogenesis decreased as expected therefore, the lowest number of DCX + cells were found in the 2 year old mouse ($p<0.0001$, 2 year vs 1 year and 5 month old mice). Regarding the PR receptor, there was a decrease in the production of new neurons in the PRKO mouse at all ages analysed ($p<0.05$ PRKO vs WT for 5 month, $p<0.001$ PRKO vs WT for 1 and 2 year old mice). Our results suggest that Progesterone is required during adulthood and ageing to support an age-dependent level of neurogenesis. Furthermore, Progesterone effects on this parameter is acting through its classic nuclear receptor.

391. (095) **REGULATION OF SYNAPTOSOMAL 2-ARACHIDONOYLGLYCEROL HYDROLYSIS BY CANNABIS EXTRACTS DURING AGING**

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The availability of the main neuroprotective endocannabinoid, 2-arachidonoylglycerol (2-AG), was found to decrease in rat cerebral cortex (CC) synaptosomes (syn) during aging. It was also demonstrated that 2-AG metabolism can be modulated by its own receptors. Moreover, phytocannabinoids (PC) present in Cannabis extracts (CE), have been proposed as neuroprotective agents and are being used in several studies to treat neurodegenerative processes. The aim of this study was to evaluate if THC present in CE could modulate 2-AG hydrolysis by monoacylglycerol lipase (MAGL). To this end, delta-9-tetrahydrolcannabinol (THC) enriched CE were obtained from Cannabis Y Griega female flowers, previously grinded and heated 115°C for 40 min. Extractions were performed by adding 5% (w/v) ethanol, vortexed, sonicated and agitated. The mixture was centrifuged and the supernatant was evaporated under stream of N_2. THC, cannabidiol (CBD) and cannabinol (CBN) were quantified by HPLC. CC syn from adult (4-6 months) and aged (24-26 months) rats were isolated by differential centrifugation and purified in ficoll gradients. MAGL activity was assessed by incubating syn with either THC enriched CE (10^{-3}-50 µM THC) or 1 µM pure THC, and [^{3}H]-MAG, simultaneously. It was observed that CE containing 1 µM THC decreased MAGL activity in aged ($p=0.0166$) but not in adult syn ($p>0.05$). However, 1 µM pure THC failed to modulate 2-AG hydrolysis ($p>0.05$). This findings, could suggest that THC enriched CE could attenuate the deregulation of 2-AG metabolism observed in aging, and that this effect could only be generated when syn are exposed to the PC in the presence of the entire CE.

392. (122) **MOLECULAR ALTERATIONS CAUSED BY CHRONIC COCHLEAR DEPOLARIZATION IN A MOUSE MODEL OF HEARING LOSS**

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The voltage-gated potassium (K+) channel KCNQ4 is the main responsible for the extrusion of the K+ that enters the cochlear sensory cells upon sound stimulation. Besides, outer hair cells (OHC) excitability is under control of the efferent neurons of the Medial Olivocochlear (MOC) system. In response to overstimulation, MOC cholinergic neurons activate the calcium-induced K+ channels BK and SK2, which extrude K+ out of the cell repolarizing the membrane. Intracellular accumulation of K+ leads to a chronic depolarization that may damage hair cells causing hearing loss (HL). KCNQ4 activity impairment is the main cause of DFNA2, a non-syndromic progressive HL. Using a mouse model lacking Kcnq4 (*Kcnq4-/-*), we reported that OHC death begins at the basal turn progressing to the apex in 3-6-week-old (W) animals. We hypothesized that the KCNQ4 absence causes MOC chronic overstimulation leading to activation of death pathways. Using immunofluorescence (IF), we evaluated the MOC terminals and observed a lower synaptic density and mislocalization of the efferent terminals contacting OHC in 4W *Kcnq4-/-* mice. In addition, we analyzed by qPCR the gene expression of the efferent components located in the MOC terminals. We detected a ~3.5-fold decrease in the mRNA expression of the nicotinic receptor α10 subunit with no changes in the α9 subunit, and a ~8-fold decrease in the mRNA expression of BK and SK2 in 4W *Kcnq4-/-* animals. Finally, we studied the possible pathways involved in OHC death. By IF, we found an increase of cleaved-caspase 3 expression in the OHC at the basal turn and gene expression analysis by qPCR revealed that the pro-apoptotic *Bax* transcript was upregulated while anti-apoptotic *Bcl2* was downregulated in *Kcnq4-/-* mice. These results demonstrate an alteration of the efferent transmission in OHC that could contribute to the activation of the apoptotic pathway driving to OHC death.

393. (123) **EPIGENETIC MECHANISMS UNDERLYING ASTROGLIAL HETEROGENEITY IN REACTIVE ASTROGLIOSIS: TARGETING CHROMATIN REMODELERS AS A POSSIBLE THERAPY TO REDUCE DAMAGE AFTER BRAIN INJURY**

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Astrocytes respond to brain injury through a phenomenon called reactive astrogliosis in which a pro-inflammatory and pathological subpopulation of astrocyte has been described, capable of promoting neuroinflammation and neuronal death. Astrocyte pathological conversion with a pro-inflammatory gain of function involves dramatic and stable transcriptomic changes, probably following activation of transcription factor NF-kB. NF-kB interacts with chromatin remodelling enzymes and recruits them to regulatory regions of target genes promoting epigenetic changes in other cell types.
We aim to address the epigenetic mechanisms that are associated with NF-kB activation in reactive astrocytes that might lead to the establishment of an astrocyte pathological identity.
Using immunofluorescence microscopy and PCR analysis in primary cultures of mouse cortical astrocytes with different microglia abundance and exposed to pro-inflammatory stimulus LPS (Lipopolysaccharide), we observed that LPS significantly promoted: 1) Sequential NF-kB activation in microglia>>astrocytes together with morphological and transcriptional changes, 2) A variable intensity of initial NF-kB activation in astrocytes depending on microglial abundance and the release of microglial soluble factors and 3) A microglial-dependent increase in gene activating histone marks H3K9K14ac and H3K27ac and a decrease in the repressive mark H3K9me3. *In vivo* brain ischemia recapitulated the increase in H3K27ac specifically in reactive astrocytes from ischemic penumbra and inhibition of histone deacetylases exacerbated astrogliosis and brain damage.
Our results showing changes in histone mark abundance are highly indicative of chromatin remodeling events in a subpopulation of pro-inflammatory reactive astrocytes. Such epigenetic mechanisms may represent plausible therapeutic targets to reduce astrocyte pro-inflammatory phenotype, neuroinflammation and neuronal loss after brain injury.

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394. (125) **SPLENECTOMY: SHEDDING LIGHT ON THE SPLEEN-BRAIN INFLAMMATORY COUPLING IN A MODEL OF TEMPORAL LOBE EPILEPSY**

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A high percentage of patients with temporal lobe epilepsy (TLE), one of the most frequent neurological diseases, refer an initial precipitating event (IPE), such as complex febrile seizures during childhood, followed by a silent latency period (LP), until the onset of the chronic seizures phase. Using the lithium-pilocarpine rat model of TLE we previously showed that neurodegeneration, reactive gliosis and macrophages brain infiltration occur during the LP and that early interventions limiting immune activation during the LP increase epileptic threshold during the chronic phase (Rossi et al., 2013; 2017). The model consists of the administration of lithium-pilocarpine (127 mg/kg /30 mg/kg, 20h apart) to male Wistar rats. Animals develop Status Epilepticus (SE) that mimics human IPE and SE is limited to 20 min by 20 mg/kg i.p. diazepam. In this work, we have found morphological evidence of early spleen white pulp activation 1 day post SE (DPSE), while increased CD3+ and CD4+ lymphocytes in the choroid plexus, without changes in the gut-associated lymphoid tissue (GALT), followed by a decreased abundance of naïve lymphocytes in blood and spleen smears were found at 2-3DPSE.
In order to evaluate the relevance of the spleen-brain inflammatory coupling in the LP that follows pilocarpine-induced SE, we performed splenectomy (n=6) to 210-220 g male Wistar rats anesthetized with ketamine-xylazine (90 mg/kg/10 mg/kg) 1 week before the lithium-pilocarpine treatment. Sham animals were used as controls. Our loss of function studies showed that splenectomy decreased astrogliosis, neuroinflammation and deep cervical lymph nodes size at 7DPSE. We conclude that peripheral immune system is responding to specific brain-derived clues triggered by the SE and the spleen is involved in the modulation of neuroinflammation that follows SE. Supported by PICT 2017-2203; UBACYT; and FONCYT fellowship (PS).

395. (152) **UNDERSTANDING THE SPATIO-TEMPORAL DISTRIBUTION OF REACTIVE ASTROCYTES AFTER FOCAL BRAIN INJURY AND THE ROLE OF TOLL-LIKE RECEPTOR SIGNALING**

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Astrocytes are essential homeostatic cells. However, reactive astrocytes can suffer a pathological remodeling that is detrimental for neuronal survival. We have previously shown that Toll-like receptors (TLR) pathway play a key role in the astroglial proinflammatory phenotype induced by LPS. However; the spatio-temporal clues, the localization of pathologically remodeled astrocytes and whether the TLR/NFkB pathway is involved, are unknown facts. Using a model of traumatic brain injury (TBI) by performing a cortical stab-wound lesion (2 mm posterior and lateral to Bregma; 1 mm depth) in C57BL/6 mice; we evaluated the spatio-temporal distribution of six GFAP+ astroglial phenotypes described by Sholl analysis. At 1DPI astrocytes were similar to the non-injured hemisphere (type 0, resting astrocytes), while highly hypertrophied astrocytes (type V) significantly increased at 3-7DPI and were surrounded by degenerating neurons with altered NeuN distribution. At longer recovery times (14-28DPI) type V population was reduced concomitantly with reduction of altered neurons. Microgliosis was also present, starting at 1DPI and peaking at 7DPI. Interestingly, decreased expression of homeostatic AQP4 channel was determined at 7DPI compared to 3DPI. Also, we observed a decreased exploratory activity by open field assay at 7DPI compared to 3DPI. Stimulation of TLR pathway by administering LPS (5 mg/Kg i.p) resulted in a larger number of type V astrocytes with a population of proinflammatory C3+ astrocytes, increased microgliosis associated with a decreased survival neuronal at 7DPI. Loss of function achieved by administration of the chemical NFkB blocker sulfasalazine (150 mg/kg i.p) significantly reduced astrogliosis, microgliosis, and neuronal death at 7DPI. We conclude that an exacerbated astrogliosis is associated with neuronal impairment and behavioral deficit and the TLR/NFkB pathway is involved in this pathological astroglial conversion. Supported by PICT 2019-0851, UBACYT.

396. (168) **GENDER DIFFERENCES IN A FEBRILE SEIZURE MODEL IN YOUNG POSTNATAL RATS**

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A significant percentage of patients with Temporal Lobe Epilepsy report a history of complex febrile seizures during childhood. Using an animal model of hyperthermic seizures (HS), we have previously shown that male HS-exposed rats have lower convulsive threshold, microgliosis and moderate reactive gliosis with an atypical astrocytes distribution in the pyriform cortex (CP) and other brain structures, while female have higher convulsive threshold and exhibit lower reactive gliosis and microgliosis. Here, we extended the study by analyzing neuronal survival and the response of peripheral immune system. Rat pups (10-11 days old) were placed in a glass chamber, and their core temperature was raised by a regulated stream of heated air (39-42°C). Body temperature was measure at baseline, seizure onset and every 2 min during the seizures. Hyperthermic temperatures (39.5-42°C) were maintained for 30 min. The seizures onset was monitored behaviorally, and consisted of an acute sudden arrest of hyperthermia-induced tonic freeze postures and occasional oral automatism (biting and chewing) and often body flexion. Rats were then placed on a cool surface, monitored for 5 min before being returned to their mothers. At 35 days old rats were deeply anesthetized, fixed and brains and spleen processed for histological techniques, immunohistochemistry and morphometrical studies. We observed that there was a significant increase in the proinflammatory phenotypes of Iba-1+ microglia in males while females showed a decrease in the area occupied by Iba-1+ cells. Cortical neurons presented altered NeuN+ profiles in layer II of CP, being more prominent in females. Both sexes showed an activation of the white pulp, evidenced by an intense disorganization of Malpighi corpuscles. Our results strongly suggest that males are more susceptible to HS exposure and this could be related to their future susceptibility to develop epilepsy. Supported by PICT2017-2203 and UBACYT

397. (169) **PROGRESSION OF REACTIVE ASTROGLIOSIS IN MALE AND FEMALE HETEROZYGOUS A RAT MODEL OF ALZHEIMER DISEASE (AD)**

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Astrocytes are highly heterogenic, and pathological gain-of-function occurs in different brain diseases. We previously determined that highly complex astroglial phenotypes are associated with neurotoxic-proinflammatory astrocytes. Using the AD rat model McGill-R-Thy1-APP that expresses the human amyloid-beta precursor protein (AbetaPP) with Swedish and Indiana mutations, we studied by immunohistochemistry the progression of reactive astrogliosis and the accumulation of human Abeta (hAbeta) in male and female heterozygous transgenic rats of 7 and 13 months old (7mo; 13mo). Ex-vivo cultures of transgenic or wild type hippocampal explants were also used. Our results showed that transgenic rats have intracellular hAbeta accumulation at 7mo, while extracellular hAbeta plaques occur at 13mo. Significant differences in hAbeta accumulation between males and females were not observed. Interestingly, reactive astrocytes were present in the 7mo animals and image analysis showed

statistically significant increased area of astrocytes, which persisted into the 13mo group. The reactive gliosis induction at 7mo animals was confirmed in the ex-vivo cultures that showed a significant increase in stellated GFAP+ astrocytes. Sholl analysis showed that astroglial profiles are not statistically different between transgenic and wild type males or females at 7mo. However, at 13mo Sholl analysis defined three different astroglial cell populations in male transgenic rats, with increasing complexity determined by their localization: plaque astrocytes>periplaque>distant. Interestingly, distant astrocytes recapitulated Sholl profiles of wild type animals and males showed increased complexity and size in their astrocytes. We conclude that the three different astroglial phenotypes may represent different cell populations and further studies will attempt to characterize the gene expression profile in these groups.
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398. (171) **HERPESVIRUSES AND THE RISK OF MULTIPLE SCLEROSIS DISEASES**

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Multiple Sclerosis (MS) is a demyelinating, chronic, autoimmune disease that affects the central nervous system (CNS). Is the leading cause of non-traumatic neurological disability among young adults and there is no definitive treatment for it. Its etiology is not absolutely clear, but *Herpesvirus* infection, Epstein Bar virus (EBV) in particular, has been suggested as an environmental factor associated with MS risk and severity. We sought to compare antibody (Ab) titers against EBV, Cytomegalovirus (CMV) and Varicella Zoster virus (VZV) between MS patients and healthy controls (HC) from Argentina and to characterize EBV polymorphisms associated with disease risk.
Serology was assessed in 146 MS patients and 129 HC by indirect ELISA (Vircell).
In a subset of MS cases, EBV EBNA-2 and vIL-10 genes were amplified and Sanger sequenced and RNAseq was performed in EBV+ and EBV- sorted B cells.
All MS patient and HC were IgG positive for EBV and VZV, while 60% of the HC and 71% of the MS patient were seropositive for CMV. A significant difference was found between HC and MS patients for EBV-VCA titer ($P<0.01$) and CMV Ab titer ($P< 0.05$). RNA-seq of cell-sorted EBV+ B cells showed down-regulation in IQGAP1 mRNA, a B cell proliferation regulator molecule, in comparison to EBV- B cells. Sequencing of EBNA-2 revealed a CTC insertion at nucleotide position 633 in 5/8 MS samples but not in HC (0/4). No MS specific polymorphisms were found in vIL-10 gene (12 MS samples vs 8 HC).
MS is a complex disease with strong interaction between genetic and environmental factors, and different *Herpesviruses* may have an effect on MS pathology, as suggested by differences in titers for EBV-VCA and CMV Ab, RNAseq results and an EBV EBNA-2 gene variant potentially linked to MS patients. Further work is needed to validate sequencing results and confirm this association.

399. (192) **DICER ACTIVATION REDUCES LPS-INDUCED PROINFLAMMATORY PATHOLOGICAL REMODELING OF ASTROCYTES IN VITRO**

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Astrocytes undergo molecular and morphological changes in response to acute injury or chronic pathologies of the Central Nervous System (CNS) in a process called reactive gliosis. Reactive astrocytes may suffer a pathological remodeling with proinflammatory gain-of-function that facilitates neurodegeneration. Strategies to prevent this conversion are important to prevent secondary neuronal death in different CNS pathologies. Repurposed drugs such as Metformin and Enoxacin have shown neuroprotective effects in different experimental models and, although their mechanisms of action are very different, they share the ability to induce miRNA processing enzyme Dicer. Here, we pre-incubated primary glial mixed cultures containing astrocytes and microglia for 24 h with Metformin (MET, 10-30 mM) or Enoxacin (ENO, 50 μM), and then we exposed them to 25 ng/ml Lipopolissacharide (LPS), a molecule that is a powerful inducer of astroglial pathological remodeling. Astroglial and microglial morphology were analyzed at 6 and 24 h using immunofluorescence microscopy and Image analysis. Nuclear localization of the p65 NFKB subunit was evaluated as a parameter of NFKB activation by immunostaining (p65/GFAP/DAPI or p65/Iba-1/DAPI). Our results showed that 30 mM MET or 50 μM ENO significantly decreased LPS-induced NFKB activation and reduced reactive gliosis in this in vitro paradigm. Although the effect was significative in both astrocytes and microglia, astrocytes showed a trend to be more sensitive to MET and ENO effects. Expression of proinflammatory cytokines (IL-1B, TNFa) was also significantly reduced by MET treatment. Although the detailed molecular mechanisms involved have not been elucidated yet, we speculate increased Dicer activity is probably expanding the repertoire of astroglial anti-inflammatory miRNA and future work will attempt to identify these miRNA expanded by MET and ENO treatments. Supported by grants PICT 2019-0851; PICT 2017-2203; UBACYT; PIP CONICET

400. (197) **HISTONE MODIFICATIONS AND CHROMATIN REMODELING AS AN EPIGENETIC MECHANISM MODULATING ASTROGLIOSIS IN BRAIN ISCHEMIA**

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Acute brain injuries such as stroke have great impact in developing countries like Argentina. Commonly, patients suffer a worsening in their pathological condition as a consequence of neuroinflammation, a cellular response in which astrocytes and microglia are major players. Astrocytes respond to injury through a phenomenon known as reactive astrogliosis showing functional and phenotypic changes that are stable in time. We have recently shown that pro-inflammatory stimulus with lipopolysaccharide leads to increased levels of acetylated histone 3 in astrocytes suggesting that chromatin remodeling events might modulate reactive pro-inflammatory phenotype.
In this work we aim to study possible changes in histone modifications at early time points after brain ischemia since such events may "prime" astrocytes for acquisition of specific reactive pro-inflammatory phenotype. We exposed Wistar male adult rats to a unilateral ischemic brain injury by cortical devascularization and analyzed abundance of histone modifications in astrocytes using fluorescence microscopy and labelling with specific antibodies to detect astrocytes (anti-GFAP) and acetylated histone 3 (anti-H3ac). We observed a significant decrease of acetylated histone 3 in the ischemic "core" region. A similar result was observed in immunoblots from primary cultures of astrocytes exposed to oxygen and glucose deprivation. Contrarily, penumbral astrocytes surrounding the ischemic core showed increased levels of histone 3 acetylation. Interestingly, broad histone deacetylase inhibition with valproic acid, immediately after onset of injury, promoted exacerbated astrogliosis and neuronal death at longer time points.
Our results indicate that reactive astrocyte epigenetic changes after brain focal ischemia are regionally-dependent and that overriding that fine tunned control with hyperacetylation by VPA increases neuroinflammation and expands neuronal death.
Grants: UBACYT, FONCYT, ISN-CAEN, APBIOTECH

401. (206) **BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATION ASSOCIATED WITH DIFFERENTIAL DIAGNOSES IN AGEING PATIENTS WITH LEUKOARAIOSIS**

AND ALZHEIMER DISEASE

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Previous results indicate that oxidative stress (OS) and damage (OD) are associated to the etiopathogenesis of neurodegenerative disorders. Leukoaraiosis (L) is related to the small cerebral vessel disease and mainly associated with aging, dementia, hypertension, cognitive impairment and Alzheimer disease (AD). The aim of this research is to evaluate whether OS and OD are associated with inflammatory response in L and AD patients. Biomarkers of OS, OD and inflammation were evaluated in 33 ageing patients: 17 with diagnosis of L (magnetic resonance imaging and brain computed tomography) and 16 with AD (neurological and cognitive tests), and healthy subjects (controls, n=17). The following parameters were assessed in plasma: protein oxidation measured as carbonyl groups (CO), phospholipid oxidation, measured as the content of thiobarbituric acid reactive substances (TBARS), interleukin 6 (IL-6), total antioxidant (TRAP) and protein content; and in red cells: protein content, superoxide dismutase and catalase activities. A 100% increase of TBARS was observed in L ($p<0.001$) and AD ($p<0.01$), whereas CO was increased 60% ($p<0.05$) and IL-6 (52%, $p<0.05$) in AD. Total hydrosoluble antioxidants (TRAP) and proteins were not affected in plasma. In red cells, the antioxidant enzyme activities of SOD was increased in both L (3-fold, $p<0.001$) and AD (2.5-fold, $p<0.01$), while in L catalase increased 3-fold ($p<0.05$) and protein content decreased 24% ($p<0.01$). These results indicate that in ageing patients, with differential diagnosis of dementia and cognitive impairment, lipid oxidation and activation of the antioxidant response for the detoxification of the superoxide anion are common mechanisms in L and AD. In L, hydrogen peroxide (H_2O_2) decomposition is associated with a decrease in the content of total proteins in red blood cells, while in AD, the excess of H_2O_2 cannot be degraded and would be responsible for lipid and protein oxidation associated with inflammatory response.

402. (214) **STABLE CHANGES IN GLOBAL DNA METHYLATION IN REACTIVE ASTROCYTES FROM ANIMALS EXPOSED TO EXPERIMENTAL TEMPORAL LOBE EPILEPSY (TLE)**

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Neuron-centric literature considers epilepsy as the result of an alteration of excitatory/inhibitory balance; however a growing body of evidence shows the glial role in this disease. Usually, TLE patients refer a initial precipitating event (IPE), followed by a silent period of latency before initiating chronic seizures. We hypothesize that IPE releases DAMP that produce the alteration of astroglial proinflammatory and homeostatic genes during the latency period, and that long lasting effects are due to epigenetic cues. In the present work we used the Lithium-Pilocarpine model of TLE (Li-Pi Model) in male Wistar rats that were analyzed at 3, 7, 21 or 35 days post-status epilepticus (DPSE). Status epilepticus mimics the IPE events in humans. In vitro, astrocyte enriched culture were exposed to DAMP (HMGB-1) or PAMP (LPS) to induce a proinflammatory burst that imitates an in vivo IPE. Using immunohistochemistry, quantitative image analysis and RT- PCR, we observed that animals presented a long-lasting increase in global methylation in astrocytes (from 7 to 35DPSE) and coexisted with an increase in the DNMT3a/DNMT1 mRNA expression. A concomitant reduction of homeostatic astroglial Kir4.1 and glutamine synthetase (GS) expression were also observed during the latency period. In vitro, acute exposure to DAMP or PAMP was able to recapitulate the in vivo profile, by producing long- lasting increased global methylation, reactive gliosis and decreased expression of homeostatic genes in the astroglial cultures. The increased global methylation in astrocytes was also confirmed in hippocampal sections resected from TLE patients. We conclude that long-lasting astroglial alterations during the latency period induce a profound disbalance in neuronal homeostasis and K+ buffering that contributes to increased neuronal excitability. The altered pro-epileptogenic astroglial profile is sustained by increased global methylation of these genes. Supported by PICT 2017-2203 and 2019- 0851.

403. (226) **EXPRESSION, PURIFICATION OF RECOMBINANT APOLIPOPROTEIN E4 UNDER NON-DENATURING CONDITIONS, AND MITOCHONDRIAL MORPHOLOGY ANALYSIS IN A NEURONAL CLONAL CELL LINE**

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Apolipoprotein E (ApoE) is the key risk factor for Alzheimer disease. The *APOE4* allele increases the probability of developing the disease up to 15 times in homozygote carriers. Among other effects, ApoE4 has been associated with alterations in mitochondrial dynamics. In order to learn about the effects of ApoE4 on neuronal cells, we aimed at purifying the recombinant protein expressed in *E. coli* and assaying it on mitochondria of a neuronal clonal cell line, CNh. *E. coli* BL21 strain expressing the ApoE4 (pET32-E43C, containing His and Trx-tags) and 3C-protease were grown in LB medium and induced with 1 mM of IPTG for 1.5 and 4 h at 37 and 30Cº, respectively. Crude soluble ApoE was purified by affinity chromatography using a Ni-NTA resin. The 3C-protease was purified by FPLC (Superose-12 size exclusion). ApoE4 and 3C-protease were incubated at 4ºC in a 25:1 ratio to release the His-Trx tag. ApoE4 was next purified by size exclusion chromatography, with a yield of ~5 mg/L without denaturing/renaturing steps and no apparent contaminant bands in SDS polyacrylamide gels. We next studied mitochondrial morphology in neuronal CNh cells using confocal fluorescence microscopy. Cells were incubated with17μg/mL ApoE4 and mitochondria labelled with MitoTracker Orange and imaged in vivo. Mitochondrial morphology was analyzed with the Mitochondrial Network Analysis plugin for Fiji. Individual counts (<1 branch), network counts (>1 branch), network length and mitochondrial footprint (area covered) were quantified. ApoE4 induced an increase in mitochondrial footprint ($p<0.001$) and mitochondrial network ($p<0.001$) indicating an ApoE4-induced increase in mitochondrial proliferation.

404. (239) **IN SEARCH OF THE ROLE OF MICROGLIA IN THE SEX-DEPENDENT BEHAVIORAL PROFILE IN AUTISM SPECTRUM DISORDER**

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Autism spectrum disorder (ASD) is characterized by impairments in social interaction and repetitive-stereotyped behaviors. The incidence is higher in boys, and girls present different type and grade of symptoms. As microglia show sex-dependent features and reactive microgliosis has been reported both in patients and animal models of ASD, they are proposed to play a key role in ASD sex-differences. The experimental model of ASD by prenatal exposure to valproic acid (VPA) mimics the main behavioral and neuroanatomical alterations found in patients. The aim of this work was to study microglia features in male and female VPA rats in the prefrontal cortex (PFC) and the hippocampus (HP). Microglia were immunostained for Iba1 on tissue slices of juvenile control and VPA rats of both sexes. The PFC of both male and female VPA rats showed microgliosis characterized by a higher proportion of iba1 (+) unramified cells. However, in the HP, microgliosis was only evident in female VPA rats. To evaluate microglia reactivity and response to different stimuli we performed microglia primary cultures from control and VPA neonates.

Microglia were immunolabeled for Iba1 to study morphology under basal conditions and after exposure either to a pro-inflammatory (lipopolysaccharide) or a phagocytic (synaptosomes) stimulus. While cortical microglia from male VPA animals showed a pro-inflammatory profile and an intrinsic resistance to phagocytic stimuli, hippocampal microglia from male VPA animals matched microglia from controls under basal condition and showed a preserved response to pro-inflammatory and phagocytic stimuli. In the case of microglia isolated from females, both cortical and hippocampal microglia from VPA rats evidenced morphological changes under basal conditions but both were able to respond to pro-inflammatory and phagocytic stimuli. To sum up, microglia from male and female VPA rats show sex-dependent changes which may contribute to sex-differences in ASD.

405. (256) **A DEFICITARY MODEL OF CDK5 DOES NOT IMPAIRS NEURONAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS**

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CDK5/P35 is a complex involved in neuronal homeostasis and development that was described as a critical player for neuronal survival. Besides, its deregulation is linked with neurodegenerative pathologies such as Alzheimer Disease and Parkinson Disease. For that reason, we generated a deficitary CDK5 genetic model in neurons derived from human pluripotent stem cells. For this purpose, we used CRISPR/Cas9 technology to generate human embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively) KO-CDK5 lines. CDK5 protein expression levels were analyzed by western blot in samples obtained from clones where indels caused by CRISPR/Cas9 editing were detected by DNA sequencing. We obtained CDK5-/- clones for H9 hESCs and FN2.1 hiPSCs lines and a CDK5+/- clone for H9 hESCs line. Then, neural stem cells (NSC) were derived from the CDK5 KO clones using a commercial neural induction medium and their phenotype was validated by immunofluorescence staining using antibodies that recognize specific lineage markers (SOX-1, SOX-2, NESTIN and PAX-6). Finally, NSC obtained from the heterozygous CDK5+/- KO H9 hESCs clone were differentiated into neurons using a 2D-based protocol and their phenotype was validated by immunofluorescence staining of neuronal specific markers (TUJ-1 and MAP2). In conclusion, we managed to obtain NSC-neurons from CDK5-/- and CDK5+/- clones, determining that CDK5 is not essential for NSC generation. Besides, neuronal differentiation was achieved for H9 CDK5+/- clone, indicating that the CDK5 deficiency does not impair the generation of NSC-derived neurons. This result allows us to account with a CDK5-deficient model to further study its participation in neuronal homeostasis dysfunctions.

406. (266) **ASTROCYTIC INSULIN SIGNALING AND INFLAMMATION IN EXPERIMENTAL ALZHEIMER'S DISEASE**

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Insulin resistance (IR) and chronic inflammation are associated with the development of cognitive disorders and neurodegenerative diseases such as Alzheimer's (AD). However, it is not clear whether there is a causal link between these factors, which one appears earlier in the pathology or if either one of them is triggered by the increasing circulating levels of Aβ or amyloid deposits in early AD. Our objective was to study the metabolic and inflammatory status of a model of AD, the PDAPP-J20 mouse at the age of 8 months. We also treated a WT group with a high fat diet (HFD) as a positive control for IR. Our hypothesis was that in early stages of AD, the brain develops IR with astrocytes showing reactivity and impaired insulin signaling. Final body weight, glycemia and insulinemia were not affected by genotype or HFD. The open-field test showed an anxious-like behavior in transgenic and in HFD-fed mice. Insulin signaling measured by pAkt/Akt ratio was decreased in the hippocampus of AD mice ($p<0.05$) but not in the hypothalamus or the liver. Pancreatic IL1β and COX2 levels were unchanged. Insulin receptor puncta colocalizing with GFAP+ cells in the hippocampus by fluorescent immunolabeling showed a decreasing tendency in transgenic animals while astrocytic reactivity markers GFAP and S100b were increased ($p<0.05$). Finally, we evaluated the effect of fibrillar Aβ or palmitate on C6 astrocytes in vitro. Astrocytes exposed to Aβ showed increased nuclear translocation of NFkB and decreased AKT phosphorylation ($p<0.05$), suggesting inflammatory activation and impaired insulin signaling, respectively. Our results show that inflammation and insulin signaling impairment in the hippocampus are found in an early stage of experimental AD. The inflammatory context triggered by increased circulating Aβ or amyloid deposits in the brain could affect astrocytic insulin receptors, hence decreasing insulin signaling and affecting their neuroprotective capacity.

407. (267) **DIETARY RESTRICTION AS A FASTING MIMETIC IN AGED MICE. METABOLIC, COGNITIVE, AND NEUROINFLAMMATORY EVALUATION.**

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Aging is a physiological process that involves cognitive decline, decreased autophagic flux, and increased oxidative stress. Dietary restriction is a multitarget strategy that has been linked to several benefits, inducing autophagy flux, decreasing oxidative stress and inflammation, and improving metabolism. These effects establish dietary restriction as a possible approach to delay physiological aging and to prevent or treat aging-related diseases. In a previous work, we evaluated a protocol of periodic dietary restriction (PDR) in an animal model of familial Alzheimer's disease. Now, we have studied the effects of this strategy on aged female mice (16 month-old), evaluating metabolic, cognitive, and neuroinflammatory changes.
PDR involved 5 days of dietary restriction (DR) alternated with 9 days of ad libitum (AL) food intake for 7 weeks. During the DR period, mice ate 60% of their habitual intake.
Animals under PDR showed similar body weight and glycemia to AL mice. During DR periods, circulating ketone bodies increased (1WAnova-Sidak, basal vs DR $p<0.001$) suggesting a fasting-like effect. Additionally, we evaluated cognitive performance by the novel object location recognition test. No changes were observed between AL and DR animals, but both groups' performance was worse than that of 5 month-old mice, evidencing an age-related cognitive decline. We assayed S100b/GFAP by immunofluorescence in the hippocampus and analyzed morphological astrocytic parameters. S100b, an astrocytic pro-inflammatory marker, was diminished in DR mice (vs AL). However, GFAP immunoreactivity was unchanged. These preliminary results evidenced fasting-like effects in mice exposed to DR. Further, cognitive impairment in aged mice was corroborated, and a possible modulation of the pro-inflammatory S100b with DR. Future perspectives point to evaluating glial morphology in depth, and autophagy as a possible main mechanism for DR.

408. (268) **ADMINISTRATION OF ANASTRAZOLE, AN AROMATASE INHIBITOR, REDUCES THE PROTECTIVE EFFECTS OF TESTOSTERONE TREATMENT IN AN ANIMAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of upper and lower motoneurons leading to muscle weakness and motor impairment. The Wobbler (WR) mouse, a recognized model of ALS, shows a selective loss of motoneurons, astrocytosis and microgliosis in cervical spinal cord (CSC). ALS presents in men at younger ages than women, but increases after menopause. Testosterone (T) exerts its effects via androgen (AR), or estrogen receptors after bioconversion into several metabolites. Previous work has shown that T reduces gliosis and improves clinical score in male WRs. Now, we investigated the effects of cotreatment of T + anastrazole, an aromatase inhibitor (AI), on: 1) mRNA expression of myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP)- 2) CD11B mRNA, a marker of microglia, 3) % AR-immunoreactive (IR) cells in ventral horn. T was implanted in 10mm silastic tubes for 2 months. AI was given in DMSO 10% by Alzet osmotic pumps (1mg/kg/day) s.c. starting 1 week before T. Four groups were prepared: a) WRs or (b) controls receiving empty silastic tubes + vehicle-pumps, c) WR+T (silastic tubes filled with T) + vehicle-pumps and d) WR+T+AI. Pituitary weight, a gland sensitive to estradiol, is greater in WRs ($p<0.05$ vs. control) and smaller in WR+T+AI ($p<0.05$ vs WR). MOG mRNA rose in WR+T ($p<0.05$ vs WR) but not PLP. However, both myelin genes were significantly reduced in WR+T+AI ($p <0.01$ vs. WR+T). CD11B was reduced by T in WRs ($p<0.05$ vs. WR), but WRs and WR+T+AI showed higher expression ($p<0.05$ vs. controls or WR+T). The % AR-IR cells were low in WRs and WRs+T+AI ($p<0.01$ vs. controls), but increased in WR+T ($p<0.01$ vs WR). The mRNA for the steroidogenic acute regulatory protein (StAR) increased in WRs ($p<0.05$ vs control) and was still higher in WR+T and WR+T+AI ($p <0.05$; $p <0.01$ vs WRs). These data support that estrogen-derived aromatase may play a role in androgen neuroprotection.

409. (271) **SHORT AND LONG-TERM ASSESSMENT OF NOISE EXPOSURE ON HIPPOCAMPAL OXIDATIVE STRESS IN ADOLESCENT FEMALE AND MALE RATS**

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Previous studies from our laboratory have shown that noise exposure was able to trigger behavioral and neurochemical alterations in the Central Nervous System (CNS) of adolescent male rats when evaluated at short-term, whereas results in females have not been obtained yet. In addition, long-term measurements have not been performed. Therefore, the aim of the present work was to investigate the effects of noise exposure in the hippocampus (HC) of adolescent female and male rats on biochemical parameters evaluated at short and long-term. Male and females PND28 Wistar rats were separated into different cages and at PND 33 a subgroup was exposed to noise (2h, 95-97 dB). HC was dissected at short (PND 33) and long-term (PND 39) to assess reactive oxygen species (ROS) levels and catalase activity (CAT). Results showed an increase in ROS levels in females (sham: 0.004±0.001; noise: 0.123±0.005) and males (sham: 0.019±0.004; noise: 0.054±0.003) and an increase in CAT activity only in males (sham: 0.0004±0,0002 noise: 0.001±0.0001) when evaluated at short term. In contrast, long-term results showed a decrease in CAT activity in females (sham: 0.001±0.0004; noise: 0.0003±0.0007), whereas no significant differences were found in males. No differences were found in either group in ROS levels. These results suggest that noise exposure may induce short-term changes in oxidative markers that seem to disappear at long-term and to be sex-specific. In conclusion, adolescence seems to be a period of vulnerability to different stimuli capable of generating oxidative imbalance in the hippocampus, which could underlie some of the behavioral changes previously observed.

410. (276) **INFLUENCE OF PERSONALITY TRAITS, ALCOHOL EXPECTANCIES AND COVID-19 LOCKDOWN ON ALCOHOL CONSUMPTION IN STUDENTS OF THE UNIVERSITY OF BUENOS AIRES**

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University students (US) often consume alcohol for recreational purposes being exposed to various negative consequences that this substance produces. Different factors have been associated with increased alcohol consumption (AC), such as personality, alcohol expectancies and social isolation (e.g. COVID-19 lockdown). Thus, the aim of this work was to evaluate the AC pattern and the potential influence of personality, alcohol expectancies and lockdown in students from the University of Buenos Aires.
A sample of 1776 US completed an online survey that assessed the amount and frequency of AC before and during the first year of COVID-19 pandemic. In addition, US responded to the BIG-5 and CEA-A questionnaires to assess personality traits and alcohol expectancies.
Results showed that students' AC was highly prevalent both before and during the lockdown. In addition, men consumed significantly more alcohol than women per occasion ($F1,3523=12.83$) and month ($F1,3523=21.10$), but women had more episodes of heavy drinking ($\chi2=40.68$). When comparing both time periods, the amount and frequency of AC decreased during lockdown (F3,3523= 36,67 and F3,3523= 14,15). Moreover, positive and significant correlations were observed between AC and personality traits such as agreeableness (rs= 0.08 to 0.11) and responsibility (rs=
0.06 to 0.13) in women, and extraversion (rs= 0.13) and agreeableness (rs= 0.16 to 0.21) in men. Finally, results showed positive and significant correlations between AC and all alcohol expectancies evaluated (positive and negative), in both sexes (rs= 0.11 to 0.37).
In conclusion, this study suggests that AC is highly prevalent in US, which is worrying given the negative consequences associated with it. Furthermore, factors such as personality and alcohol expectancies could promote AC, whereas lockdown decreased it. Finally, the knowledge about risk and protective factors for AC is important for the development of interventions aimed at preventing and reducing AC.

411. (282) **ASSOCIATION OF FOLATE PRODUCTION AND IMMUNE MODULATION BY SELECETED BACTERIA IN PARKINSON'S DISEASE MODELS**

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Parkinson's disease (PD) is a neurodegenerative process that affect motor functions and involves an inflammatory response and B group vitamins' deficiency. Folate depletion with hyperhomocysteinemia are related with immune activation; however, the association between folate (vitamin B9) and the immune system in PD requires further research. Aim: To evaluate the effect of folate-producing and immunomodulatory lactic acid bacteria (LAB) in PD models. Methods: *Streptococcus thermophilus* (*St.*) CRL808 (folate producer

strain), and *St.* CRL807 (immunomodulatory strain) were evaluated individually using *in vitro* and *in vivo* PD models. N2a neuronal cells were differentiated to dopaminergic neurons with di-butyryl cyclic AMP and then exposed to the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) in presence of intracellular extracts from LAB or the commercial vitamin B9. Cell viability, IL-6 production and reactive oxygen species (ROS) were determined. *In vivo*, LAB were administered to mice injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Motor capacity, tyrosine hydroxylase (TH) in brain, and cytokines in serum were evaluated. Results: Neurotoxic effect of MPP+ decreased in cells cultured with both LAB intracellular extracts. This result was related with significant ($p<0.05$) decrease of ROS formation and IL-6 release by the neurons. Mice given LAB improved motor skills altered by MPTP and significantly ($p<0.05$) increased the number of TH+ neurons in the brain. The LAB effect was associated to decreased pro-inflammatory cytokines such as IL-6 and TNF-alpha and increased levels of IL-10 in the serum of the mice. Conclusions: LAB selected as folate producers and as immunomodulators have the potential to be used as adjuvants in PD, improving both vitamin deficiency and the inflammatory state associated with this pathology, which translates into less loss of dopaminergic neurons and better motor skills.

412. (290) **GLUN2A REDUCED EXPRESSION CHANGES MOLECULAR COMPOSITION AND STRUCTURE OF SYNAPSES WHICH WOULD BE RELATED TO CERTAIN TYPES OF EPILEPSIES**

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For several years, NMDA receptors (NMDAR) have been involved in memory and learning processes as well as in a wide range of neurodevelopmental disorders, as epilepsies. NMDAR are heterotetramers composed by two GluN1 obligatory subunits and two regulatory subunits, being GluN2A and GluN2B the most expressed in hippocampus and other cognitive related structures. During development and synaptic maturation, there is a shift in GluN2A/GluN2B expression ratio. This change was called the developmental switch and modifications in this relationship were associated to learning and memory as well as to different pathologies. Recently, grin2a (the gene that codifies for GluN2A) mutations were related to complex syndromes that include the development of seizures and or epilepsy. In this work, we induced a knockdown in GluN2A expression (GluN2A-KD) after developmental switch *in vitro* and *in vivo*. Results showed that NMDAR total amount and GluN2A/GluN2B ratio was decreased in GLuN2A- KD cultures. Moreover, downregulation of GluN2A *in vitro* increased dendritic branching and the number of dendritic spines, which in consequence, rise neuronal excitability. On the other hand, in vivo, GluN2A silenced expression in hippocampus, induced an impairment in contextual fear conditioning memory and a change in spatial-exploration. In addition, rats where GluN2A hippocampal expression were silenced, showed increased seizure susceptibility, both in time and intensity. Altogether, these results led us to conclude that the decrease in GluN2A expression would be related to epileptogenic mechanisms.

413. (367) **GLATIRAMER ACETATE REVERTED CHRONIC STRESS-INDUCED ALTERATIONS IN BEHAVIOUR, REGULATORY T CELLS IN SPLEEN AND TGF-β LEVELS IN HIPPOCMPUS OF BALB/C MICE**

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In previous reports we found that chronic mild stress (CMS) exposure induces a decrease in learning and memory in female BALB/c mice. This cognitive deficit correlated with a decrease in CD4+CD25+FOXP3+ and an increase in CD4+CD25-FOXP3+ regulatory T cells (Tregs) in CMS mice spleen. No differences in the CD4+CD25+FOXP3+ and CD4+CD25-FOXP3+ Tregs were found in lymph nodes in stressed mice. Tregs have an important role in maintaining self-tolerance through the inhibition of effector T cells. The main cytokines involved in this mechanism are IL- 10 and TGF-β, both released by Treg cells. Moreover, glatiramer acetate (GA) (synthetic amino acid polymer that can safely simulate the protective and reparative effects of autoreactive T cells) reverted the behavior and the neuroimmune alterations induced by CMS. In this context, the aim of this work was to evaluate the GA effect on the Treg cells in spleen and ARNm levels of TGF-β and IL-10 in hippocampus of CMS mice. Here, we show that CMS mice presented a poor learning performance in Y-maze and open field test. The Treg were evaluated by flow cytometry. The decrease of CD4+CD25+FoxP3+ (control-PBS vs. CMS-PBS: $p<0.05$) and the increase of CD4+CD25-FoxP3+ (control-PBS vs. CMS-PBS: $p<0.01$) cells in spleen of CMS mice were reverted by GA treatment (CMS-PBS vs. CMS-GA: $p<0.05$ and CD4+CD25-FoxP3+= CMS-PBS vs. CMS-GA: $p<0.01$, respectively). The mRNA expression by qRT-PCR indicated an increase in the mRNA levels of TGF-β in hippocampus in CMS mice respect to control mice ($p<0.05$). This levels were reverted by GA in CMS mice ($p<0.05$). No differences in the mRNA levels of IL-10 were found in hippocampus of stressed mice. Our findings indicate that GA revert the chronic stress effects on the immune system through a mechanism that involves Treg cells possibly by the release of TGF-β. This suggests Treg cells participation in the cognitive deficit observed in chronic stressed female mice and this can be reverted by GA.

414. (393) **REGIONAL DIFFERENTIAL EFFECTS OF HORMONE-REPLACEMENT TREATMENTS ON MITOCHONDRIAL DNA REPAIR MECHANISM ARE NOT EXERTED THROUGH GENE EXPRESSION REGULATION IN THE BRAIN**

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The brain is highly susceptible to mitochondria dysfunction and oxidative stress due to its high demand of energy and low antioxidant capacity. Mitochondrial DNA (mtDNA) is specially vulnerable to oxidative damage and Base Excision Repair (BER) is the main mtDNA repair mechanism. Ovarian hormone loss during natural or induced reproductive senescence is associated with mitochondrial alterations, synaptic decline and increased risk of age-related diseases. The aim of this work was to assess whether hormone-replacement treatments affect the expression of BER enzymes to explain previous results regarding the differential activity of such enzymes in the hippocampus (Hp) and cerebral cortex (Cc) of hormone-treated ovariectomized (OVX) rats.

To this aim, adult OVX or sham-operated (SHAM) rats were s.c. with empty or containing estradiol (E) and/or progesterone (P) silastic capsules. After 12 weeks, cDNA was obtained from total RNA extracted from the Hp and Cc and amplified by qPCR using specific primers for BER enzymes.

The expression of DNA glycosylases was either lower o similar to SHAM group in both Hp and Cc of OVX rats (NEIL1 $p<0,01$; NEIL2 $p<0,05$; UNG1 $p<0,01$; OGG1 ns; Student´s t test). Similar results were obtained for the rest of the enzymes of the pathway (AP endonuclease1, γ-polymerase and lygase3 $p<0,05$, Student´s t test). Hormone treatments did not affect the expression of OGG1, NEIL2 or UNG1 in any brain region, but increased the expression of NEIL1 only in the Hp (E+P $p<0,05$; ANOVA). On the other hand, P alone or combined with E, increased the expression of the rest of the enzymes of the pathway in both brain regions ($p<0,05$; ANOVA).

Our results show that OVX decreases the expression of BER en-

zymes in both brain regions and that there is no association between ARNm levels and the activity of such enzymes in hormone-treated rats. Thus, hormones exert their regional differential action on BER pathway through a mechanism not involving gene regulation.

415. (397) **METFORMIN TREATMENT IS ASSOCIATED WITH IMPROVED COGNITION AND REDUCED PATHOLOGICAL BIOMARKERS IN DIABETIC PATIENTS WITH PRODROMIC ALZHEIMER'S DISEASE ENROLLED IN THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE (ADNI) STUDY**

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive decline, with no effective treatment available to this day. AD hallmarks include aberrant Amyloid-β and Tau accumulation in the brain, the presence of chronic neuroinflammation and alterations in brain metabolism. Evidence suggest a possible shared pathophysiology between Type 2 Diabetes Mellitus (T2D) and AD, as impaired insulin signaling. Some therapeutic strategies employed on T2D subjects could be beneficial on AD patients. Here, we evaluated the effect of the antidiabetic drug metformin on patients enrolled in ADNI, an observational and longitudinal study including patients from all around the world. We employed data from patients diagnosed with mild cognitive impairment (MCI) due to AD and we performed a principal component analysis focusing on biomarkers associated to AD measured in cerebrospinal fluid (CSF). We concluded that MCI metformin-treated patients were globally characterized as subjects with a better CSF biomarkers profile than the mean population of MCI patients ($p<0.05$). On the other hand, control subjects and T2D patients were paired by age, gender, ApoE allele and years of education, defining three groups: MCI, MCI+T2D and MCI+T2D+metformin. We evaluated the effect of T2D and metformin treatment employing the PACC score, and composites defined from standardized ADNI variables to evaluate the memory and learning function. We found that MCI+T2D patients have a worse cognitive performance than MCI patients ($p<0.01$), but this deleterious effect was not observed in MCI+T2D+metformin patients. These cognitive variations were associated with changes in cortical thickness and hippocampal volume obtained from Magnetic Resonance Images ($p<0.001$). To summary, our study shows a beneficial effect of metformin treatment on cognitive performance, CSF biomarkers profile and neuroanatomical measures in MCI due to AD patients.

416. (419) **PLASMA BIOMARKERS FOR THE EARLY DETECTION OF ALZHEIMER'S DISEASE USING A MACHINE LEARNING BASED LOGISTIC REGRESSION MODEL**

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Alzheimer's Disease (AD) is the most common type of dementia. Clinical and basic research hope for early interventions to cure or stop the progression of the disease. However, patients seeking help mostly present mild to advanced cognitive decline. While it would be helpful to look for early signs of AD, specific tests are not routinely done and present elevated costs. Notably, evidence shows that blood biomarkers are altered through the progression of the disease. Here, we used patients' plasma biomarkers data available from Alzheimer's Disease Neuroimaging Initiative (ADNI) from healthy controls (HC), patients with mild cognitive impairment (MCI) and AD to develop an automated classifier. The total of patients selected was 544 (55 HC, 379 MCI, 110 AD) all having associated demographic and cognitive information. We used a total of 146 routine blood tests results, such as transferrin and CRP, and did not include AB, Tau of=r pTau.
There was no difference in representativity of females, age, education level or ethnia between groups. We separated the data into a train set and a hold-out set. We performed cross validation with the train sets with 5 folds and a grid search for hyper parameters optimization to train a logistic regression model. Employing the train set, our model presented a ROC curve with an AUC for HC prediction of 0.86, MCI
0.81 and AD 0.79. When tested with the hold-out, the AUC of the ROC curves were HC: 0.81, MCI: 0.77 and AD: 0.77. Only 2% of MCI patients were misclassified as HC, and none AD patient was classified in the HC group. Feature importance analysis showed pregnancy associated plasma protein as the most relevant parameter, in accordance with literature. While the number of patients between groups is unbalanced, our classifier has a very good predictive power and successfully minimizes type 2 errors. In the future, it would be important to increase the number of subjects to train and test the model with balanced groups.

417. (424) **MYELINATION PROCESS DURING THE EMBRYOGENESIS OF *LAGOSTOMUS MAXIMUS*, A PRECOCIAL RODENT**

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Myelin is a protein synthesized by oligodendrocytes forming a multilaminar membrane around the axons of the CNS. This membrane acts as an insulator increasing the speed of stimulus transmission. Myelination is a continuous process that begins when axons reach a diameter of approximately 1 μm and follows a structural sequence with a caudo-rostral, dorso-ventral and center-to-periphery progression which varies between species. The aim of this study was to analyze the myelination process during the embryogenesis of the South America plains vizcacha, *Lagostomus maximus*, an hystricomorph rodent native from Argentina, and to compare it with this process in rat, mouse and guinea pig. Brains of 24 embryos, distributed between 51 embryonic days (e.d.) and 2 days postnatal, were used and studied by Klüver-Barrera histological technique. The onset of the myelination process was observed around mid-pregnancy. Inside the brain, the myelination process began around 72 e.d. up to 118 e.d. in the cortex; at the same time, the myelination extended towards connection fibers beginning in the external cingulate at 106 e.d. and gradually progressing towards the internal cingulate from 112 e.d. onwards. At 124 e.d., the internal and external capsule, and optic chiasm were myelinated. After that, striosomes of the corpus striatum and the fornix columns were myelinated at 133 e.d. At birth time (155 e.d.), all the intra-hemispheric white matter structures were myelinated, but the inter-hemispheric connections of the corpus callosum and the anterior commissure were not myelinated yet. These results show that both the onset of the myelination process and its progression during embryonic development are framed in the precocial character of *L. maximus.* The onset of this process in vizcacha agreed with that in guinea pig, and was in contrast to the postnatal onset in rat and mouse. Grant: Fundación Científica Felipe Fiorellino.

418. (428) **EVALUATION OF CIRCULATING MONOCYTES AND PROINFLAMMATORY CYTOKINES IN PATIENTS WITH MOOD DISORDERS**

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Mood Disorder (MD) affects more than 300 million people globally, and its etiology is unknown. In recently published data, MD has been correlated with the inflammation and the immune system. This study aims to determine if there is a specific activation profile of monocyte-macrophage in patients with an MD that differentiates them from healthy control (HC), and to analyze if this activation profile in patients with MD is present only during a clinical episode of active depression (AD) or during no clinically active depression (NAD).
Patients and HC were recruited and evaluated by psychiatrists using the International Psychiatry Interview MINI to diagnose the MD and the Hamilton Depression Rating Scale (HADRS) to define the 3 groups: AD, NAD, or HC. Blood samples were obtained and directly stained in 100 uL with the following cocktail of antibodies CD11b, HLA-DR, CD86, CD14, and CD16. IRB approved the study; each participant gave written consent. MD sample was 23% male, 77% female with a 25-62-year age range.
Circulating monocytes and the proportion of the three subtypes were analyzed by flow cytometry based on $CD16^{neg}CD14^{++}$ (classical), $CD16^{+}CD14^{+}$ (intermediate) and $CD16^{++}CD14^{neg}$ (non-classical). Additionally, the level of 16 cytokines was measured in plasma employing two panels of Legend Plex system by flow cytometry. Each panel was evaluated in a single sample of 50 uL of plasma. Our preliminary analysis shows that patients with MD (n=26) have a significantly reduced proportion of classical monocytes (p=0.02) and increased intermediate (p=0.02), and an upward trend in non-classical subsets (p=0.07) compared to the HC group (n=7). Moreover, we observed that patients with MD have higher concentrations of IL-1β (p=0.002), IFNγ (p=0.019) and IL-17 (p=0.004) than HC samples. After segregation of patients in AD (N=17) vs. NAD (N=12), no significant differences were observed yet, showing that some patients with MD have an inflammatory compromise even in remission.

419. (439) **MIGRATION OF GNRH-I NEURONS DURING THE EMBRYONIC DEVELOPMENT OF THE PLAINS VIZCACHA (*LAGOSTOMUS MAXIMUS*)**

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The neurons that express gonadotropin-releasing hormone type-I (GnRH), the key hormone that regulates the reproductive axis function, are originated in the olfactory placode, outside the CNS. External and internal factors guide GnRH neurons migration during embryonic development from the olfactory bulb up to the hypothalamus, specifically at preoptic area (POA) and arcuate nucleus (ARC). In the adult South American plains vizcachas, *Lagostomus maximus* (Rodentia, Hystricomorpha), GnRH neurons are located in POA, ARC, suprachiasmatic (SCN), and supraoptic (SON) nuclei. This work aimed to characterize the migration of GnRH neurons up to their colonization of the hypothalamus in the vizcacha during the embryonic development. Thirty-six vizcachas from 52-embryonic days (ed) up to birth (~155-ed) were used. Complete heads or brains were removed and used for histological and immunohistochemical localization of GnRH neurons. GnRH cells without dendritic processes appeared firstly as early as 52-ed outside the CNS, in the olfactory epithelium, with increased number at 60-ed. At 75-ed, GnRH cells were found in the olfactory bulb tract within the brain, and at 80-ed, they began to colonize the hypothalamus at the POA, following a ventro-dorsal migration route from the piriformis area, going through the SCN. Then, at 112-ed, was observed a decrease in the number of GnRH neurons in the POA together with an increase in the amount of GnRH varicosities. Comparison of GnRH cell migration between vizcacha and other rodents, identifies differences in the migration stages between vizcacha and mouse. The migration of GnRH neurons in vizcacha from the olfactory epithelium supports the extra-CNS origin in Rodentia. This is the first study of Central neurogenesis in Hystricomorpha, with GnRH cells as the main elements. The signaling molecules that participate in the ventro-dorsal migration to the hypothalamus remain to be described in further investigations.

420. (454) **BRAIN SELECTIVE NANOCARRIERS LOADED WITH TRIAMCINOLONE PREVENTED EMOCIONAL SEQUELAE AND OXIDATIVE STRESS INDUCED BY TRAUMATIC BRAIN INJURY**

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Traumatic brain injury (TBI) causes a variety of neuropathological manifestations including cognitive, emotional and physiological deficits, probably related to early neuroinflammatory processes. We have previously shown that TBI increases levels of protein (AOPP) and lipid (MDA) peroxidation, considered oxidative stress (OS) biomarkers that persisted over a week. Regardless the research investment on the development of anti-inflammatory and neuroprotective treatments, most pre-clinical studies did not report significant effects, probably because of the limited blood brain barrier permeability of clinically available anti-inflammatory drug formulations. **Objective:** to evaluate the effectiveness of brain selective nanocarriers, loaded with the synthetic glucocorticoid triamcinolone (NA-TA), to prevent oxidative stress (OS) processes and to reduce the emotional sequelae induced by TBI. **Materials and methods**: TBI was induced in anesthetized adult male Wistar rats, which 15 min and 24 h later received a dose of NA-TA. Animals were sacrificed 1 or 7 days after treatment to measure AOPP and MDA levels in different brain areas. Other groups of animals were exposed to contextual fear conditioning 6 days after TBI and treatment. Twenty-four h (test 1) and 6 days (test 2, 12 days after TBI) after conditioning, fear memory expression was evaluated. **Results**: TBI induced a significant decrease in the % freezing only in test 2 compared to the controls (two-way ANOVA). Interestingly, preliminary data suggest that animals treated with NA-TA did not show changes in fear memory. Also, TA prevented increments in AOPP and MDA levels (one-way ANOVA). **Conclusions:** TBI induced neuroinflammatory mediators that could have detrimental actions on fear memory retention, probably through emotional processing alteration. NA-TA administration could be a therapeutic alternative for the early treatment of neuroinflammation mediated by TBI, that contribute to the emotional sequelae prevention.

421. (465) **INCREASED ACTIVATION MARKERS ON CIRCULATING CD4 LYMPHOCYTES OF PATIENTS WITH MOOD DISORDERS**

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Mood Disorders (MD) are highly prevalent psychiatric conditions occurring early in life and follow a relapsing and remitting course. During the last years, emerging literature associated MD with increased inflammatory status and unbalanced immune response. This was particularly evident in patients with no response to the conventional treatment, giving rise to the inflammatory theory of depression. This study aimed to determine the activation status of circulating CD4 lymphocytes in patients with MD coursing a clinical episode of active depression (AD) or during no clinically active depression (NAD).
Patients with MD were evaluated by psychiatrists using the International Psychiatry Interview MINI to diagnose the MD and the Hamilton Depression Rating Scale (HADRS) to define AD (N=8) and NAD (N=18) status. MD sample was 26% male and 74% female with a 25-62 year age range.
Blood samples were obtained and directly stained using the following antibodies against human (CD3, CD4, CD8, CD69, CD44, PD1, LAG3 and viability dye) and analyzed by flow cytometry.
This preliminary and partial analysis of the current study shows that patients with active MD have increased levels of CD69 activation marker on circulating CD4 T cells compared with patients with NAD ($p<0.01$). No significant difference was observed on the other activation marker, CD44. When we analyzed the exhaustion markers, PD1 and LAG3, we found a significant increase of LAG3 on circulating CD4 lymphocytes of patients with AD vs. NAD ($p<0.05$). Furthermore, most of the circulating CD4 T cells from patients with MD showed increased levels of activation markers compared with CD4 T cells from the mononuclear fraction of healthy volunteers.
These results clearly show that the lymphocyte compartment of patients with MD presents an unbalanced active condition, more skewed in patients with AD. It is also interesting that some patients with MD show an inflammatory compromise even in remission of clinical symptomatology.

422. (497) **POSITIVE EFFECTS OF ORCHIECTOMY ON SWIMMING BEHAVIOR IN HEMIPARKINSONIAN ANIMALS TREATED WITH THE NEUROACTIVE STEROIDS ESTROGEN AND PROGESTERONE**

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Parkinson's disease is the second most frequent neurodegenerative pathology after Alzheimer's disease, with a predominance in men over women. Unilateral neurotoxic injury with 6-hydroxydopamine (6-OHDA) induces selective dopaminergic neurodegeneration and hemiparkinsonism in rats with contralateral predominant motor impairment. The aim was to evaluate whether the decrease in testosterone concentration by orchiectomy (ORX) would be a positive factor on the neuroprotective effect induced by estrogen (E) and progestrone (P) administration on the motor and depressive-like behavior alterations observed in the injured animals. Male Sprague-Dawley rats (280-320 g) aged 60 days ORX and non-ORX were used. At 10 days post ORX, neurodegeneration was induced by microinjection of 6-OHDA into the left striatum. The experimental groups were 1- non-ORX: control (C), E and P-treated control (CEP), hemiparkinsonian (HP), E and P-treated hemiparkinsonian (HPEP) and 2-ORX: control (CO), E and P-treated control (COEP), hemiparkinsonian (HPO), E and P-treated hemiparkinsonian (HPOEP). Signs of motor impairment and hopelessness, were assessed by a forced swim test (PNF). Data were expressed as mean+SEM and analyzed by ANOVA 2 and Student Newman-Keuls.
ORX induced a significant increase in swimming time in HP animals with respect to group C ($p<0.0001$). This significant increase was increased in the HPOEP group with respect to the COEP group ($p<0.05$). Likewise, the magnitude of the increase was greater in HPOEP animals with respect to HPO animals ($p<0.0001$). We conclude that the decrease of testosterone by ORX is a positive inducing factor on the alterations in the motor and hopelessness signs, at the same time that the administration of E+P potentiates the positive response on the evaluated variables, proposing this combination of neuroendocrine modifications as a potential neuroprotective and neuroregenerating inducing treatment on neurodegenerative noxas.

423. (500) **OXIDATIVE STRESS IN HUNTINGTON DISEASE MODELS: BDNF ANTIOXIDANT EFFECT**

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Huntington disease (HD) involves oxidative stress and mitochondrial dysfunction which can be mimicked by 3-nitropropionic acid (3NP), a phenotypic model of HD. Reactive oxygen species (ROS) generate oxidative stress which is associated with neuronal death. Glutathione (GSH) is an antioxidant molecule secreted by astrocytes that can protect neurons from death by reducing ROS levels. Superoxide dismutase 2 (SOD2) is a mitochondrial antioxidant enzyme that reduces ROS levels and its overexpression provides neuroprotection. We have shown that brain-derived neurotrophic factor (BDNF) reduces ROS levels induced by 3NP in astrocytes and increases intracellular GSH while 3NP reduces extracellular GSH levels. Now, we have studied BDNF effect on ROS production in cortical astrocytes, and GSH levels and SOD2 expression in cortical and striatal astrocytes. We found that BDNF reduces ROS levels induced by 3NP in cortical astrocytes ($p<0.05$ DCFH-DA assay). Intracellular GSH levels were increased by BDNF in both astrocyte populations ($p<0.05$ NDA assay). 3NP reduced extracellular GSH levels regardless of the presence of BDNF ($p<0.05$ cortical, $p<0.01$ striatal astrocytes). SOD2 expression, although not modified by BDNF, was increased by 3NP in striatal astrocytes ($p<0.05$). We also evaluated motor performance, ROS and GSH levels in Q175 (HD mice), a knock-in model of HD which has not been tested for oxidative stress. We observed reduced motor performance in the open field test in 4-month-old ($p<0.05$) and 8-month-old ($p<0.0001$) HD mice compared to WT mice. 4-month-old HD mice cortex presented higher ROS levels ($p<0.05$) and 8-month-old HD mice striatum showed lower GSH levels ($p<0.05$) than WT mice. In brief, BDNF antioxidant effect could be a protective mechanism for neurodegeneration by reducing ROS levels and increasing intracellular GSH. Q175 mice model of HD exhibits signs of oxidative stress as early as 4 months.

424. (505) **LIPOTOXICITY- INDUCED METABOLIC INFLAMMATION: POTENTIAL ROLE OF CERAMIDES IN GLIAL CELLS INTERACTION AND INFLAMMATORY-DAMAGE PROPAGATION**

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Western diet is associated with the elevated rates of obesity and related metabolic disorders, also considered important risk factors for brain dysfunction. Chronic inflammation and insulin resistance comprise the most relevant shared pathways, promoting alterations in the plasticity of limbic structures such as the hippocampus. With the aim to study the impact of metabolic disturbances on the brain we focused on the role of glial cells in two different approaches: *in vivo*, in the hippocampus of C57BL/6 mice exposed to a high fat diet (HFD) and *in vitro*, emulating the lipotoxic context with the saturated fatty acid palmitate (PA) as an insult to microglia and astrocyte cell lines and the role of ceramide pathway.
We have previously found that HFD mice exhibited decreased neurogenesis and structural synaptic alterations, together with spatial memory impairment and neuroinflammation, with increased expression of hippocampal TNFα and IL1β cytokines and enlarged microglia. Here, we show that astrocytes also respond to the inflammatory status with a trend to increased S100b staining and a higher cell complexity (assessed by GFAP labeling and Sholl analysis RM-ANOVA $p<0.05$). Microglial cells exposed to PA showed induced

phagocytic ability and the expression of IL1β, but this effect was prevented if ceramide synthesis was inhibited by Cambinol ($p<0.05$).
Regarding the interaction between glial cells, while PA failed to induce IL1β expression in astrocytes, conditioned media (CM) from PA-exposed microglia did ($p<0.001$) and this effect was absent when microglia was pretreated with Cambinol. Interestingly, preliminary data showed that exosomes derived from PA-microglia exerted the same effect as the complete PA-derived CM, suggesting a relevant function .
Our results suggest a role of ceramide pathway in the induction and propagation of the inflammatory context induced by PA in glial cells.

425. (532) ***OXALIS ERYTHRORHIZA*** **MODIFIES THE ANXIOUS TYPE BEHAVIOR LEVELS IN RATS SUBJECTED TO A CHRONIC SUCROSE BEVERAGE**

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In the Cuyo region (Argentine), *Oxalis erythrorhiza Gillies* ex Hooker et Arnott *(*Oxalidaceae*;* Oe) is popularly consumed as a "medicinal plant" to regulate the level of glucose and cholesterol. The recent chemical characterization of Oe revealed the presence of an antidiabetogenic compound. We have previously reported that Oe produces beneficial properties on rats with experimental induced diabetes. The sustained sucrose consumption during the early developmental stage may cause insulin resistance. Also, we have recently reported that this consumption increases the anxious type behavior in adulthood. Here we studied the preventive effects of Oe on insulin resistance development and anxiety of rats subjected to sucrose consumption during the juvenile stage (childhood-adolescence). Male rats (SD) received as drinking solution sucrose (10% W/V; group SUC) or sucrose + Oe decoction (5% W/V; group SUC+Oe) from PND 21 to PND 61. A glucose tolerance test was performed on PND 62 and the values of the area under curve (AUC) were obtained. An open field test was realized on PND 63 and the recorded sessions were analyzed using the ANY-maze© software. This program determines the entrance number (EN), the distance traveled (DT) and the permanence time (PT) in three zones of the device (central: zone 1; intermediate: zone 2; peripheric: zone 3). The AUC levels of SUC+Oe were 25% lower than those of SUC ($p<0.001$). The EN and PT the zones 1 and 2 from SUC+Oe animals were higher than those of SUC ($p<0.05$). Also, the PT in zone 3 of SUC+Oe was lower than that of SUC ($p<0.05$). These results revealed that rats drinking SUC+Oe had lower levels of anxiety than those drinking only SUC. Thus, the Oe administration may prevent the development of insulin resistance and reduces the anxious behavior. Additional studies are required to propose Oe as a new source of therapeutic phytocompounds and to extrapolate these effects to humans. (PIP-0243, PICT2019-623).

426. (576) **EVALUATION OF THE ANTINOCICEPTIVE EFFECT OF MORPHINE IN A MODEL OF NEUROPATHIC PAIN IN MALE AND FEMALE MICE**

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Morphine is one of the most widely used analgesics in the treatment of moderate and severe pain. However, its clinical use in long-term chronic pain treatment is limited by the enormous addictive potential. The co-administration of morphine with other drugs that enhance the analgesic effect and reduce its reinforcing properties, could be an alternative in pain treatment with opioids.
In the present study, we propose to determine the lower effective dose of morphine to ameliorate the nociceptive threshold by using the partial sciatic nerve ligation (PSNL) in male and female Balb/C mice, a widely used model of neuropathic pain. The Von Frey test (VFT) was performed in order to evaluate mechanical allodynia by calculating the nociceptive threshold (g). First, mice were habituated to the environment of the experiment during 4 days. After the habituation period, baseline responses were measured and surgery of the right paw was performed in a group of animals with PSNL (PSNL group), and surgery without PSNL was executed in another group (Sham group). On day 9 after surgery, VFT was performed after administration of morphine (1, 3, 9 mg / kg, i.p.) or saline solution as vehicle.
Finally, three-factor ANOVA (sex, surgery, treatment) was applied with Tukey's post-hoc test, using a $p<0.05$ as statistically significant. Our results showed that morphine (1 mg/kg and 3 mg/kg) was able to reduce neuropathic pain in male and female mice, respectively ($p < 0.05$). The sexual dimorphism observed herein, confirms the lower sensitivity of females compared to males in the antinociceptive response of morphine.
The lower effective doses of morphine determined in male and female mice by a neuropathic pain model, will allow us to continue with our research in order to evaluate potential therapeutic targets to enhance the analgesic effect of opiates, reducing or preventing the addictive properties.

427. (584) **MANGANESE-EXPOSED BV-2 MICROGLIA INDUCES DAMAGE IN N27 DOPAMINERGIC NEURONAL CELLS**

Adriana María Belén Abiuso[1], Soledad Porte Alcon[1], Angeles Vinuesa[2], Flavia Saravia[2], Mónica Lidia Kotler[1], Roxana Mayra Gorojod[1].
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[2]Laboratorio de Neurobiología, QB-FCEN-UBA. IBYME-CONICET

Manganese (Mn) intake is essential at physiological concentrations. However, prolonged exposure produces a neurodegenerative disease called manganism, whose symptoms are often confused with idiopathic Parkinson´s disease. Even though the harmful effect of Mn in different cell types has been described, much remains to be understood regarding the outcomes of glial activation on neuronal death. Objective: To evaluate the effect of soluble mediators released by microglial cells after Mn exposure on neuronal integrity. For this purpose, we first characterized the direct effect of Mn exposure on neuronal and microglial cells, and then explored the influence of microglia-conditioned medium (MCM) on neurons viability. Methodology: MCMs were generated by BV-2 cells incubation with 250-1000 μM Mn for periods of 3 or 6 h, and were used to stimulate N27 neuronal cells for 24h; cell viability was assessed using MTT reduction assay; the study of ROS production was performed using DCFDA; the change in mRNA expression was quantified by RT-qPCR. Results: In N27 neuronal cells, Mn exposure induced a concentration-dependent decrease in cell viability after 24h (100-1000 μM, $P<0.05$), which was associated with an increase in ROS production. On the other hand, BV-2 cells increased ROS production after 3 and 6 h of Mn exposure, with no change in their viability ($P<0.05$). MCM of cells exposed to Mn induced a decrease in N27 viability ($P<0.01$), consistent with an increase in ROS production and a change in N27 morphology. Finally, we observed a higher expression of IL-1β and TNF-α mRNA in Mn exposed BV-2 cells (6 and 4 fold-increase, respectively, $P<0.001$, 750 μM). Conclusion: In microglial cells, Mn exposure induces cytokines expression and ROS generation, probably responsible for decreasing neuronal viability. The advance in the knowledge of Mn toxicity mechanisms will facilitate its diagnosis and the design of effective therapeutic strategies to avoid neurodegeneration.

428. (586) **DEVELOPMENT OF A CELLULAR MODEL TO EXPLORE NEURONAL PATHOLOGY IN SANFILIPPO DISEASE BY CRISPR/CAS9 GENOME EDITING**

Marcos Gabriel Francia[1], Soledad Porte Alcon[2], Adriana María Belén Abiuso[2], Alejandra Sonia Guberman[1], Mónica Lidia Kotler[2], Roxana Mayra Gorojod[2].
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Sanfilippo syndrome type IIIA (Mucopolysaccharidosis IIIA; MPSIIIA) is a rare intractable disease characterized by an early-onset, severe, progressive neurodegeneration. It is caused by mutations in the gene encoding for the lysosomal hydrolase N-Sulfoglucosamine Sulfohydrolase (SGSH), which is crucial in the stepwise degradation of the sulfated glycosaminoglycan (GAG) heparan sulfate (HS). Nowadays, there is a lack of cellular models to explore the mechanisms of disease in the central nervous system (CNS). In this work, we aimed to develop a novel neuronal model by employing CRISPR/Cas9 technology. We designed two sgRNAs targeting exon 1 of the mouse *sgsh* gene and cloned these sequences in the pX330 vector. Insert-containing plasmids were amplified and checked by PCR and sequencing. HT-22 hippocampal neurons were transfected with PEI, and transfected cells were selected with puromycin. Subsequently, clones were isolated and amplified. We determined the lack of enzyme activity by employing the fluorogenic substrate 4-MU-GlcNS and obtained five putative knock-out cell lines where we tested lysosomal and mitochondrial integrity. Once confirmed the successful knockout by sequencing, these cell lines will constitute reliable reporters of the cellular context of disease and great models to study cell-type-specific damage. The strategy used is versatile and will be employed in other cell lineages to study cell-cell interactions of different cell types within the CNS. Further research may lead to the identification of relevant signaling pathways to investigate candidate drugs for novel therapies.

429. (596) **EXTRACELLULAR MATRIX ALTERATIONS IN MÜLLER GLIAL CELLS IN A RETINAL DEGENERATION MOUSE MODEL**

Harmonie Vallese-Maurizi[1], Georgina Pamela Coló, Luis Politi, Lorena German

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Müller glial cells (MGC) are retinal stem cells, although their regenerative capacity is very low in mammals. We recently demonstrated that these cells in the *rd1* retinal degeneration mouse have decreased regenerative potential, an excessive number of neurons interacting with MGC, and a notable reduction in their lamellipodia, respective to their wild type (*wt*) counterparts. This suggests that extracellular matrix (ECM) protein synthesis and/or secretion could be altered in *rd1*, interfering with the substrate adhesion and lamellipodia extension, and thus affecting *rd1* MGC morphology and functionality. The aim of this work was to study *rd1* ECM protein expression and localization, and determine whether ECM pretreatment could restore the *rd1* MGC morphology and functionality. Using mixed neuron-glial cultures obtained from postnatal day two *rd1* and *wt* mice retinas, we analyzed by immunocytochemistry, osteonectin and fibronectin (FN) expression at 6 days in vitro, and we quantified focal adhesions with paxillin. On the other hand, *rd1* mixed cultures were seeded on culture dishes previously treated or not with ECM enriched conditioned medium (CM). We analyzed *rd1* MGC morphology, proliferation and photoreceptor survival (using BrdU and DAPI respectively). Our preliminary results showed a decrease in osteonectin expression, an alteration in FN expression, and a decrease in number and length of focal adhesions in *rd1* MGC when compared to the *wt* condition. Instead, the pretreatment with CM promoted *rd1* MGC cytoplasmatic extension, increased glial cell proliferation, decreased the number of neurons with pyknotic and fragmented nuclei, and decreased the Neuron/MGC ratio by 26.9%. These results indicate that *rd1* MGC display alterations in EMC protein synthesis and/or secretion, and that EMC supplementation improves MGC morphology and functionality.

430. (600) **EVALUATION OF THE ANTINOCICEPTIVE EFFECT OF MORPHINE IN A MODEL OF NEUROPATHIC PAIN IN MALE AND FEMALE MICE**

Perez V., Canero E., Balerio G.

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Morphine is one of the most widely used analgesics in the treatment of moderate and severe pain. However, its clinical use in long-term chronic pain treatment is limited by the enormous addictive potential. The co-administration of morphine with other drugs that enhance the analgesic effect and reduce its reinforcing properties, could be an alternative in pain treatment with opioids.

In the present study, we propose to determine the lower effective dose of morphine to ameliorate the nociceptive threshold by using the partial sciatic nerve ligation (PSNL) in male and female Balb/C mice, a widely used model of neuropathic pain. The Von Frey test (VFT) was performed in order to evaluate mechanical allodynia by calculating the nociceptive threshold (g). First, mice were habituated to the environment of the experiment during 4 days. After the habituation period, baseline responses were measured and surgery of the right paw was performed in a group of animals with PSNL (PSNL group), and surgery without PSNL was executed in another group (Sham group). On day 9 after surgery, VFT was performed after administration of morphine (1, 3, 9 mg / kg, i.p.) or saline solution as vehicle.

Finally, three-factor ANOVA (sex, surgery, treatment) was applied with Tukey's post-hoc test, using a $p < 0.05$ as statistically significant. Our results showed that morphine (1 mg/kg and 3 mg/kg) was able to reduce neuropathic pain in male and female mice, respectively ($p < 0.05$). The sexual dimorphism observed herein, confirms the lower sensitivity of females compared to males in the antinociceptive response of morphine.

The lower effective doses of morphine determined in male and female mice by a neuropathic pain model, will allow us to continue with our research in order to evaluate potential therapeutic targets to enhance the analgesic effect of opiates, reducing or preventing the addictive properties.

431. (605) **THE PRESENCE OF CO SPECIFICS DURING NICOTINE EXPOSURE ALTERS DRUG PREFERENCE IN A DOSE DEPENDENT MANNER IN ZEBRAFISH (DANIO RERIO)**

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Reinforcing drugs such as nicotine have been proven to alter the way group behaviour takes place. Interestingly, there have not been studies that show whether group dynamics alter the way individuals react to a drug. These dynamics may be highly influential on the possibility of an individual becoming addicted to a certain substance. Nicotine rewarding properties have been assessed in zebrafish using a biased conditioned place preference (CPP) protocol. In the present study, we aimed to evaluate whether individuals exposed to nicotine as a group developed different responses to those of individuals exposed to the substance in isolation ("classic" CPP) and whether these responses varied in accordance to the concentration of nicotine to which they were exposed.

By exposing fish to either a grouped or an isolated CPP Protocol our preliminary results seem to show that Nicotine elicits a stronger, more robust CPP when being exposed to the drug as a group (Nicotine 15mg/L). When Nicotine concentration is raised to 50mg/L, however, the animals exposed as a group show negative CPP scores in comparison to their isolated exposure counterparts. These results may indicate that grouped exposure enhanced the effects of nicotine to a point that higher concentrations resulted in an exacerbation of its negative, anxiogenic effects, outweighing it's rewarding, anxiolytic properties. When nicotine exposure was coupled with Phenylbutirate, an HDAC inhibitor that has been proven to arrest the development of CPP in isolated animals, blocking the unfolding of CPP in a group-enhanced CPP protocol resulted in a positive CPP score at higher concentrations (50mg/L) whereas the isolated CPP

protocol still showed negative results regardless of concentration.
In conclusion, our results seem to elucidate a novel approach to alter nicotine rewarding properties that is neither invasive nor pharmacological but solely through social stimuli.

ONCOLOGÍA

432. (030) **SMYD2 INHIBITION AS A NEW THERAPEUTIC STRATEGY FOR HEPATOCELLULAR CARCINOMA**

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Introduction: The development of therapies for hepatocellular carcinoma (HCC) remains a topic of interest since the impact of available therapies on patient survival is still poor. The methyltransferase SMYD2 is an epigenetic modifier frequently upregulated in tumors that has been pointed out as a potential therapeutic target. Our aim was to explore the therapeutic potential of SMYD2 pharmacological inhibition in HCC.
Methods: SMYD2 expression, its correlation with clinical prognosis and transcriptional programs relevant to the disease were explored in HCC using TCGA, ICGC and GSE1542 databases. The effect of LLY507, an SMYD2 inhibitor, on HCC cells survival, cell cycle and apoptosis were assessed by standard MTT assay and flow cytometry. RNA-Seq analysis of HuH7 cells treated with LLY507 and their correlation with HCC datasets were used to characterize the underlying mechanism upon SMYD2 inhibition.
Results: We observed that SMYD2 is upregulated in the tumoral vs non-tumoral tissue and correlates with a poor prognosis when highly expressed in HCC. Additionally, we found that SMYD2 expression negatively correlates with a set of genes linked to immune-related processes, apoptosis, and MAPK pathway, and that are downregulated in HCC. The pharmacological inhibition of SMYD2 by LLY507 showed a potent *in vitro* antitumoral effect, induced cell cycle arrest and apoptosis. Finally, RNA-seq of LLY507-treated HCC cells revealed the downregulation of aggressive, cell cycle-related genes, as well as the upregulation of immune genes that correlate negatively with SMYD2.
Conclusions: The bioinformatic analysis of public HCC datasets showed that SMYD2 can be considered a new therapeutic target for HCC. The targeted inhibition of SMYD2 by LLY507 has a potent antiproliferative effect on HCC cells and reverts an oncogenic transcriptional program. These data suggest that inhibition of SMYD2 merits further investigation as a therapeutic target in HCC.

433. (032) **DOWNREGULATION OF DNA DAMAGE REPAIR GENES BY VALPROIC ACID RADIOSENSITIZE ANAPLASTIC THYROID CANCER CELLS**

Perona M[1,2], Ibañez I[3,4], Rosemblit C[5], Grissi C[3,4], Campos Haedo M[5], Thomasz L[1,2], Dagrosa MA[1,2], Cremaschi G[5], Durán H[3,4], Juvenal G[1,2]

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Introduction: Anaplastic thyroid cancer (ATC) is one of the most aggressive malignancies in humans. Novel strategies to control it, like radiotherapy as altered fractionation or in combination with chemotherapy, are therefore necessary. We have previously shown an increase in the DNA damage by the histone deacetylase inhibitors (HDACi) valproic acid (VA) in irradiated ATC cells. HDACi have emerged recently as promising anticancer agents. Their antitumor activity has been linked to their ability to induce gene expression through acetylation of histone and nonhistone proteins. DNA double-strand breaks (DSBs) are the lethal lesions induced by ionizing radiation and the majority is repaired by either non-homologous end-joining (NHEJ) or homologous recombination (HR). The modulation of DNA damage repair by HDACi could be one of the underlying mechanisms for the radiosensitizing effect in cancer cell lines.
Objectives: To study the mechanism of the radiosensitizing effect of valproic acid in an anaplastic thyroid cancer cell line (8505c).
Methods: Cells were incubated with 1 mM VA and irradiated with a source of gamma rays. Radiation response was analyzed by clonogenic assay. Gene expression was assessed by real time PCR at 30 min and 4 hours after irradiation. **Results:** A radiosensitizing effect was observed with a reduction of survival fraction at 2 Gy from 0.28 to 0.20 in the treated cells ($p<0.05$). The expression of the genes of the NHEJ pathway Ku80 ($p<0.01$), Ku70 ($p<0.001$) and DNA-PKc's ($p<0.01$) was downregulated 4 hours after irradiation by VA. Moreover, when we studied the expression of the HR´s genes, we observed a significant downregulation in Mre11 ($p<0.01$) and RPA2 genes ($p<0.05$). On the contrary, no significant changes were obtained for the expression of BRCA2 and Rad51. **Conclusion:** Our results suggest that VA could lower the cell´s capacity to repair radiation-induced DNA damage by affecting the DNA repair pathways, NHEJ and HR.

434. (039) **IL-6 INTRACELLULAR SIGNALING IN NATURAL AND INDUCED SENESCENT CELLS**

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Senescence is defined as a growth arrest program that preserves physiological cell functions. It has been associated with tumor suppressor mechanisms as well as a resistance mechanism to evade tumor eradication. Interleukin-6 (IL-6) is part of the secretome of senescent cells denominated senescence associated secretory phenotype (SASP).
In previous work we demonstrated the relevance of the intracellular IL-6 signaling *in vitro* and *in vivo* in a model of senescent pituitary tumor cells. In this work we focus on the characterization of this signaling in a natural and therapy-induced senescent model.
In MtT/S cells, a naturally senescent pituitary somatotroph cell line, to discriminate the effect of intracellular action of IL-6 we inhibited secretory pathway using two independent approaches: a) overexpression of a dominant negative form of Rab11 which is a key molecule in anterograde transport, involved in IL-6 associated vesicle exocytosis and b) treatment with brefeldin A (BFA) 100 ng/ml, a drug that blocks intracellular vesicle transport from endoplasmic reticulum to Golgi.
In both cases we observed an increase in the senescent biomarkers pRb and $p16^{INK4}$ (measured by western blot) and the activity of senescence associated β galactosidase (SA-β-Gal) ($n=4$, $p<0.05$). To investigate the subcellular localization of the molecules involved, we performed confocal images of MtT/s cells transfected with fluorescent tagged plasmids of IL-6 and IL-6 receptor and observed spatial association of both with structures related to anterograde traffic.
We next studied another model of senescence, therapy-induced senescent pulmonary A549 cells treated with doxorubicin (Dox) 132 nM. In these cells stimulation and retention of IL-6 (verified by western blot) with IL-1β 20 ng/ml and BFA also increases the senescent profile according to the biomarkers: increase in $p21^{Cip1}$ and decrease in pRb.
We conclude that IL-6 regulates senescence through a specific intracellular signaling pathway that could represent a general mecha-

nism for tumoral senescent cells.

435. (048) **DELTA-TOCOTRIENOL ENHANCES THE ANTITUMORAL EFFECTS OF INTERFERON ALPHA THROUGH ROS PRODUCTION AND ERK PATHWAY IN HEPATOCELLULAR CARCINOMA CELLS**

Lucci A[1,2], Vera M[2], Comanzo C[2], Palma NF[2], Ferretti A[1], Ceballos MP[1,2], Quiroga AD[1,2], Alvarez ML[1,2], Carrillo MC[1,2]
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Our group has postulated the supplementation with δ-tocotrienol (E) to interferon alpha (IFN) therapy as a strategy to treat liver cancer because we have shown that the combined treatment inhibits cell growth and metastatic properties, and increases apoptosis in SK Hep-1 (SK) and Huh7 hepatocarcinoma (HCC) cell lines.
In this work we explored the signaling pathways involved in the studied processes.
Our hypothesis is that reactive oxygen species (ROS) and Erk activation participate in the effects produced by E supplementation to IFN therapy.
We treated SK and Huh7 cells with 10000 and 20000 U/l IFN, respectively, 25 uM E, or the combination of both drugs (IFN-E-group). Also, we used 4 mM N-acetyl cysteine (NAC), a reductor compound, and 5 uM PD98059 (PD), an inhibitor of Erk phosphorylation .Treatments were carried out for 72 h. We performed: a) dichlorofluorescein assay to evaluate ROS production, b) western blot studies to analyze phospo and total Erk expression, and c) MTT assay to determine cell viability. Results: IFN-E-group showed a higher increase in ROS production in SK (+500%*) and Huh7 (+350%*) cells, an increase in phospo Erk/ total Erk ratio (SK: +82%* and Huh7: +95%*), and a significant decrease in cell viability (SK:-58%* and Huh7:-52%*). The decreases in viability were parcially restored with PD (IFN-E-PD-group, SK: -25%#, Huh7: -24%#) and with NAC (IFN-E-NAC-group, SK: -27%#, Huh7: -15%#). *p<0.05 vs control; #p<0.05 vs IFN-E-group. In summary, we demonstrate that the effects produced by the addition of E to IFN therapy are mediated, at least in part, by ROS production and Erk pathway. Knowing the mechanisms that operate when cytokines and vitamins are combined to reduce tumorigenesis opens potential clinical targets against HCC.

436. (049) **DELTA TOCOTRIENOL POTENTIATES THE INHIBITORY EFFECTS OF INTERFERON ALFA 2-B ON PROLIFERATION, MIGRATION AND INVASION IN HUMAN EA.HY926 UMBILICAL VEIN CELLS**

Lucci A[1,2], Capocasa J[1,2], Vera M[2], Comanzo C[2], Palma NF[2], Ferretti[1] A, Ceballos MP[2], Quiroga AD[1,2], 1,2Alvarez ML[1,2], Carrillo MC[1,2]
[1]Área Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario y CONICET
[2]Instituto de Fisiología Experimental (IFISE), CONICET

Our group has postulated supplementation with δ-tocotrienol (E) to interferon alpha (IFN) therapy as a strategy to treat liver cancer because the combined treatment inhibits cell growth and metastatic properties and increases apoptosis in hepatocarcinoma cells.
In this work we studied the effects of the combination of IFN and E on normal angiogenesis, using EA.hy926 human umbilical vein endothelial cells.
Our hypothesis is that E supplementation to IFN therapy has significant advantages in the angiogenesis process compared to individual IFN treatment.
We treated EA.hy926 cells with 10000 U/l IFN (IFN-group), 12,5 uME (E-group), the combination of both drugs (IFN-E-group) or vehicles (C-group). We performed the MTT assay to determine cell viability at 72 h, constructing the dose-response curves to calculate the IC50 values. Combination index was also calculated (Compusyn). Besides, we performed the wound healing assay to determine migration at 6 h and invasion assays in transwell chambers at 24 h. The results were tested by one-way ANOVA, followed by Tukey's test (3 independent experiments; n=4 in each one). As expected, IFN-E-group showed a higher decrease in cell viability (-63%*#) compared to monodrug therapy: IFN-group (-22%*), E-group (-21%*). Combination index showed synergism between IFN and E. In the migration assay, IFN-E-group did not show a significant decrease (-16 %*) compared to monodrug therapy: IFN-group (-16%*) and E-group (-14%*). Finally, IFN-E-group showed a significant diminution in the invasion assay(-96 %* #) compared to monodrug therapy: IFN-group (-33%*) and E-group (-87%*). * p<0.05 vs C-group; #p<0.05 vs monotherapies. In summary, we demonstrate that addition of E to IFN therapy reduces proliferation, migration and invasion of human EA.hy926 endothelial cells, processes that are necessary for normal angiogenesis. In this regard, combined treatment might open a potential clinical target against angiogenesis in the future.

437. (053) **GEF-H1 DRIVES TUMOR FORMATION AND METASTASIS IN BREAST CANCER CELLS**

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RhoGTPases family is involved in several biological process including gene transcription, cell polarity, migration and invasion. RhoGTPases switch between on and off states and are regulated by several GEFs (activators) and GAPs/RhoGDIs (inactivators).
The aim of this work is to study the role of a particular RhoA-GEF, GEF-H1, in breast cancer (BC) progression. We observed by immunostaining a significant increase in GEF-H1 protein expression in BC human biopsies compared with non-tumoral tissue (n=76, p=0.0287). In addition, GEF-H1 expression correlated with the invasive potential of human and murine BC cell lines. To further study the role of GEF-H1 in tumor development, we generated GEF-H1- knock out (KO) BC cells using CRISPR/Cas9 technology. A decrease in proliferation, migration, invasion and anchorage-independent colony formation rates was observed in GEF-H1-KO cells compared to wild type (WT) cells (p<0.001). These results correlated with reduced focal adhesion formation and its downstream signalling. Furthermore, BALB/c mice were subcutaneously inoculated with GEF-H1 KO cells, showing a significant delay in tumor formation (p<0.01) and lung metastasis development compared with mice inoculated with WT cells.
These results demonstrate that GEF-H1-RhoA activation mediates the signalling pathways involved in controlling cell proliferation, migration and invasion of BC cells. In vivo assays and human biopsies studies suggest that GEF-H1 expression in BC cells might indeed contribute to tumor progression.

438. (057) **ANTICANCER ACTIVITY OF NOVEL COPPER(II) COMPLEX WITH A SCHIFF-BASE LIGAND ON IN VITRO AND IN VIVO OSTEOSARCOMA CANCER MODELS**

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Osteosarcoma (OS) is the most common bone malignant tumor, affecting mainly children and young adults. Cisplatin has been effective for the treatment of different solid tumors, including OS. However, cisplatin treatment often results in the development of chemoresistance and several side effects, leading to therapeutic failure. In this sense, copper compounds have shown to be potentially effective as antitumor agents, attracting increasing interest as alternatives to usually employed platinum derived drugs.
The aim of this work is to evaluate the *in vitro* and *in vivo* antitumoral activity against MG-63 cells of the nitrate salt of a novel Cu(II) cationic complex containing a tridentate hydrazone ligand, Cu(HL)

for short.
Cytotoxic activity on MG-63 cell line was evaluated in 2D (monolayer) and 3D (spheroids) models. Cu(HL) significantly reduced cell viability after 24 h treatment in both models (IC_{50} 2D: 1.98 ± 0.51 μM; 3D: 9.05 ± 1.0 μM) ($p<0.001$). Further studies demonstrated that Cu(HL) inhibits cell proliferation and conveys cells to apoptosis, determined by flow cytometry. Cu(HL) showed a great genotoxicity, evaluated by comet assay.
Finally, we assessed *in vivo* anticancer activity in animals bearing growing OS s.c. xenografts. Treatment during 4 weeks with Cu(HL) (2 mg/kg i.p. three times per week) markedly impaired tumor progression, enhancing necrosis and reducing tumor growth rate and mitotic index ($p<0.01$). Treatment with an equivalent low dose of reference metallodrug cisplatin (2 mg/kg i.p. three times per week) failed to inhibit tumor growth.
Taken together, these results show that Cu(HL) has a promising anticancer activity against *in vitro* and *in vivo* OS models.

439. (058) **IMPACT OF IDH MUTATIONS ON THE IMMUNOLOGICAL LANDSCAPE OF GLIOMAS: A TCGA META-ANALYSIS BASED ON THE 2021 WHO CLASSIFICATION OF BRAIN TUMOURS**

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Mutations in the enzyme isocitrate dehydrogenase genes (mIDH) are currently used to classify diffuse gliomas, the most common malignant primary brain tumours in adults. Additional genetic lesions led to the most recent WHO classification that allows stratification in four tumor entities, mIDH gliomas [Oligodendrogliomas (OD) and Astrocytomas (AA)], and wtIDH gliomas: [Glioblastoma (GBM)-like and GBM]. While mIDH clearly correlates with better prognosis, the role of this mutation in antitumor immunity remains controversial. First, we predicted the level of infiltrating immune and stroma cells by ESTIMATE scores and both were significantly lower in mIDH patients. We also found reduced expression of immunoregulatory genes (PD-LI, PD-1, CTLA-4, IDO1, IL-10, LAG3 and TIM3) in mIDH biopsies ($p<0.05$, vs. wtIDH). Moreover, PD-L1 exhibited a strong negative correlation with mIDH1 in the whole set of mIDH gliomas ($p<0.05$). However, within this group, this correlation is completely lost in AA (Spearman r: 0.06) in comparison with OD (Spearman r: -0.46, $p<0.05$). The analysis of gene signatures of tumor-infiltrating immune cells indicated that lymphocyte populations and antigen presenting cells were downregulated in mIDH tumors ($p<0.05$, vs. wtIDH). However, once again, we found differences within the mIDH group, suggesting that OD exhibit an even colder immunophenotype than AA. Finally, we analyzed the correlation between the expression of IDH1 and MGMT or ATM, DNA repair enzymes that affect chemo- and radio-resistance, respectively. We found a significantly negative correlation between IDH1 and MGMT that is lost in GBM biopsies. In contrast, IDH1 exhibited a significantly positive correlation with ATM in mIDH gliomas but a negative one in GBM. Our observations suggest that the immune landscape of gliomas not only differs due to mIDH status, but also within glioma subtypes, supporting the idea that the overall effect of this genetic lesion depends on the cellular context.

440. (069) **cAMP EFFLUX INHIBITION BY NSAIDS: DRUG REPOSITIONING FOR PDAC TREATMENT**

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In a previous work, we validated the inhibition of MRP4-dependant cAMP extrusion process as a promising therapeutic strategy for Pancreatic Ductal Adenocarcinoma (PDAC). In view of the therapeutic challenge associated with PDAC, we set out to search and characterize approved drugs that inhibit cAMP transport with the goal of establishing a repositioning strategy. Based on the results of this screening, we selected the Non-steroidal anti-inflammatory drugs (NSAIDs) as an interesting pharmacological family to inquire for rational drug repositioning. NSAIDs have been tested in the past as co-adjuvants in the therapy of various types of cancer, in many cases with positive results. Although their effects that depend on cyclooxygenase-2 inhibition are well described, the effects that are independent of this inhibition are far for being clear. We hypothesize that MRP4 inhibition could be a missing link in the overall action on tumor progression of these compounds. In this work, we measure the intracellular cAMP response upon treatment with 13 different NSAIDs using a technique developed in our laboratory in which we use HEK-293T cells stably transfected with the EPAC-SH187 sensor. Ibuprofen, acetyilsalicylic acid, Naproxen, Indomethacin, Diclofenac, Dexketoprofen and Ketorolac have shown to increase intracellular cAMP concentrations upon treatment ($p<0.01$). The concomitant significant reduction of extracellular cAMP upon treatment with these NSAIDs was also measured using a Radio-Binding Protein assay (RBP), which confirmed cAMP transport inhibition as one of the mechanisms that triggers intracellular cAMP increment ($p<0.05$). On the other hand, Celecoxib, Acetaminophen, Dipyrone, Phenacetin, Meloxicam and Piroxicam failed to increase intracellular cAMP upon treatment. These emerging results, together with an exhaustive literature search will allow us to select our repositioning candidates to continue with its characterization regarding its therapeutic potential in PDAC.

441. (070) **iNOS INHIBITOR S-METHYLISOTHIOUREA AFFECTS GLIOBLASTOMA STEM CELL NICHE, WITHOUT AFFECTING DIFFERENTIATED TUMOR CELLS**

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Introduction: Glioblastomas (GBM) are the most common and aggressive brain tumors. Despite the traditional chemotherapy with temozolomide (TMZ) and radiotherapy, patients' survival does not exceed 2 years. It has been suggested that the selective inhibition of inducible nitric oxide synthase isoform (iNOS) enzyme is related to a decreased proliferation of GBM cells.
Objective: Evaluate the effect of iNOS specific inhibitor, S-methylisothiourea (SMT), alone or in combination with TMZ, on GBM stem cell (GSC) niche (spheres) and on more differentiated cells (monolayer and spheroids).
Methodology: Human GBM cell lines LN229, U251 and U87 were seeded in monolayer, as spheroides (hanging drop aggregation assay) and under sphere conditions (low adhesion and high dilution). Viability in 2D was determined by MTS. For spheroids' growth monitoring, they were measured in their diameter every week using imageJ. The number of GSC was established by sphere forming efficiency (SFE) in relation to the seeded cells, and the diameter of the spheres was also measured.
Results: In monolayer and in spheroids, SMT (50 μM) marginally reduced LN229 cell line growth (20-5% inhibition); however, in GSC niche, it decreased significantly the SFE in the three lines (inhibition, LN229 42%; U251 61%; U87 48%) and their diameters (inhibition, 33%, 17% and 28%, respectively). The combination of SMT (50 μM) with TMZ (250 μM), further inhibited SFE (LN229 57%, U251 70%, U87 50%) compared to SMT or TMZ alone.
Conclusion: iNOS inhibitor in combination with TMZ therapy, could be useful in reducing GSC growth . Further studies on mechanisms of action will establish the differences observed between different GBM cell lines.

442. (079) **BRAF AND HISTONE 3 ALTERATIONS IN THE INTEGRATED DIAGNOSIS OF PEDIATRIC GLIAL AND GLIONEURONAL TUMORS: A SINGLE CENTER EXPERIENCE**

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While tumors of the central nervous system account for over 20% of pediatric tumors, gliomas represent more than 55% of them. Their classification into low (LGG) or high grade gliomas (HGG) may reflect survival odds. Molecular techniques enable more accurate diagnostic results and risk stratification. Molecular alterations in *BRAF* gene and histone 3 genes (H3) were evaluated by FISH, IHC and Sanger sequencing, in 102 pediatric glial and glioneuronal tumors. BRAF and/or H3 were assessed in LGG or HGG according to WHO recommendations. Results were correlated with clinical and histological findings to evaluate them as diagnostic and prognostic tools. The *KIAA1549-BRAF* gene fusion was relevant as a diagnostic tool for Pilocytic astrocytoma, a LGG,(43/64 cases), but was not related to progression free survival (PFS) and overall survival (OS) (Kaplan Meier, Log-rank
(Mantel-Cox) Test; P>0.05). This fusion showed no association with different age groups (10
< years old ≥ 10, Fisher's exact test; P>0.05), but was more prevalent in the cerebellum (Chi square test; P=0.04). The BRAFV600E mutation occurred preferentially in the brain hemispheres 7/10 cases (Fisher´s exact test; P=0.004) and was associated with a shorter OS (P=0.0082), but not with a decreased PFS (P=0.14) in LGG. When only considering Pilocytic astrocytomas, it was associated with a decreased OS and PFS (P<0.0001 and P=0.0135, respectively). All HGG of the midline were positive for H3K27M mutation, while the H3G34R mutant cases were located in brain hemispheres. The H3K27M mutation in HGG was associated with decreased PFS and OS (P=0.0124 and P=0.006, respectively).
Assessing druggable molecular markers with prognostic value is of paramount importance particularly in those cases where complete resection or further radiation therapy is not possible due to critical-location of the tumor.

443. (081) **ADDITION OF β-BLOCKER PROPRANOLOL OR ANTIPARASITIC DRUG IVERMECTIN ENHANCES METHOTREXATE ANTICANCER ACTIVITY IN HUMAN OSTEOSARCOMA CELLS**

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Osteosarcoma (OS) is considered a clinical challenge due to its rapid progression and limited response to therapy. Despite being a first-line therapeutic strategy for OS, methotrexate (MTX), specially at high doses, can cause significant toxicity, including myelosuppression, nephro- and hepatotoxicity. In this context, the addition of cost-effective repurposed agents such as the non-selective β-blocker propranolol (PPN) or the antiparasitic drug ivermectin (IVM), could potentiate the therapeutic anticancer activity of MTX in OS, at lower and better tolerated doses.
The aim of this work was to evaluate the *in vitro* antitumoral activity of MTX in combination with PPN or IVM on highly aggressive MG-63 and U-2OS human OS cells.
Sensitivity to PPN, IVM and MTX was assessed obtaining IC50 values of 45.6 μM, 8.3 μM and 8.6 nM for MG-63 and 47 μM, 12.8 μM, and 58.6 nM for U-2OS cells, respectively. After 72h treatments, exponential growth of OS cells was significantly abrogated after combining different optimal and suboptimal concentrations of both PPN or IVM plus MTX. However, stronger synergistic cytostatic effects (CI<1) and consistency among experimental OS models were observed after the addition of PPN to MTX. Furthermore, a potent inhibitory effect on MG-63 cell chemotaxis was observed combining PPN (50 μM) with MTX (10 nM), reducing migratory capacity by 50% in comparison to vehicle-treated cells (p<0,0001). At a metabolic level, lactate production rates were also inhibited, reducing final lactate concentration by nearly 30% after a 24h treatment using PPN+MTX (p<0,0001). Finally, by using suboptimal concentrations of PPN (10 μM) with MTX (1 nM), colony-forming ability and clonogenic growth were inhibited by 30% and 60%, respectively (p<0,0001).
These preliminary studies show synergistic activity of MTX plus IVM or specially PPN in OS human cells. Further preclinical research evaluating different dosing schemes *in vivo* is warranted.

444. (087) **CHARACTERIZATION OF AHCYL1 AND KI67 EXPRESSION IN NON-SMALL-CELL LUNG CANCER**

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Lung cancer is an extremely malignant disease due to its metastasis and recurrence. In particular, non-small cell lung cancer (NSCLC) is characterized by high cellular and molecular heterogeneity. Recent research has reported that AHCYL1 (adenosylhomocysteinase-like 1) protein could be involved in the regulation of autophagy and tumor cell proliferation processes. We have previously shown that AHCYL1-depleted cells have higher tumorigenic capacity; however, the expression and the potential role of AHCYL1 in lung cancer remain unclear. We explored the expression of AHCYL1 and Ki67 (a cell proliferation marker and indicator for poor prognosis in many cancers) in patients with NSCLC by immunohistochemical analysis; along with transcriptomic data analysis of public databases. RNA-seq data revealed that AHCYL1 is expressed in all stages of lung cancer, and notably, it shows an inverse correlation with the expression of stemness-related genes. Proliferation marker Ki67 also showed an inverse correlation with AHCYL1 (p<0.0001). At the protein level, the examination of 26 primary NSCLCs samples, mainly adenocarcinomas, revealed that AHCYL1 expression was inversely correlated with the histologic tumor grade score (p=0.011) and Ki67 expression (p=0.002). These results indicate that AHCYL1 is mainly expressed in low-grade and less proliferative tumors. Future investigation of the role of AHCYL1 will give an insight as a potential therapeutic target for the treatment of lung adenocarcinomas.

445. (093) **REGULATION OF SPHINGOSINE KINASES AND SPHINGOSINE-1-PHOSPHATE RECEPTORS EXPRESION IN MELANOMA CELLS RESISTANT TO VEMURAFENIB**

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Over the last years, the incidence of melanoma, the deadliest form of skin cancer, has risen faster than any other cancer type. Considering that half of the patient's exhibit the BRAFV600E mutation, therapies with BRAF and MEK inhibitors (BRAFi/MEKi) showed an impressive success rate. Unfortunately, treatments are marginally effective since tumors quickly become resistant. In that regard, accumulating evidence supports that sphingosine-1-phosphate (S1P) is linked to multiple mechanisms leading to cancer progression and resistance. Thus, the aim of this study was to evaluate how vemurafenib (BRAFi) resistance affects the expression of sphingosine kinases (SphK) and S1P receptors (S1PR) in melanoma. To this end, we generated vemurafenib-resistant melanoma cells by continuous exposure of parental sensitive cells to increasing concentrations (0,01μM – 1μM) of the drug for 3 months. Previously, we showed that vemurafenib-resistant Lu1205 melanoma cell (Lu1205R) exhibit higher IC50 and pERK levels than their sensitive parents (Lu1205S). Here, we extended those studies to A375 melanoma cells. Certainly, A375R cells also showed increased resistance and pERK, but not pAKT levels. Expression of SphK and S1PR in both cells lines was evaluated by RT-qPCR. Surprisingly, the modulation of SphK1, S1PR1 and S1PR3 expression differ in Lu1205R and A375R cells. In addition, a bioinformatic analysis was performed using the public Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24862). This study showed a differential gene expression pattern in other cell lines, including A375, supporting the concept that melanoma heterogeneity may trigger different mechanisms of resistance to BRAFi therapy. In summary, although our results indicate that S1P signaling may have a role in vemurafenib resistance, further studies will be necessary to elucidate its importance.

446. (103) **PIN-POINTING THE KEY PLAYERS IN METABOLIC REWIRING OF PROSTATE TUMOR CELLS TOWARDS PROGRESSION IN THE BONE NICHE**

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Metabolic rewiring is associated with the metastatic cascade, and communication between tumor cells and the bone niche is determinant for tumor progression. Here, we sought to identify key metabolic genes that fuel prostate cancer (PCa) bone metastasis. By an indirect transwell co-culture system of PCa (PC3) and bone progenitor cells (MC3T3 or Raw264.7) we assessed the transcriptomic profile of PC3 cells modulated by soluble factors released from bone precursors. Strong activation of lipid metabolic pathways including PPAR and PI3K-Akt (P<0.05) was observed in PC3 cells. Next, we selected the altered metabolic genes for an unsupervised clustering analysis using transcriptomic data from human PCa and bone metastasic samples (GSE74685). Interestingly, those genes could cluster PCa patients in two defined groups: primary PCa and bone metastasis, highlighting that the early transcriptional metabolic alterations triggered in our co-culture model could discriminate primary tumors from bone metastatic samples. Further, the expression levels of four lipid associated genes (*VDR*, *PPARA*, *SLC16A1* and *GPX1*) could be independent risk-predictors of death (HR: 4.96, 2.85, 3.93 and 3.67, respectively; P<0.05), and that the combined expression of these four genes correlates with a worst outcome in metastatic patients (HR: 2.65, P<0.05) (SU2C-PCF data set). Further, we identified PKA as a master regulator of this lipid-associated signature (Ingenuity Pathway Analysis). Secretome analysis (ESI MS/MS) of conditioned media from these co-cultures revealed critical soluble factors secreted by bone progenitors (Col1a2, Fn1 and Cacna2d1) which could regulate PKA activity to promote the metabolic rewiring of PCa cells. Overall, we identified a novel lipid gene signature triggered during the communication between PCa and bone cells that appears to be critical for survival in PCa patients, pointing out to new attractive druggable targets for the disease.

447. (104) **MECHANISM OF ACTION OF A TRIAZOLYL PEPTIDYL PENICILLIN IN MELANOMA CELLS AND SYNERGISTIC ANTITUMOR EFFECT OF ITS COMBINATION WITH THAPSIGARGIN**

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In a previous study, we demonstrated that TAP7f, a synthetic triazolylpeptidyl penicillin, behaves as an effective antitumor agent and induces an apoptotic response in murine melanoma cells. In this work, we comparatively examined its mechanism of action in murine B16-F0 and human A375 melanoma cells. We first studied the contribution of an endoplasmic reticulum (ER) stress response to the apoptotic effect induced by the derivative. To this end, the expression levels of different ER stress-related proteins were evaluated by Western blot assays in both melanoma cell lines. A significant increase in the amount of ATF4 (≈1.5-2 fold), GADD153/CHOP (≈1.5-2.5 fold), Calnexin (≈2 fold) and GRP78/BIP (≈2-3.5 fold) was observed after incubating B16-F0 cells for 3 h or 6 h with a 20 μM concentration of TAP7f (p<0.05). A similar effect was observed in A375 cells for some of these ER markers. It was also demonstrated that TAP7f-induced activation of p38, JNK and PI3K-I/Akt signaling pathways occurred downstream ER stress. Based on the effectiveness of combined therapies for cancer treatment, we decided to investigate the *in vitro* antiproliferative effect of TAP7f with thapsigargin, a well-known ER stress activator. The simultaneous incubation of different concentrations of the two compounds showed a higher inhibition of cell growth with respect to the effect of each individual agent, both in B16-F0 and A375 melanoma cells. The quantitative analysis of dose-effect curves obtained by using Compusyn software rendered combination indexes lower than 1 (0.48-0.59 for B16-F0 and 0.41-0.76 for A375), indicating synergism (p<0.01). In conclusion, we showed that induction of ER stress and activation of p38, JNK and PI3K-I/Akt pathways are involved in the antitumor effect induced by TAP7f in melanoma cells. The efficacy of the combination of TAP7f with thapsigargin suggested that this therapy could be considered an auspicious tool for melanoma treatment.

448. (105) **SOLUBLE GUANYLYL CYCLASE BETA1 SUBUNIT OVEREXPRESSION DECREASES CELL CYCLE PROGRESSION, PROLIFERATION AND MIGRATION IN ECC-1 AND HELA TUMOR CELL LINE**

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Soluble guanylyl cyclase (sGC) is a heterodimeric enzyme constituted by two subunits, alpha1 (a1) and beta1 (b1). Previously we showed that a1 subunit promotes tumor cell growth, survival and migration in ECC-1 and HeLa tumor cell lines, while b1 role remains unclear. b1 subunit protein levels are slightly reduced in most biopsies from human hormone-dependent malignant tumors. Here we investigate the effects of b1 on cell cycle, migration and protein expression in ECC-1 and HeLa cells.

a1 and b1 were silenced through siRNAs. b1 was overexpressed by using an adenoviral vector carrying the complete sequence of b1 (sGCbeta1-GFP) or empty virus as control (C). Multiplicity of infection (MOI) ranging from 100 to 750 was used. 6 h after virus infection, cells were incubated with complete media for 24-48 h. After treatments, cell cycle was studied by flow cytometry. Cell migration was determined by scratch motility assay. Protein expression was studied by western blot.
In ECC-1 cells, a1 knock-down increased b1 protein levels (2.9 fold-increase vs C, p<0.05). b1 knock-down increased PCNA protein expression (1.97 fold-increase vs C, p<0.05). b1 overexpression augmented the percentage of subG0/G1 DNA content (48h; % of cells as % of C; ECC-1, MOI 250:193.4±8.3*; MOI 750:232±16**; HeLa, MOI 250:151.2±11.2*, MOI 750: 150±9*, *p<0.05, **p<0.001 vs C) and in G2/M phase (ECC-1, MOI 250:130.3±8.1*; MOI 750:120.3±11.2**; HeLa, MOI 250:156.67±10.4*, MOI 750:136.8±9.5*, *p<0.05, **p<0.001 vs. C). Additionally, b1 overexpression decreased cell migration measured by wound closure (24h, ECC-1, % of closed wound as % of C; MOI 250:71.9±2.3**; MOI 250:32.05±1.78***, **p<0.01, ***p<0.001 vs C).
Our results suggest that b1 negatively regulates cell proliferation and migration. Besides, a1 and b1 subunits expression seem to be inversely related supporting the opposite role of each protein in tumor biology.

449. (107) **KETONE BODY METABOLISM PROMOTES PROSTATE CANCER CASTRATE RESISTANT PROGRESSION**

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The mechanisms underlying prostate cancer (PCa) castration resistance (CRPC) after androgen deprivation therapy (ADT) include those that reactivate androgen receptor (AR), or those that are entirely independent or cooperate with androgen signaling to underlie PCa progression. Several studies have discussed the fact that a dietary restriction, such as in ketogenic diets (KD), impairs tumor growth rate, however, the intricacy of metabolic pathways associated with PCa progression remains elusive. Here, using PCa patient-derived xenografts (PDXs) mimicking the response of the human donor to ADT, we performed a comprehensive metabolomic analysis of PCa PDXs that relapsed following castration. We discovered a metabolic shift from high glycolytic activity to exacerbated ketone body (KB) metabolism. These results indicated that a subpopulation of CRPCs that progresses with partial or complete loss of AR dependence are fueled by KB. We confirmed (IHC) that the expression of critical ketolytic enzymes (*ACAT1*, *OXCT1*, *BDH)* was significantly augmented after castration-resistant progression in both the PDX tumor and its human donor tissue (P<0.05). Further, in an *in silico* approach (n=986), increased expression of ketolytic enzymes was also observed for a subset of PCa patients that relapsed with low AR and ERG expression. Moreover, expression of these factors was also associated with decreased time to biochemical relapse and decreased progression-free survival.
In summary, our studies reveal KB as key metabolites fueling castration resistant progression in the context of a partial or complete loss of AR dependence. This subpopulation of PCas with high ketolytic enzymes may influence the effectiveness of a KD. Thus, assessing in combination both, the KB content and the expression levels of these enzymes, may be crucial in stratifying PCa patients for which a KD may prove unsuccessful to halt disease progression.

450. (110) **REPURPOSED HEMOSTATIC AGENT DESMOPRESSIN (DDAVP) IMPAIRS TUMOR AGGRESSIVENESS AND METASTATIC PROGRESSION IN TRIPLE-NEGATIVE BREAST CANCER (TNBC)**

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Desmopressin (dDAVP) is a repurposed hemostatic drug in oncology that acts as a selective agonist for the AVPR2 receptor present in blood microvessels and some tumor cells. Preclinical data show that compound triggers cytostatic mechanisms in malignant cells, impairing angiogenesis and metastatic progression. It is known that triple-negative breast cancer (TNBC) is associated to poor prognosis due to limited response to therapy and metastatic relapse. Considering the unsatisfied clinical needs of TNBC, we evaluated the antitumoral activity of dDAVP on aggressive preclinical models of TNBC alone or in addition to chemotherapy. For that purpose, we used human (MDA-MB-231; MDA-MB-468) and murine (F3II) TNBC cells. MCF-7 cells were used as a hormonedependent less aggressive model.
In vitro, dDAVP significantly reduced clonogenic and 3D growth, viability and chemotaxis of AVPR2-expressing TNBC. Cytostatic effects of dDAVP were associat-ed to altered actin cytoskeleton dynamics and differen-tial expression of migration, angiogenesis and metastasis-related genes. In the MCF-7 cell line we ob-tained equivalent results.
In silico, we demonstrated a positive prognostic impact of AVPR2 expression in Basallike BC and we assessed the AVPR2 target expression in TNBC xenografts and clinical BC samples. Using syngenic and xenogenic animal models synergistic effects were observed after combining dDAVP with taxane or alkylating therapy. In mice bearing TNBC tumors combined therapy resulted in greater inhibition of tumor progression, reduction of skin infiltration and metastatic spread to lungs. Statistical analyses were performed using the PRISM8, T-test or ANOVA, p<0.05
In conclusion, agonist activation of AVPR2 using dDAVP represents an achievable and interesting therapeutic approach to modulate TNBC aggressiveness. dDAVP could be used as a coadyuvant agent for treating this disease, not only in combination with chemotherapy but also administered during the perioperative setting.

451. (113) **IN VITRO EXPLORATION OF DIFFERENT REPURPOSED AGENTS FOR COLORECTAL CANCER TREATMENT: SYNERGISTIC COMBINATIONS AS POTENTIAL CO-ADJUVANT STRATEGIES**

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Metastatic colorectal cancer (mCRC) still stands as a challenge for public health. In this setting, repurposed drugs arise as cost-effective co-adjuvants strategies to the standard chemotherapy regimens for mCRC. Considering the wide variety of therapeutic benefits previously reported for other tumor types, we aimed at exploring the antitumor activity of five rationally selected repurposing drugs; ivermectin (IVM), atorvastatin (ATV), benznidazole (BDZ), propran-

olol and losartan in aggressive CRC CT-26 cells. *In vitro* sensitivity to the selected compounds was assessed on rapidly growing cell cultures finding heterogenous IC50 values ranging between 10-800 μM. Combinational studies were carried out with IVM, ATV and BDZ based on their clinically-relevant IC_{50} values and their known impact on drug resistance reversal, immune modulation and hypoxia-activated cytotoxicity. The addition of IVM to ATV, exerted a synergic inhibitory effect (*combination index* <1, Compusyn software) over the proliferation of CRC cells, while combined treatment of IVM and BDZ in a hypoxic setting did not provide further therapeutic benefit. Combined concentrations of IVM and ATV at a low μM range were also found to reduce 75% of transwell cell migration, to completely abrogate low-density cell growth, as well as significantly decrease glucose uptake and lactate production. IVM (1 μM) plus ATV (5 μM) dramatically impaired clonogenic growth, alone or combined with oxaliplatin (1 μM), improving the cytotoxic monotherapy by nearly 25% (ANOVA, $p<0.05$) On a preliminary safety protocol in Balb/c mice, the administration of concomitant i.p. IVM (10 μg/kg) and ATV (20 μg/kg) thrice-weekly, was well tolerated and no signs of toxicity were observed. As a conclusion, IVM and ATV are interesting candidates for further in vivo preclinical exploration as co-adjuvant agents for CRC management, focusing their potential to modulate the tumor stroma and metastatic spread.

452. (114) **NICOTINIC MODULATION OF THE RESPONSE TO PACLITAXEL IN TRIPLE NEGATIVE BREAST CANCER CELLS**

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Previous evidences pointed to the participation of nicotinic acetylcholine receptors (nAChRs) in the effect of paclitaxel (PX) as an antitumor agent in lung cancer. However, less knowledge is available regarding the actions of PX on nAChRs in nearby organs such as those of the breasts. The addition of nicotine (NIC) confirmed the presence of functional nAChRs in mammary cells which mediated an increment in cell viability measured by MTT assay. This effect was more potent in MDA-MB231 triple negative tumor cells (189±2%) than in MCF-10A non-tumor cells (145±7%; $p<0.001$) and was blunted by nicotinic antagonists (10^{-6}M). We also confirmed that PX triggers tumor breast cell death in a concentration dependent manner, but the presence of NIC (10^{-10}M) totally reverted the action of PX CE50 ($4x10^{-7}$M) ($p<0.001$). The effect of NIC was also reduced by the presence of non-selective nicotinic antagonist, or α7 and α9 selective nicotinic antagonists, and also by the inhibition of protein kinase C, MEK1/2, ERK1/2 or NF-kB pathways linked to nAChRs ($p<0.05$). Apoptosis contributes in part to death action of PX on tumor cells. By flow cytometry we confirmed that PX increased apoptosis (control:6.1±0.7%, PX:16.7±1.4%; $p<0.05$) in MDA-MB231 cells which was significantly reduced by NIC treatment (10.3±1.6%; $p<0.05$). In conclusion, the activation of nAChRs increases breast cell proliferation and could be responsible either of tumorigenesis or malignization; it should be considered that NIC reduces PX effectiveness in tumor treatment probably due to an increment in the resistance to chemotherapy.

453. (127) **INHIBITION OF HO-1 ENZYMATIC ACTIVITY IMPAIRS HEAD AND NECK CANCER CELL SURVIVAL**

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We have previously reported that, in human HNSCC samples, hemeoxygenase-1 (HO-1) mRNA expression is up-regulated and it is associated with worst survival. We also reported an up-regulation of HO-1 protein levels and that it is localized in the cytoplasmic and nuclear compartments. Moreover, we demonstrated that pharmacological activation of HO-1 by hemin and genetic full-length HO-1 (FL-HO-1) overexpression increases HN13 cells survival and cell cycle progression, suggesting a protumor role of HO-1 in HNSCC. However, whether byproducts of HO-1 enzymatic activity are involved in FL-HO-1 mediated-effects remains unknown. In this study, we aimed to elucidate if inhibition of HO-1 enzymatic activity impacts on head and neck cancer cells behavior. To that end, HO-1 activity was inhibited pharmacologically using ZnPP and an enzymatically inactive FL-H25A-HO1-overexpressing HN13 cell line was established. We evaluated HO-1 expression by western blot and indirect immunofluorescence, cell viability by crystal violet, cell proliferation by manual cell counting, cell cycle progression by propidium iodide staining and flow cytometry, and cell migration by wound healing assay. We found that 10 μM ZnPP impaired cell viability ($p<0,001$ vs DMSO) at 48h and 72h as well as it diminished cell number at 72h ($p<0,01$ vs DMSO). Also, in such conditions, ZnPP induces overexpression of HO-1, which is localized in the cytoplasm. In line with the previously mentioned, we found that FL-H25A-HO1 HN13 cells have a lower growth rate ($p<0,001$) than FL-HO1 HN13 cells. We also found that the population of FL-H25A-HO1 HN13 cells have an increase in Go/G1 phase ($p<0,01$) and a decrease in G2/M phase ($p<0,05$) compared with FL-HO1 HN13 cells. Related to cell migration, FL-H25A-HO1 failed to alter migratory capacity ($p>0,05$ vs FL-HO1). In conclusion, our results show that the enzymatic activity of HO-1 plays a role in the FL-HO-1-mediated effects on head and neck cancer cell survival.

454. (130) **ROLE OF RACOTUMOMAB IMMUNOTHERAPY AND N-GLYCOLYLNEURAMINIC ACID (NEUGC)-RICH DIET IN CYTIDINE MONOPHOSPHO-N-ACETYLNEURAMINIC ACID HYDROXILASE (CMAH) KNOCKOUT HUMANIZED MICE BEARING LUNG CANCER TUMORS**

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NeuGc is a sialic acid molecule found in animal cell membranes as terminal components of glycoproteins and glycolipids. In most mammals including mice and apes, NeuGc synthesis is catalyzed by CMAH. However, humans lack such enzyme due to gene inactivation, being NeuGc obtained from dietary sources. Although healthy human tissues contain negligible levels, tumor cells are able to incorporate large amounts of NeuGc. Aggressive human neoplasms such as non-small cell lung cancer (NSCLC) can express NeuGc-containing gangliosides as cell surface neoantigens. The anti-NeuGc anti-idiotype monoclonal antibody racotumomab is approved for switch maintenance immunotherapy in patients with advanced NSCLC. We used CMAH knockout (CMAH-/-) mice to study the antitumor activity of racotumomab in the context of a humanized model and to further analyze the role of NeuGc-rich diet. CMAH-/- female mice were inoculated i.v. with 3LL Lewis lung carcinoma cells (10^5 cells/mouse) and surface lung nodules were counted 25 days later. Therapeutic immunization with weekly s.c. doses of racotumomab at 200 µg/dose formulated in aluminum hydroxide (racotumomab-alum) exhibited a significant antitumor activity against lung tumor nodules (Control: 14 ± 7 versus Racotumomab-alum: 6.5 ± 2.5, median lung nodules per mouse ± interquartile range; $p<0.01$, Mann-Whitney test) in CMAH-/- mice fed ad libitum with a NeuGc-rich diet, containing beef fat and cattle meat bone powder. On the contrary, no significant antitumor effects of racotumomab-alum immunization were observed in animals receiving a standard rodent chow with low NeuGc content. The present data suggest the importance of dietary intake of NeuGc-rich foods in the effectiveness of racotumomab immunotherapy using a humanized mouse model of NSCLC.

455. (132) **HEMEOXYGENASE-1 PLAYS A PROTUMORAL ROLE IN THYROID CANCER**

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We have previously demonstrated that hemeoxygenase-1 (HO-1) mRNA is overexpressed in papillary (PTC) and anaplastic (ATC) thyroid tumor compared to non-malignant areas to the tumor (NMT). We also demonstrated that HO-1 protein levels are up-regulated in human PTC samples, showing cytoplasmic localization, and such HO-1 tumor expression correlates with histological subtype. Now, we aim to study the role of HO-1 in thyroid cancer (TC) biology. To that end, we evaluated HO-1 expression by indirect immunofluorescence, cell viability by crystal violet method, cell cycle progression by propidium iodide staining and flow cytometry, and cell migration by wound healing assay. We found that pharmacological activation of HO-1 using 80µM hemin increased cell viability in TPC-1 ($p<0.001$) and 8505c ($p<0.001$) cell lines at 72h. In TPC-1, we found that hemin increased cell number in S- ($p<0,05$) and G2/M ($p<0,001$) phases and diminished cell number in Go/G1 phase ($p<0,001$) at 48h. In 8505c, hemin increased cell number in S- ($p<0,001$) phase and diminished cell number in Go/G1 ($p<0,01$) and G2/M ($p<0,001$) phases at 48h. In Nthy-Ori-3-1, a normal thyroid cell line, we found that 80µM hemin decreased ($p<0.001$) cell viability at 72h. Also, we found that 80µM hemin increased cell migration ($p<0.001$) in TPC-1 and 8505c cell lines. On the contrary, inhibition of HO-1 activity using 16µM ZnPP decreased cell viability in TPC-1 ($p<0.001$) and 8505c ($p<0.001$) cell lines at 72h while no differences were observed in Nthy-Ori-3-1 cells. In TPC-1, ZnPP diminished cell number in Go/G1 phase ($p<0,01$) and increased cell number in G2/M ($p<0,05$) at 48h. However, in 8505c, cell cycle progression remained unaltered after ZnPP treatment. Also, 16µM ZnPP reduced TPC-1 cell migration ($p<0.001$), but in 8505c ZnPP failed to alter migratory capacity. In conclusion, our results demonstrate that HO-1 plays a protumoral role in TC cells by altering cell survival, cell cycle progression and cell migration.

456. (133) **ORAL TONGUE SQUAMOUS CELL CARCINOMAS CAN BE DIFFERENTIATED BY TWO PATHWAY- SPECIFIC SIGNATURES THROUGH GENOMIC DATABASES ANALYSIS**

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Head and neck (HN) SCC show variations in aetiology, incidence rates, clinical progression and prognosis based on the anatomical site affected. Oral tongue squamous cell carcinoma (OTSCC) is one of the most common malignant tumors of the oral cavity and have a significantly worse prognosis than other sites. Recently we have reported that two stemness factors, KLF4 and SOX2, exhibit opposite expression profiles in oral SCC. We found that KLF4 has a differential expression pattern within OTSCC that depends on the subsite location of the SCC. Most high-throughput omic studies have consider OTSCC as a homogenous group. Aiming to identify putative subtypes of OTSSC based on differential activated signaling pathways we first performed an unsupervised cluster classification using TCGA-HNSC database (Firehose Legacy, 522 cases). Using the MD Anderson NG-CHM we inspected the RNA expression vs sample heatmap. 4 statistically different clusters were identified (1-4). Cluster 1 (168 cases) represents over 60% of the non-oral cases in the dataset. Only 7.7% OTSCC mapped in cluster 1, remaing ones are distributed on clusters 2, 3 and 4 (30, 70 and 17%). Next, OTSCC were grouped by cluster assignment and analyzed independently. Cluster assignment cannot be attributed to patient age, tobacco or alcohol consumption or any other clinical features (cBioPortal). OTSCC from clusters 2, 3 and 4 significantly differ in histologic grade (Chi-squared test, q-value <0.05). We selected all DEG with q-value <0.05. Three independent GO analyses were performed using Reactome Pathway Finder for DEG overexpressed in each cluster. DEG highly expressed on cluster 2 (1756 genes) are significantly enriched on keratinization pathway while cluster 3 (3752) fit to PD-1 signaling. DEG highly expressed on cluster 4 were not significantly integrated on a GO pathway. In summary, our study has identified 2 putative OTSCC subtypes based on differential activation of PD-1 and keratinization signaling pathways.

457. (134) **IMPACT OF HPV-16 E6/E7 SELECTIVE OVEREXPRESSION IN THE ANAL SQUAMOUS EPITHELIA OVER THE ANAL CARCINOGENESIS**

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Anal SCC is a rare malignancy of the lower gastrointestinal tract. However, it is one of the most common reported malignancy among people with HIV infection. Moreover, infection with human papillomavirus (HPV) has been demonstrated to be the primary causative agent in the development of SCC of the anogenital tract, including other cancers. HPV infection has been reported to be associated with 75% to 90% of anal SCC. The exact pathogenesis of anal SCC remains unknown. Previously, we have validated the K14CreER^{TAM} driver mouse line to target the anal mucosa. Aimed to selectively explore the role of HPV-related oncoproteins in anal carcinogenesis, without HPV virus infection, we developed a specific HPV-16 E6/E7 genetically engineered mouse model (GEMM) by crossing the K14CreER^{TAM} mice with the linker line Rosa26-rtTA-IRES-EGFP-rtTA^{flox} and then with Tet-E6/E7 mice (E6/E7-GEM). In this model expression is achieved, in a Cre-dependent manner, only after doxycycline (DOX) administration. Up to 2 months after induction of the system E6/E7-GEM (N=8) and their control littermates (N=8) did not present any overt phenotype without significant differences in survival. However, histological evaluation revealed an abnormal anal epithelium as well as tongue and vaginal mucosa changes. The anal epithelium developed hyperplasia with hyperorthokeratosis, acanthosis and increased proliferative activity in the basal layers. DOX withdrawal reverts the above mentioned proliferative phenotype. Also, we confirmed the expression of EGFP specifically in the anal mucosa targeted by this system upon tamoxifen treatment as well as E6 expression by immunohistochemistry in E6/E7-GEM. In addition, the increase in basal proliferation was confirmed through labeling with bromodeoxyuridine. We conclude that this GEMM can be further combined with pathway-specific manipulations to unveil the contribution of different regulatory elements involved in the anal carcinogenesis.

458. (138) **MIR-19B-3P AND MIR-101-3P: KEY PROSTATE CANCER ONCOMIRS WITH DIAGNOSTIC AND PROGNOSTIC VALUE**

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Prostate cancer (PCa) is the most commonly diagnosed male malignancy worldwide and the third cause of death by cancer in Argentinian men. High fat diet (HFD) and metabolic syndrome (MeS) increase PCa risk and aggressiveness. MiRNAs are small non-coding RNA molecules that regulate gene expression and can be secreted by tumor cells into the bloodstream. Our aim was to identify miRNAs-based biomarkers for diagnosis and prognosis of PCa associated with MeS. NSG mice chronically fed with HFD or control diet (CD) were s.c. inoculated with PC3 cell line. After tumor growth, mice were sacrificed, plasma and tumors were collected. PC3 xenograft from HFD-fed mice significantly increased the expression of hsa-miR-19b-3p,-320a-5p,-101-3p,-3613-3p,-1207-5p,-5095 and -2277-5p compared to CD-fed mice. Using DIANA-miRPath v3

and DIANA-TarBase v7 tools we found that these miRNAs modulate specific metabolic and cancer related pathways and more than 1,100 validated targets involved in proteoglycans in cancer and fatty acid biosynthesis. Furthermore, using bioinformatic tools and human datasets we demonstrated that these 2 miRNAs were significantly increased in the bloodstream of PCa patients compared to non-PCa volunteers, in prostate tumors compared to normal adjacent tissues (NAT) and in tumors of metastatic patients compared to tumors of non-metastatic patients. In addition, miR-101-3p was increased in exosomes isolated from blood of PCa patients. Finally, we established that 6 target genes that negatively correlate with miR-19b-3p in prostate tumor samples from patients were involved in Proteoglycan in cancer and Focal adhesion pathways and were down-regulated in tumors compared to NAT. No significant changes in the expression of miR-101-3p target genes were found in tumor compared with NAT. In summary, we propose miR-19b-3p and miR-101-3p as oncomiRs at tumor and metastatic site level, respectively, that can be useful as prognosis and diagnosis biomarkers.

459. (142) **HEME OXYGENASE 1 (HO-1) MODULATION OF METASTASIS AND STEMNESS PROCESSES IN PROSTATE CANCER**

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Within prostate cancer (PCa) tumors, there is a sub-population of cancer stem cells (CSCs) that enhances the tumor's capacity to proliferate and metastasize. Thus, stemness represents a therapeutic challenge in PCa. Heme oxygenase 1 (HO-1), the rate-limiting enzyme in heme degradation, is an essential player in cellular responses to pro-oxidative and pro-inflammatory insults. We have reported HO-1 strong anti-tumoral effect *in vivo* and *in vitro*, but its association with metastasis-stemness (M-S) is still poorly elucidated. In this work, we aimed at describing the effects of HO-1 overexpression on M-S processes in PCa. Clonogenic assays were performed to evaluate the effect of HO-1 induction on the stem properties of PCa cells. Results showed a significant reduction in colony formation after HO-1 pharmacological induction with hemin. RNA-Seq analysis was performed to assess differential gene expression of 144 M-S associated genes in PC3 hemin vs. PC3 control. We found a significant modulation of 32 markers related to these processes under HO-1 induction. We assessed the clinical significance of these genes through integrative bioinformatics analyses using public data repositories. The Oncomine database showed that 15/32 significantly HO-1-modulated genes were differentially expressed in prostate adenocarcinoma vs. normal prostate gland. Hemin treatment reverted the expression profile observed in PCa tumors. The TCGA-PRAD dataset revealed that *ADAM15, BCL2L1, LTBR, MBNL2* and *SPINT1* showed the same expression profile as Oncomine. Moreover, PCa patients with high *MBNL2* expression showed a significant increase in the disease progression free interval, which is interesting as it is upregulated in PC3 overexpressing HO-1. This was validated by RT-qPCR. This study may cast a new light on PCa treatment, highlighting a multifaceted role for HO-1 in altering these processes by modulating cell plasticity, thus supporting it as a potential therapeutic target for the disease.

460. (147) **CHARACTERIZATION OF THE TUMOR MICROENVIRONMENT (TME) OF A TRIPLE NEGATIVE MAMMARY ADENOCARCINOMA, GROWING IN ANIMALS WITH DIFFERENT GENOTYPES**

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The TME plays a critical role in tumor growth, invasion and metastasis. One of its components are the cancer associated fibroblasts (CAF). The type I collagen, secreted by CAF regulates tumor sensitivity to chemotherapeutic drugs. Solid tumors progress in a hypoxic environment, inducing the hypoxic inducible factor (HIF-1α) which activates genes related to cell survival, angiogenesis and metastasis. The CBi$^-$ mice line was selected by artificial selection from the CBi line. M-406 is a triple negative (ER$^-$,PR$^-$,HER2$^-$) mammary adenocarcinoma which grew spontaneously in a CBi female mouse and it is maintained *in vivo* by i.p. injections. It grows and kills 100% of CBi mice. On the contrary, in CBi$^-$ mice, it grows briefly in all the animals, and then, the tumor is rejected in 100% of the mice. Our aim was to characterize the M-406 TME when growing in CBi mice and in the F1 generation of the parental lines CBi (susceptible) and CBi$^-$ (resistant). Mice were challenged $^s/_c$ with M-406, and tumor volume evolution and lung metastasis N° were determined. At the end of the experiment it was evaluated the CAF presence through the % of tumor collagen (Picro-Sirius Red) and α-SMA$^+$ (marker CAF) (IHQ) areas and, also, the N° of HIF-1α$^+$ cells (IHQ). The tumors behaved as seen previously, growing exponentially in CBi and F1 mice. While CBi mice did not develop metastasis, 36/37 of F1 mice did. The % of collagen area was lower ($P<0.0001$) and of α-SMA$^+$ area was higher ($P<0.01$), and the N° of HIF-1α$^+$ cells was lower ($P<0.0001$) in CBi growing tumors compared to those growing in F1 mice. Conclusions: 1) M-406 shows a different TME when grows in genetically different hosts; 2) Only F1 mice developed metastasis; 3) F1 mice tumors showed more HIF-1α$^+$ cells and less % of collagen and % of α-SMA$^+$ areas, than CBi mice; 4) In F1 mice, the dynamic interaction between tumor cells and their environment led to more aggressive tumors, shown by the extremely high % of animals developing lung metastasis.

461. (151) **CORRELATION BETWEEN P-REX1 WITH CLINICAL AND HISTOPATHOLOGICAL FEATURES IN BREAST CANCER PATIENTS**

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Breast cancer is a multifactorial disease and current therapies often fail. At present, treatment decisions should be based on the underlying molecular pathways and biological mechanisms. P-Rex1 is a Rac-GEF essential for Rac1 activation that plays an important role in regulating cell migration. It has been reported that P-Rex1 is overexpressed in luminal breast tumors and, when silenced, the migration of cancer cells is inhibited. We evaluated the levels of P-Rex1 and its association with clinical- histopathological parameters in Argentine patients with breast cancer at the Marie Curie Oncology Hospital. We analyzed the expression of P-Rex1 in biopsy material and in tumors and their corresponding healthy adjacent tissues from patients not subjected to neoadjuvant therapy. P-REX1 mRNA expression was analyzed by quantitative PCR (Q- PCR), and protein by Western blot (WB) or immunohistochemistry (IHC). These data were validated by an *in silico* analysis made from public access databases (Metabric, GEO repository of NCBI and UCSC Xena).
P-REX1 mRNA levels were significantly overexpressed ($p<0.05$) in

luminal and HER2+ tumors types compared to triple negative breast cancer (TNBC). Moreover, 50% of TNBC Argentine patients overexpressed P-REX1
The P-Rex1 protein in TNBC was also significantly increased (observed by WB and IHC), showing specific staining exclusively in tumor cells. These new findings add relevance to the field of cancer therapies, as the TNBC subpopulation could benefit from P-Rex1 inhibition therapy.
We found significant correlations ($p < 0.05$) of P-Rex1 with: estrogen and progesterone receptors, tumor size, Ki67 proliferation marker, age, histological subtype, clinical stage, number of regional lymph nodes affected, menopausal status, histological grade, ethnicity, immunological subtype, and disease recurrence. Overall, our results contribute to establish that PREX1 represent a possible therapeutic target for TNBC that exhibit PREX1 overexpression.

462. (153) **ROLE OF CYTOKINE UPREGULATION AND ENDOTHELIAL LINEAGE DIFFERENTIATION IN KSHV DRIVEN MESENCHYMAL STEM CELL KAPOSI'S SARCOMAGENESIS IN A PRO-ANGIOGENIC ENVIRONMENT**

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Cumulative evidence shows the importance of bone marrow-derived human Mesenchymal Stem Cells (hMSC) in Kaposi's sarcoma (KS) Herpesvirus (KSHV) induced tumorigenesis; including the increased expression of chemokines, cytokines, and endothelial markers after infection. These data reinforce the hypothesis that KS may derive from KSHV-infected MSCs migrating into an inflammatory and angiogenic site enhancing the reprograming and transformation capacity of KSHV. **Aim:** uncover specific mechanisms and conditions involved in KSHV-induced transformation and reprograming of hMSCs. **Methods:** We carried out KSHV infection of hMSCs then subjected to MEM or KS-like pro-angiogenic culture conditions. Three days after infection, we performed RNA-sequencing and pathway analysis of the Differentially Expressed Genes (DEGs) between these different environmental conditions. **Results:** We found that differentially regulated pathways related to Extracellular Matrix Organization, Cytokine Activity and Cell Cycle showed twice the amount of DEGs (LogFC>1.5, $p<0.001$) in KS-like compared to MEM conditions. Differential pathways ($q < 0.05$) like MAPK cascade, PI3K-Akt and Angiogenesis appear to be activated only in hMSCs infected in a KS-like environment with increased expression of endothelial markers such as: PECAM1, VEGFR1, ROBO4, XDH and ESM1. These correlates with increased expression of KSHV lytic genes and highlights the importance of this condition in KSHV reprograming of hMSC towards endothelial differentiation and transformation. Using a KS gene signature and data from 24 KS patients and contralateral control skin, we showed that infection of hMSC in KS-like conditions reprograms hMSC closer to KS lesions compared to infection in MEM conditions, which cluster with normal skin. **Conclusion:** This analysis shows that KSHV infection in KS-like conditions reprograms hMSC closer to KS expression profiles reinforcing the notion of these cells as most likely KS precursors.

463. (157) **USE OF MICROFLUIDIC DEVICE TO EVALUATE BREAST CANCER STEM-LIKE CELLS IN RESPONSE TO CHEMOTHERAPY**

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Introduction: Breast cancer (BC) is the most common tumor in women and a significant cause of cancer-related morbidity and mortality worldwide. The standard treatment depends on several factors including stage, histology, and molecular markers expression. Chemotherapy (CT) is a widely approach used many times as concomitant neoadjuvant therapy with radio, endocrine or immunotherapy. CT is widely used for cancer treatment as a neoadjuvant therapy to reduce tumor volume prior to surgery and as a post-surgical adjuvant. However, in some cases tumors do not respond to treatment. Cancer Stem Cells (CSC) are a minority tumor cell population, associated to chemoresistance. Thus, the development of an in vitro sphere-forming assay that can predict CSC treatment response, is an important issue for these patients. Microfluidic devices (MD) have surged in the last decade, as a powerful tool for cell individual study, with the benefit of using a low amount of biological samples. Objective: Evaluate CSC response post-treatment with CT drugs like doxorubicin (Doxo) and paclitaxel (Ptx) using a murine BC mixed cell line (LM38-LP) in conventional culture conditions and in Microfluidic approach. Results: Cancer sphere forming assay, was carried out with or without Doxo or Ptx in both culture support. CT decreased the number of CSCs, measured as sphere formation efficiency ($p<0,005$), spheres size in 40% ($p<0,005$) and decreased the remaining survived cells by 65% with Doxo and 35% with Ptx ($p<0,005$). Also, CT induced an increase of pluripotent markers Oct4, Sox2, Nanog and CD44 determined by qPCR ($p<0,0001$ vs control) and immunofluorescence. Conclusion: Both CT drugs inhibited the number and survival rate of CSC but increases pluripotent capacity in remanent cells. The use of MD provides the additional advantage of evaluating small samples with statistic relevance and a translational possibility for therapeutic prediction in patients.

464. (173) **GLYCOSYLATION-DEPENDENT CIRCUITS SYNCHRONIZE THE PRO-ANGIOGENIC AND IMMUNOREGULATORY FUNCTIONS OF MYELOID-DERIVED SUPPRESSOR CELLS IN CANCER**

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Myeloid-derived suppressor cells (MDSCs) favor tumor progression

and therapy resistance by reprogramming antitumor immunity and promoting angiogenesis. To elucidate the mechanisms that synchronize these functions, we investigated the role of glycosylation-dependent, galectin-1 (Gal1)-driven circuits in coupling immunoregulatory and pro-angiogenic activities of MDSCs. Flow cytometry and HPLC-HILIC/WAX revealed an activation-dependent glycan profile in monocytic and polymorphonuclear MDSCs (p=0.03) that controlled Gal1 binding and was more prominent in tumor microenvironments. Exposure to Gal1 led to concomitant activation of immunosuppression and angiogenesis programs in bone marrow derived MDSCs. Flow cytometry of Gal1-conditioned MDSCs showed higher expression of immune checkpoint molecules, including programmed death ligand-1 (PD-L1) (p=0.005) and indoleamine 2,3-dioxygenase (IDO) (p=0.037) and greater production of reactive oxygen species (ROS) and nitric oxide (NO) (p=0.02). *In vitro,* Gal1-conditioned MDSCs showed greater T-cell suppressive capacity (p=0.03) and higher IL-10 (p=0.04) and IL-27 (p=0.003) secretion. These effects were accompanied by enhanced endothelial cell migration, tube formation, 3D-sprouting and vascularization (p<0.05). *In vivo*, Gal1-conditioned MDSCs accelerated tumor growth (p=0.001) and fostered immune evasion and vascularization programs in Gal1-deficient colorectal tumors. Mechanistically, mass spectrometry, immunoblot and blocking assays identified the CD18/CD11b/CD177 complex as a bona fide Gal1 receptor and STAT3 as a key signaling pathway coupling these functions. Accordingly, a combined algorithm that integrates Gal1 expression and MDSC phenotype, showed critical prognostic value by delineating the immune landscape and clinical outcome of human cancers. Thus, glycosylation-dependent Gal1-driven circuits favor tumor progression by coupling immunoregulatory and pro-angiogenic programs of MDSCs via CD18- and STAT3-dependent pathways.

465. (180) **KAEMPFEROL, A PHYTOESTROGEN, IMPEDES SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT-DRIVEN MIGRATION AND CELL CYCLE PROGRESSION IN ESTROGEN-RESPONSIVE AND UNRESPONSIVE TUMOR CELL LINES**

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Kaempferol (Kf) is a bioflavonoid that suppresses cell proliferation in human cancers. However, the effects of Kf on endometrial and cervical carcinoma remain unknown. Soluble guanylyl cyclase (sGC) is a heterodimeric enzyme constituted by two subunits, alpha1 (a1) and beta1. We showed that a1 overexpression increases proliferation, migration and survival in cervical (HeLa) and endometrial (ECC-1) cancer cells. High levels of a1 correlate with worse survival in cervical cancer. Our aim was to study the effects of Kf on ECC-1 and HeLa cells overexpressing a1.

a1 was overexpressed by using an adenoviral vector carrying the complete sequence of a1 (a1-myc) or empty virus as control. Multiplicity of infection (MOI) ranging from 0 to 500 was used. After 6 h virus infection, cells were incubated with complete media and 40 μM Kf. Determinations were carried out at 24 and 48 h. Optimal Kf concentration was calculated by cell membrane integrity fluorescence assay. Cell cycle was studied by flow cytometry. Migration was determined by scratch motility assay.

Kf induced cell cycle arrest at G2/M phase (% of cells from respective control (C); HeLa, Kf: 207.97±2.5*, MOI250+Kf: 230.81±8.2*; MOI500+Kf: 234.62±7.6*; ECC-1, Kf: 172.13±14*; MOI250+Kf: 152.88±15*; MOI500+Kf: 135.24±13.3*) and increased SubG0/G1 DNA content (HeLa, Kf: 377.8±12**; MOI250+Kf: 268.7±16.3*; MOI500+Kf: 436.11±25.1**; ECC-1, Kf: 470.22±20**; MOI250+Kf: 445.73±23.5**; MOI500+Kf: 229.4±11.3*; *p<0.05, **p<0.01 vs C). Besides, Kf reduced the migration measured by wound closure (% of closed wound vs C; HeLa, MOI500: 174.12±10**; Kf: 48.53±13.4*; MOI500+Kf: 52.26±8.6*, *p<0.05, **p<0.01 vs C)

Our results show for the first time the anti-tumoral effect of Kf on ECC-1 endometrial cancer cells. Moreover, Kf impeded basal and a1-driven cell cycle progression and migration in both cell lines. These results highlight the potential therapeutic use of Kf in uterine malignancies, which needs to be further addressed.

466. (215) **IN SILICO IDENTIFICATION OF ADRENERGIC RECEPTOR-ASSOCIATED miRNA EXPRESSION AS BIOMARKERS FOR 100% DISEASE-FREE SURVIVAL IN BREAST CANCER SUBTYPES**

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Breast cancer (BC) is the most frequently diagnosed and leading cause of cancer death among women worldwide. We have previously described (Buenos Aires Breast Cancer Symposium 2020) that some miRNAs that bind to ADRA2A can predict better disease-free survival (DFS). The present work aimed to find some miRNAs that bind to one or more of the 4 adrenergic receptors expressed in breast cancer and predict 100% DFS in the different breast cancer subtypes (luminal A, LA, luminal B, LB, HER2-enriched H and basal-like, B).

We interrogated in the public TCGA database the expression of mature miRNAs able to bind to the receptor 3´UTR. The cutoff points for disease-free survival (DFS) were selected using Evaluate Cutpoint. Survival analysis was performed by Kaplan-Meier and log-rank (Mantel-Cox).

We first analyzed all the miRNAs that bind ADRA2A, ADRA2B, ADRA2C and ADRB2, the adrenergic receptors expressed in breast cancer. We found more than 40 miRNAs whose high or low expression significantly predicted better DFS in some tumor subtypes. From them, we selected those in which a high or low expression was associated with 100% DFS. Those miRNAs are shown for the specific subtype in which the association was found. The p-value for DFS between high and low expression is also shown. The miRNA whose high expression showed 100% DFS were for LA: miR-30e-5p, p=0.0175; for LB: let-7b-3p, p=0.0138; let-7d-3p, p=0.0221; let-7i-3p, p=0.0322; miR-33a-5p, p=0.0393; miR-30c-5p, p=0.0314 and for H: miR-30d-5p, p=0.0415. The miRNA which a low expression predicts 100% survival were for LA: miR-15a-5p, p=0.0298; miR-16-1-3p, p=0.0156; miR-23b-5p, p=0.0240; miR-145-5p, p=0.0148; miR-497-3p, p=0.0124; for LB: miR-30c-5p, p=0.0384 and for H: miR-195-5p, p=0.0126. None was found for B tumors.

These miRNAs, through the several proteins whose expression they regulate, might be selectively used as prognostic biomarkers in the different BC subtypes.

467. (273) **THE INHIBITION OF THE ALTERNATIVE NF-kB PATHWAY AND BCL-2 TARGETING AS A POTENTIAL THERAPEUTIC APPROACH FOR THE TREATMENT OF CLASSICAL HODGKIN LYMPOHOMA**

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Refractory/relapsed (R/R) disease in classical Hodgkin Lymphoma (cHL) is the major challenge making necessary the development of new therapeutic strategies. We have previously reported that the non-canonical NF-kB pathway (NCP) plays a key role in human cHL cells survival, being NIK stabilization responsible for its constitutive signaling. Also, we have shown that BCL-2 sustained expression is

partially a consequence of this mechanism and should be considered as a prognosis biomarker. The aim of this work was to explore the mechanism underlying the anti-tumor effects of the NCP and BCL-2 inhibition after treatment with 4H-isoquinoline-1,3-dione (iNIK) and venetoclax (VTX), respectively, or their combination in human cHL cell lines from R/R patients. Trypan blue exclusion and MTS assays showed that treatment with iNIK decreased cell growth in all cell lines ($p<0.05$), but only increased the % of PI^+ cells in UH01 and L1236 cells ($p<0.05$) in FDA/PI staining assay by FC. Treatment with VTX induced cell death in all three cell lines ($p<0.01$). Interestingly, iNIK+VTX treatment increased % of PI^+ cells as compared to each drug alone ($p <0.05$) in all cell lines, inducing cell death at doses that did not do so as single treatments. Searching for signaling pathways that could explain the anti-tumor mechanisms, we re-visited our previous expression array data of the inhibition of the NCP in UH01 cells (GSE109803) using InnateDB. NCP inhibition was associated with "Apoptotic process" ($p=0.0006$), "B cell proliferation" ($p=0.026$) and "BH3-only proteins associate with anti-apoptotic BCL-2 members signaling pathway" ($p=0.006$). In view of this, we performed WB analysis for proteins involved in those processes after treatment with iNIK or VTX. We found that both treatments were able to modulate the expression of Bcl-XL, Bad, LC3, Beclin-1 and p-Akt/Akt. Our results led us to conclude that inhibition of the NCP and BCL-2 should be considered as potential targets in R/R cHL treatment.

468. (277) **FGFR2 AND RUNX2 PROMOTE BREAST CANCER PROGRESSION IN HUMAN BREAST CANCER MODELS**

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We have previously shown that FGF2 increases the proliferation of breast cancer cells activating FGFR2 and ligand-independent hormone receptor signaling. FGF/FGFR alterations are frequent in breast cancer patients, and for this reason, FGFR inhibitors are being evaluated to overcome endocrine resistance. In addition, a positive loop between FGF2 and RUNX2 has been shown in bone tissue. Thus, our aim was to evaluate the interplay between FGFR2 and RUNX2 in luminal human breast cancer models in response to endocrine or FGFR-targeted therapies. We stably transfected MCF7 and T47D cells with a constitutively active FGFR2 (R2CA), a RUNX2 expression plasmid, or an empty vector (Control). R2CA and RUNX2 cells grow *in vivo* without hormone supply. R2CA cells show phosphorylated estrogen (ER) and progesterone receptors (PR) and higher RUNX2 levels as compared with control cells. RUNX2 cells express lower levels of estrogen receptor (ER) and higher levels of FGFR2 as compared with control cells. Both, R2CA and RUNX2 tumors give rise to lung metastasis, absent in control mice. The antiestrogen Fulvestrant ($p<0.001$), or the antiprogestin mifepristone ($p<0.05$) decreased R2CA tumor growth compared to controls while RUNX2 tumors were unresponsive, suggesting that RUNX2 overexpression bypasses the hormone receptor-associated pathways. Then, we evaluated if RUNX2 tumors were sensitive to the FGFRs inhibitor PD173074 taking advantage of the higher levels of FGFR2 expressed by this model. RUNX2 tumors exhibited PD173074 resistance showing even a more aggressive phenotype than control tumors. We conclude that FGFR2 and RUNX2 promote tumor progression and hormone-independent growth. However, RUNX2 seems to be more important for the acquisition of endocrine resistance. Our results emphasize a) the evaluation of RUNX2 before initiating an FGFR2 directed therapy and b) the possible use of RUNX2 inhibitors in combination with standard therapy in selected breast cancer patients.

469. (283) **EFFECT OF HISTAMINE ON THE INDUCTION OF DIFFERENTIATION/APOPTOSIS BY MRP4/ABCC4 INHIBITORS IN AML CELLS: CEEFOURIN-1 EVALUATION**

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We previously described that both the cAMP transporter MRP4 and the histamine H2 receptor are molecular targets for Acute Myeloid Leukemia (AML) differentiation therapy. Increased intracellular cAMP concentrations play an important role in leukemic cell maturation, proliferation, and apoptosis. In the present study we analyzed the ability of histamine (HIST), in combination with MRP inhibitors, to induce AML cell differentiation/apoptosis, and made special focus in ceefourin-1. We first evaluated the effect of 100μM HIST on the induction of cell differentiation upon treatment with MRP4 inhibitors (500μM probenecid, 25μM MK571, or 25μM ceefourin-1) in AML cell lines, determined by the expression of CD14 (FACS), CD88 (western blot), and cell morphology (MGG staining). The combined treatment of probenecid or MK571 with HIST significantly increased the differentiation markers ($p<0.001$), while ceefourin-1, alone or in combination with HIST, did not. In contrast, ceefourin-1 induced apoptosis of AML cells, determined by caspase-3 cleavage and annexin V-FITC ($p<0.05$), and co-stimulation with HIST potentiated this effect ($p<0.05$). Since until today, there are no reports of ceefourin-1 administration *in vivo,* we assessed its potential subacute toxic effects in BALB/c mice (ip, 10mg/kg, 3 times/week, 2 weeks). Blood parameters, as well as hepatic and renal functions, showed no alterations upon treatment with ceefourin-1. We then evaluated the impact of ceefourin-1 (ip, 10mg/kg daily) and its combination with HIST (sc, 5mg/kg daily) in U937 xenografts in NGS mice treated for 2 weeks. Although tumor growth was unaltered, a significant decrease in the mitotic index and an increase in the apoptotic index was observed in ceefourin-1-treated tumors, with or without HIST, compared to vehicle or HIST groups. Taken together, our results contribute to the rational basis of a polypharmacological approach in AML using HIST and MRP4 inhibitors that needs to be studied further.

470. (286) **THE EFFECT OF SOCIAL ISOLATION ON HEALTH STATUS OF PEOPLE WITH CANCER CONTEXTS OF SOCIOECONOMIC VULNERABILITY DURING COVID-19 PANDEMIC**

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Social isolation due to the pandemic, in contexts of health and socioeconomic vulnerability impact on the neglect of people's health status. To estimate the impact on cancer populations in popular neighborhoods, a descriptive and analytical cross-sectional prevalence study was started in that population.
An oncological population in a neighborhood called Los Hornos (LH), was selected based on socio-sanitary data collected door by door during 2020. For the impact study, people were relinked by telephone or by a home visit. Type of cancer and its evolution, comorbidities and their life context in the pandemic were investigated through individual interviews. Routine biochemical determinations,

tumor markers and COVID-19 antibodies, were analyzed in blood samples. Also, clinical parameters (blood pressure, blood oxygen saturation, capillary glycemia) were registered.
In 65 blocks surveyed in 2020, 364 dwellings (1,274 inhabitants) were surveyed and 53 people declared oncological disease as comorbidity. It was able to relink and interview 22 of them and 9 were also included in the protocol. During 2020, the 25% of the people could continue monitoring their disease and comorbidities, but the 66% had to discontinue it. Also, 42% of the people did not need oncological medication during 2020, but from the remaining 58%, 25% could not access the medication. From clinical evaluations, 57% of people had one or more altered parameters, several classified as risk factors for arterial hypertension and diabetes. The COVID-19 serology was (+) for 89% of the analyzed people, with a positivity index (PI) range between 4.97 <PI <18.37. A single person, not vaccinated, had no antibodies. We preliminarily identify difficulties in adherence to treatment of comorbidities concomitant with oncological disease.
Having scientific evidence of the health status of vulnerable populations allows the generation of community actions aimed at assisting and linking the institutions and their community.

471. (294) **REGULATION OF PR, ER AND PLASMA STEROID HORMONES BY MIFEPRISTONE TREATMENT IN BREAST CANCER PATIENTS FROM MIPRA TRIAL**

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Preclinical data suggests that antiprogestins inhibit the growth of luminal breast carcinomas expressing higher levels of progesterone receptor (PR) isoform A (PRA) than isoform B (PRB). Thus, we conducted a pre-surgical window of opportunity trial to test the effect of mifepristone (MFP; 200 mg, PO, QD, 14 days) in 20 breast cancer patients selected by their high PRA/PRB isoform ratio (MIPRA; NCT02651844). The primary endpoint was to compare the Ki67 levels of biopsies and the post-therapy surgical specimens. A 49.62% decrease in the median was registered in all surgical specimens compared to baseline (p=0.0003). RNA-seq analysis showed that MFP upregulated genes related to the immune system and matrix remodeling, and downregulated genes involved in cell-cycle and proliferative pathways. Now we report data regarding the PR and estrogen receptor alpha (ER) regulation by MFP and plasma levels of MFP and 20 steroid hormones (LC-MS/MS). In western blot studies an enrichment of the PR isoforms in the nuclear compartment with upshifted bands was observed in MFP-treated samples (p = 0.002, Wilcoxon). Immunohistochemical studies comparing pre- and post-treatment samples suggested a decrease in PR, ER, pSer294PR and pSer118ER after treatment. MFP plasma levels were 683.6 +/- 28.6 nM at day 7, and 757.5 +/- 41.7 nM at day 14 after treatment initiation. An expected increase in cortisol levels (p < 0.001) and other steroids was observed in MFP-treated plasma from patients. Our results suggest that MFP is playing an active role regulating target genes in MFP-treated patients. The decrease in pSer294 PR suggest an inhibition in MAPK-mediated activation of PR. Since in a MFP-sensitive mouse model plasma levels of 20 nM are effective inducing tumor regression it may be suggested that lower MFP doses may exert similar therapeutic effects with less antiglucocorticoid side effects.

472. (296) **EFFECTIVENESS OF METRONOMIC CHEMOTHERAPY (MCT) WITH LOSARTAN (LOS) AND CYCLOPHOSPHAMIDE (CY) IN THE M-406 TRIPLE-NEGATIVE MAMMARY ADENOCARCINOMA MODEL IN FEMALE MICE with METABOLIC SYNDROME (Mets)**

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Metabolic Syndrome is a pathology that frequently coexists with breast cancer and can worsens cancer prognosis. This situation must be considered when choosing a correct cancer treatment. Our aim was to evaluate the effectiveness and toxicity of MCT with CY+LOS in mice with MetS bearing a mammary adenocarcinoma, and its effect on the tumor micro environment (TME). CBi female mice (5 weeks) were fed with a diet with 40% calories of fat (HFD) throughout the experiment. At 20 weeks, the development of MetS was confirmed by biochemical and morphological parameters. Once the MetS features were settled, mice were challenged orthotopically with M-406 tumor (day 0); when the tumor was palpable, mice were distributed into 4 groups, GI: Control, with no treatment; GII: Cy 25mg/kg/day in the drinking water; GIII: Los 150mg/kg/day in the drinking water; GIV: Treated as GII + GIII. Mice were weighted and tumor volume measured 3 times/week. When tumors were exponentially growing, mice were euthanized, tumors excised and used for immunohistochemistry (IHC). On day 19, GIV showed tumor growth inhibition compared to GI (P<0.01); on day 21 tumor volume was lower in GIV and GIII vs GI (P<0.01; P<0.05). GIII and GIV showed weight loss since day 11. IHC analysis showed lower N° of Ki67+ cells in GIV vs GI (P<0.001). The TME showed lower αSMA+ cells and collagen production and less HIF-1α+ cells in GIV vs GI (P<0.01; P<0.05; P<0.05). These results indicate that MCT with CY+LOS inhibit the growth of M-406 in a similar way that in mice feed with normal diet, as shown previously. GIII and GIV showed weight loss at the end of the experiment, a sign of toxicity caused by LOS. The therapeutic effect may be achieved, at least in part, by diminishing: cancer cell proliferation, cancer associated fibroblasts, collagen production and hypoxia. The efficacy of the therapeutic schedule herein utilized indicates the convenience of its clinical translation for patients with breast cancer and MetS.

473. (307) **ROLE OF C-FOS IN AUTOPHAGY REGULATION IN GLIOBLASTOMA**

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c-Fos is a proto-oncoprotein belonging to the AP-1 family of transcription factors; in addition to its genomic activity, it associates with components of the endoplasmic reticulum (ER) and activates the synthesis of phospholipids and glycolipids by a mechanism independent of its transcriptional activity. AP-1 proteins have been involved in several pathologies and in developing different types of cancer where their activity is often dysregulated, contributing to cell transformation, tumor progression, aggressiveness, and resistance to treatment. Autophagy is a process of degradation of cellular components and has currently been postulated as a novel therapeutic target for the treatment of tumors, playing both an anti-tumor and a pro-tumor role. Our work aims to characterize the participation of c-Fos in the regulation of autophagy and its importance in the biology of glioblastomas. We observed high levels of c-Fos expression in different tumors of the Central Nervous System (CNS), contrasting with an absent or almost undetectable expression in healthy tissues. In addition, the correlation between the degree of malignancy and the level of c-Fos expression highlights the potential of this protein as a therapeutic target. We also observed that c-Fos overexpression promotes the accumulation of the autophagy marker LC3II in CHO K-1 and T98G cells under nutrient-deficient conditions, and results of an *in silico* analysis reveal that c-Fos has a putative "LIR motif similar to ATG3". Viability assays demonstrate that c-Fos overexpression does not induce cell death under starving conditions, and

the overexpression of different c-Fos deletion mutants does not induce an increase in LC3II under nutrient-deficient conditions similar to that observed with full-length c-Fos. Preliminary results suggest that the overexpression of c-Fos in T98G cells induces a greater number of LC3 positive dotted structures than cells that overexpress a control vector.

474. (309) **PROGNOSTIC RELEVANCE OF TUMOR MICROBIOTA IN BREAST CANCER PROGRESSION**

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Microbiota are known to influence cancer development and progression. Breast tumor tissue presents an unique and diverse microbiota. In order to study the prognostic relevance of the microbiota in the evolution of early breast cancer, a study was carried out including biopsies of fresh breast tumor tissues from women with invasive ductal breast carcinoma, clinicopathological stage I/II with a 5-year follow-up minimum (*Hospital Roffo*, n=18). Samples were processed and DNA was extracted using the QIAamp-DNA-Mini-Kit. The bacterial profile was identified studying the 16S rRNA gene. OTUs classification and Alpha and Beta diversity analysis were performed to study the possible associations with the classic parameters of breast cancer and their tumor progression. Taxonomic analysis of these samples indicated that *Proteobacteria* (38%) and *Firmicutes* (28%) were the most representative phylum followed by *Bacteroidota* (12%) and *Actinobacteriota* (6%). We found a significant difference between the tumor microbial community of patients who died and those who did not, presenting differences at 16 genus level, with *Prevotella* and *Anaerococcus* being the most abundants in patients who died whereas *Escherichia-Shigella* and *Acinetobacter* in patients who did not. Twenty eight statistically significant metabolic pathways from Metacyc database were found (Shannon index p=0.02) between patients who died and those who did not, highlighting those associated with polyamine biosynthesis and hexitol fermentation to ethanol and acetate. To conclude, our preliminary findings and their concordance with previous studies would allow the establishment of a particular taxonomic and functional profile associated with breast cancer patients who died. Finally, the results found between patients who died and did not, open up new paths in the development of alternative therapies (with probiotics) to improve the patients's life-quality.

475. (311) **GPC3 MODULATES GENE EXPRESSION SIGNATURES ASSOCIATED WITH METASTASIS SUPPRESSION IN BREAST CANCER**

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We have suggested that GPC3 acts as a metastasis suppressor in breast cancer, reversing the epithelial-mesenchymal transition (EMT). To further our research, we performed in silico studies using the TCGA (The Cancer Genome Atlas) database. We determined by bioinformatics analysis that normal breast tissues exhibit high GPC3 levels while its expression decreases in tumors ($p<0.0001$, ANOVA test), mainly in the basal-like subtype ($p<0.05$, ANOVA test). Differential gene expression studies revealed that "high GPC3" breast tumors have 1651 upregulated and 299 downregulated ones (FDR<0.05, Log_2 fold change>0.5). To investigate the biological processes associated to these modulated genes, we did gene enrichment analysis. Our results showed that in "high GPC3" patients gene signatures involved in the negative regulation of cell-matrix adhesion (FDR<0.05, NES=1.67 and 1.74) and in the activation of cell-cell adhesion (FDR<0.05, NES=2) are upregulated. In association, *in vitro* assays confirmed that GPC3 overexpressing human breast cancer cells (MDA-MB231-GPC3) have low adherence to substrates like FN and LN ($p<0.01$, ANOVA test), and express less integrin β1 and β4. We also investigated the EMT process, finding an upregulation of a signature involved in its inhibition "high GPC3" patients (FDR<0.05, NES=1.58). Moreover, signatures related to the canonical Wnt signaling (FDR<0.05, NES=-1.64) and to the TGF-β pathway activation (FDR<0.05, NES=-1.51) were downregulated. In agreement, MDA-MB231-GPC3 cells exhibited reduced Smad2/3 phosphorylation, suggesting a TGF-β pathway inhibition. Finally, associated with its anti-metastatic function, here we showed that "high GPC3" patients have a longer disease-free survival time and a lower probability of relapse ($p<0.014$, Chi^2 test, Mantel-Cox Test; HR: 0.6140).
In sum, these results reinforce the central role of GPC3 in breast onco-pathology and propose its potential usefulness as a prognostic biomarker able to predict patient evolution.

476. (317) **CLINICAL SIGNIFICANCE OF HO-1 AND 14-3-3Z/Δ INTERACTION IN PROSTATE CANCER**

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Prostate Cancer (PCa) cells display abnormal expression of proteins resulting in an augmented capacity to resist chemotherapy and colonize distant organs. We have previously showed that heme-oxygenase 1 (HO-1), encoded by the gene *HMOX1*, has a strong anti-tumoral effect in PCa. **We propose that HO-1** and its interactors reprogram PCa cells, favoring a less aggressive phenotype. In this work, we undertook a mass spectrometry-based proteomics analysis to identify HO-1 molecular partners which might collaborate with its modulatory function in PCa. PCa cells were transiently transfected with GSTHO-1 or control and treated with the stressor agent H_2O_2. Immunoprecipitated protein complexes were subjected to LC-ESI MS/MS. We identified TRIM28, HNRNPA2B1, HSPB1, CBX1, CBX3, MATR3, NPM1, DDB1, HMGA1, 14-3-3ζ/δ and ZC3HAV1, among the HO-1 interactors with nuclear localization. We next performed correlation analysis using open-access PCa patient datasets between *HMOX1* and the selected candidates, in order to assess their clinical significance. Results show a significant and positive Spearman correlation between *HMOX1* and 6 of those genes, and an increased relapse-free survival in PCa patients with high expression of those genes. Alternatively, *HMOX1* and *YWHAZ* (14-3-3ζ/δ encoding gene and a strong predictor of PCa aggressiveness) showed a significant negative correlation. Moreover, PCa patients with high expression of *YWHAZ*, showed a higher risk of relapse. We then validated our proteomics approach by co-immunoprecipitation analysis, ascertaining HO-1 and 14-3-3ζ/δ interaction. Immunofluorescence assays provided evidence that HO-1 and 14-3-3ζ/δ co-localize in the cell nuclei under oxidative stress conditions. In summary, we describe a novel protein interaction between HO-1 and 14-3-3ζ/δ in PCa and highlight the clinical correlation of these two proteins pointing out to a potential inhibitory role of HO-1 on 14-3-3ζ/δ, for future therapeutic avenues.

477. (318) **HSP27 AFFECTS CISPLATIN-INDUCED DNA DAMAGE RESPONSE THROUGH ATR/CHK1 PATHWAY IN HUMAN COLON CANCER CELLS**

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Abstract: HSP27 (HSPB1) is overexpressed in many tumor cells

and has been involved with cancer progression and resistance to cancer therapy. Accordingly, HSP27 has become an attractive therapeutic target. Previously, we reported that HSP27 interacts with DNA mismatch repair (MMR) proteins after cisplatin (cPt) treatment. However, the role of HSP27 in cPt-induced DNA damage response (DDR) through ATR/CHK1 pathway in MMR deficient/proficient tumor cells remains unknown. Here, human colon cancer cell lines HCT116+ch2 (MMR deficient, MMR-) and HCT116+ch3 (MMR proficient, MMR+) were exposed to 10 μM of cPt for 24 h. Downregulation of HSP27 was performed using the antisense oligonucleotide (OGX427) and ATR/CHK1 inhibition by VE-821 (VE). Cells were collected at T0, T3, T9 and T24 (0, 3, 9 and 24 h post-cPt or cPt+VE, respectively). γH2AX (DNA double-strand breaks marker), HSP27 and phosphorylated CHK1 (pCHK1, Ser345) were analyzed by western blot and cell viability by CCK8. HSP27 downregulation significantly reduced the expression of γH2AX and pCHK1 after cPt treatment in MMR+ cells (P<0.05). Interestingly, ATR/CHK1 pathway inhibition significantly decreased HSP27 expression after cPt at T0, T3 and T9 in both MMR+/- cell lines (P<0.01), but specially in MMR- cells. CHK1 inhibition also decreased γH2AX levels after 9 hours in recover media in both cell lines and reduced cell viability (particularly in MMR- tumor cells at T0, T3 and T9, P<0.01). Nuclear colocalization of HSP27 with pCHK1 and CHK1 was demonstrated by confocal microscopy in cPt-treated MMR-/+ tumor cells. The Pearson correlation coefficient (PCC) was >0.6 and Mander's overlap coefficient (MOC) were: MOC1 >0.6 at T3 and >0.8 at T9; and MOC2 >0.7 at T3 and T9. Our data indicate that HSP27 is involved in cPt-induced DDR through ATR/CHK1 pathway and could be a promising target to enhance tumor chemosensitivity.

478. (328) **HYALURONAN METABOLISM IS ASSOCIATED WITH DNA REPAIR GENES IN BREAST AND COLORECTAL CANCER**

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The tumor microenvironment (TME) plays an important role in the progression of cancer. One of its components that is frequently altered in tumors is Hyaluronan (HA) which has been associated with the aggressiveness of cancer. Also, it has been shown that changes in TME can lead to changes in the expression of BRCA1 and BRCA2 genes, which have been extensively studied in different tumors.
Aim: The aim of this work was to compare mRNA levels of HA metabolism members and BRCA genes, between tumor (TT) and non-tumor adjacent tissue (NAT) in breast and colorectal cancer, and test its correlation with clinical biomarkers (ER, PR, HER2, KI67) and patient survival.
Methods: We compared TT and NAT between HA metabolism members and the DNA repair genes by qPCR, and tested their correlation with biomarkers. We investigated relationship between mRNA expression and patient survival using the Kaplan–Meier Plotter database. The STRING analysis was used to evaluate a protein interaction networks between HA metabolism members and BRCA genes.
Results: We show alteration in HA metabolism in colorectal but not breast cancer. We found a decrease in HYAL1 levels in breast but not colorectal cancer. We also show lower HA levels in TT compared with NAT. In both breast and colorectal cancer, CD44 and BRCA2 showed a strong positive correlation. Kaplan–Meier Plotter data showed that the mRNA expression levels of BRCA1 and 2 are higher in cancer than normal tissue and are significantly associated with the relapse-free survival (RFS). HYAL1 levels in breast cancer are higher in normal tissue but don't influence survival. STRING analysis showed two clusters: HA metabolism (HAS2, HAS3, HYAL1, HYAL2, CD44) and DNA repair and regulation (BRCA1, BRCA2, TP53, EP300) with CD44 as a link between these processes.
Conclusion: There is an association between HA metabolism and the DNA repair genes which gives us a new insight in the molecular mechanisms of the development and the progression of cancer.

479. (335) **DECIPHERING THE METABOLIC VULNERABILITY OF MALIGNANT MELANOMA CELLS**

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Aggressive melanoma is still a highly live-threatening malignancy being BRAF mutation its most frequent oncogenic driver follow by NRAS mutation. In addition, metabolic rewiring has been involved in progression and resistance of melanoma. Here we studied the response of eight human malignant melanoma cell lines treated at different levels of key metabolic pathways to address their vulnerability and to interpretate their response in terms of the metabolic and genetic background of each cell line. We found that melanoma cells exhibited a broad spectrum of sensitivities to bioenergetic modulators. Based on a previous published work, we defined a concentration for each modulator to segregate melanoma cells as sensitive (considered as some dependency on this pathway) or non-sensitive. After that, we found that the order of response to metabolic modulators was BRAFV600R≥BRAFV600E>NRASQ61K. In addition, we found a significant positive correlation between the response to DCA (Pyruvate dehydrogenase inhibitor) *vs* RAD001 (mTORC1 inhibitor) (r=0.87; p<0.0048), DCA *vs* oxamate (LDH inhibitor) (r=0.73; p<0.038) and DCA *vs* Metrotrexate (MTX, r=0.77; p<0.026). Our results suggest that sensitivity to DCA, mTORC1 inhibitor, MTX and Oxamate is related to the optimization of glucose oxidation by mitochondria. This could be an effective strategy for some specific group of melanomas, probably excluding the NRAS mutated subtype. However, additional experiments are required to validate and better understand these results.

480. (337) ***IN VITRO*** **EFFECTS OF METFORMIN IN COMBINATION WITH THE INHIBITION OF THE PENTOSE PHOSPHATE PATHWAY IN HUMAN GLIOBLASTOMA CELLS**

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The pentose phosphate pathway (PPP) enables cancer cells to adapt to oxidative stress and lipid synthesis by means of the production of NADPH. The inhibition of PPP key enzymes, including glucose-6-phosphate dehydrogenase (G6PD), strongly affects cancer cell proliferation in vitro, as well as in vivo. We have previously shown that monolayers of glioblastoma (GBM) cell lines result sensitive to PPP inhibition by 6AN. In addition, the combination of 6AN with metformin (MET, an oral drug for the treatment of T2MD) increases the effectiveness of both monotherapies. Here we investigated the impact of 6AN (10 or 25 μM) alone or in combination with MET (5 mM) on i) clonogenic cells and spheroids (3D) of two GBM cell lines (U251 and U373), ii) the AMPK/mTOR/S6 axis (by immunostaining of pACC (an AMPK substrate) and pS6 (a mTOR pathway effector)) and iii) the presence of senescent cell (by the senescent associated β-galactosidase enzyme activity). We found

that 6AN alone, and the combination of MET/6AN altered spheroids development and clonogenic efficiency of U251 and U373 GBM cells. Media supplementation with 5 mM nicotinamide (NAM, a precursor of NADPH) or 100 μM NADPH was able to counteract almost completely (NAM) and partially (NADPH) the effects of 6AN on clonogenic cells. After 24 h of treatments, we found a decrease in pS6 (decreased mTOR signaling) and an increase in pACC immunostaining (increased AMPK activity) compared to control cells with both monotherapies and combinatory approach. Finally, 7 days of treatment with 10 μM 6AN promoted the presence of β-galactosidase positive giant cells probably related with a senescent process. The presence of these cells was prevented by 5 mM NAM. In conclusion, our results indicate that the combination of MET/6AN decreases the mTOR pathway signaling affecting 3D cultures and clonogenic efficiency of glioblastoma cells.

481. (345) **PRECLINICAL ANTITUMOR EFFICACY AND PHARMACOKINETICS OF THE RAC1 INHIBITOR COMPOUND 1A-116: COMPARISON OF DIFFERENT ADMINITRATION ROUTES**

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Rac1 is a key mediator of different relevant cellular functions, such as proliferation, migration and invasion. Aberrant Rac1 signaling is associated with progression in several tumor types, including breast cancer. Previously, our team developed the antitumor compound 1A-116, a small molecule Rac1 inhibitor, using a rational design approach. We have shown that 1A-116 is able to interact with Trp56, a key residue involved in Rac1 activation by different guanine nucleotide exchange factors. Such interaction abrogates Rac1 signaling, thus generating antitumor effects *in vitro* and *in vivo*. These previous evidences supported further preclinical development of 1A-116. The aim of this work was to evaluate oral, subcutaneous (SC), intravenous (IV) and intraperitoneal (IP) administration routes of an aqueous formulation of 1A-116. We first analyzed drug effects in Balb/c mice implanted in the subcutis with F3II tumors (2 x 10^5 cells/mouse), an aggressive hormone-independent mammary carcinoma model. Daily IP treatment with 10 mg/kg 1A-116 significantly reduced *in vivo* tumor growth and similar results were observed when mice were treated once a week with 1 mg/kg IV. We also evaluated drug effects on experimental lung colonization by F3II cells injected via tail vein (10^5 cells/mouse). IP administration showed a significant reduction in the number of metastatic lung nodules, but oral and SC 1A-116 administration had no effect. The pharmacokinetic study showed that 1A-116 has a good distribution after IP or IV administration (peak plasma concentrations 0.7-1.4 µg/ml), and poor oral bioavailability with this aqueous formulation. Importantly, 1A-116 showed a favorable acute toxicology profile in mice, with maximum tolerated doses of 80 mg/kg and 8 mg/kg for IP and IV routes, respectively. Our preclinical experiments highlight 1A-116 compound as a promising candidate for the treatment of aggressive tumors with aberrant Rac1 signaling.

482. (347) **HLA-G GENE EDITING IN A CHORIOCARCINOMA CELL LINE AS A NOVEL TOOL IN CANCER THERAPIES**

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Cancer immunotherapies, based mainly on the blockade of immune-checkpoint (IC) molecules by anti-IC antibodies, offer new alternatives for treatment in oncological diseases. However, a considerable proportion of patients remain unresponsive to these therapies. Hence, the development of novel clinical immunotherapeutic approaches and/or targets are crucial. In this context, targeting the immune-checkpoint HLA-G/ILT2/ILT4 has caused great interest since it is abnormally expressed in several tumor malignancies generating a tolerogenic microenvironment. Here, CRISPR/Cas9 gene editing system was used to block the HLA-G expression in a choriocarcinoma cell line named JEG-3, which expresses high levels of this protein. For this purpose, four different single guide-RNA targeting *HLA-G* exon 1 and 2 were designed (named 1A-, 1B-, 2A- and 2B-sgRNA). Then, these sgRNAs were cloned into pSpCas9(BB)-2A-puro(PX459) vector and were transfected in JEG-3 cells, each one separately or all four plasmids simultaneously. The HLA-G expression was measured in the edited JEG-3 cells by western blotting, flow cytometry and RT-qPCR. Genomic DNA was evaluated by Sanger sequencing to analyse possible modifications. The results showed that HLA-G gene editing was achieved. Downregulation of HLA-G was reached to different degrees when JEG-3 cells were edited with each one sgRNA separately. Whereas, complete HLA-G silencing was achieved when JEG-3 cells were edited with all sgRNAs simultaneously. Most importantly, the NK degranulation assay showed that edited JEG-3 cells (HLA-G negatives) triggered a higher *in vitro* response of NK cells with respect to wild type JEG-3 cells (HLA-G positives). Altogether, we demonstrated for the first time the HLA-G downregulation through gene editing, with a concomitant effect in immune cell activation. This approach would reactivate the host immune system and help to eliminate tumor cells, thus proposing novel cancer immunotherapy.

483. (348) **TISSUE SENESCENCE AND CANCER IN A MURINE MODEL OF LIVER CARCINOGENESIS**

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Previous works have described the role of tissular damage and phenotype associated with tissue senescence as elements that favor development of tumors. However, to date, it is unknown whether senescence, in the context of tissue injury, is a necessary condition for carcinogenesis. Herein, we determine whether livers of mice that have received the carcinogen diethylnitrosamine (DEN) show, prior to the emergence of the tumor, symptoms of damage and a decreased regenerative capacity. Then we have studied if tumors are located preferentially in senescent areas. DEN (5 μg/gram, intraperitoneal route) was inoculated into 15-days old C3H male mice and, in these conditions, produced liver tumors in about 100% of animals. First tumor foci appeared about 90 days after DEN. Over time their number and size increased progressively and by six months, displayed the characteristics of trabecular carcinoma. We have observed as functional signs of liver damage an increase in alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase enzymes post-inoculation of the carcinogen over time ($p<0.001$ vs inoculation day), which correspond to histological changes compatible with chronic liver inflammation. On the other hand, we observed that tumors were placed into or very close to se-

nescent areas. Senescence was detected by both a reduction of regenerative ability after partial hepatectomy (day 36: p<0.01; day 78: p<0.05 and day 180: p<0.001 after DEN inoculation vs control mice) and a higher expression of beta-galactosidase in DEN-treated mice (p<0.001), an enzyme expressed specifically in senescent cells. These changes preceded in about 40-50 days the first appearance of tumors and thereafter they increased progressively. Results suggest that tumor cells strongly depending on the microenvironment in which they are placed raising the possibility that manipulation of a senescent microenvironment may be useful to prevent, manage and even reverse neoplastic growth.

484. (354) **THYROID HORMONES REGULATE CELL PROLIFERATION AND ANTITUMOR IMMUNITY IN MELANOMA**

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Melanoma (ME) is the most severe type of skin cancer and despite immune checkpoint inhibitors provide exceptionally durable responses, only a limited number of patients benefit from it, making crucial the study of new compounds for ME treatment. Thyroid hormones (TH) influence tumor progression by direct actions on cancer cells, tumor microenvironment and antitumor immunity. Our first aim was to evaluate TH effect on the proliferation of human (A375 and WM35) and mouse (B16F10 and B16F1) ME cells. We found that physiological levels of TH induce 15 to 35% ME cell proliferation (p<0.05 vs control). Moreover, cilengitide, a selective inhibitor of the TH membrane receptor (mTR) αvβ3 integrin, not only prevents the proliferative effect of TH, but also inhibits the basal viability of ME cells (*p<0.05 vs vehicle * p<0.05 vs TH). Additionally, the expression of both integrins was found to be present on ME patient's samples from the TCGA-SKCM project, indicating that the mTR could be a possible target to improve ME therapy. We next evaluated the effect of thyroid status on ME cells growing *in vivo* in a syngeneic mouse model. For this, C57Bl/6 mice were subcutaneously inoculated with B16F1 cells after the treatment with thyroxine (12mg/l, 30 days) or propylthiouracil (500mg/l, 15 days) in the drinking water to obtain hyperthyroid (hyper) and hypothyroid (hypo) mice. Hyper mice showed increased tumor growth rate compared to controls and hypo mice (p<0.05). To further analyze the effect of thyroid status on the anti-ME immune response we evaluated the distribution of immune subsets in secondary lymphoid organs from tumor-bearing mice. We observed an increased percentage of NK cells (p<0.05) and increased cytotoxic T lymphocyte activity (p<0.05) but also increased proportion of MDSC (p<0.05) in spleens from hyper mice. Our results suggest that TH positively regulate ME cell proliferation through αvβ3 integrin and are also involved in the systemic anti-ME immune response.

485. (360) **COUMARIN 4-METHYLUMBELLIFERONE (4MU) REDUCES THE RESISTANCE TO CONVENTIONAL CHEMOTHERAPY AND THE TUMORIGENIC CAPABILITY OF CD133+ LUNG CANCER CELLS**

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Patients with non-small cell lung cancer (NSCLC) will ultimately progress or relapse after treatment with conventional chemotherapy (Qx) with platinum-taxanes. In the tumor microenvironment (TME) cancer stem cells (CSC), which express CD133, CD44 and CD47 among other markers form residual cell niches involved in the recurrence after treatment. Hyaluronan (HA), a component of the TME, regulates, at least in part, the function of CSCs. We previously demonstrated that tissue sections from murine Lewis Lung Carcinoma (LLC) tumors present high levels of HA and it is higher on isolated LLC CSCs (CD133+) compared with the non-CSCs population (CD133-; by FACS). Our analysis of TCGA data from patients with NSLCS showed that HA Synthase (HAS) 3 expression strongly correlates with levels of transcription factors involved in CSC phenotype. We modulated HA with the coumarin 4Mu, detecting an increase in the sensibility of LLC cells to paclitaxel (Pa). We aimed to cross-validate the TCGA findings on whole LLC and isolated CD133+ cells after exposure to Pa or cisplatin (Cis), alone or in combination with 4Mu. We analyzed the expression of HAS and CSCs genes by qPCR. The expression of HA and their clonogenic and tumor-forming capability was also evaluated. Pa increases mRNA levels of HAS3 (p<0.05) and HAS2 (p<0.01) while LLC treated with Pa+ 0.25 mM 4Mu showed reduced HAS3 levels(p<0.05). 4Mu also reduced HA levels produced by LLC. CD47 and SOX2 gene expression were enhanced by Pa while they decreased with Pa+4Mu (p<0.05). About 8.53 ± 0.35% of LLC are CD133+, and express more HA compared to CD133- (p <0.05). Viability of CD133+ cells decreased when treated with 4Mu+ Qx (Pa: p<0.01, Cis: p<0.05). Remarkably, 4Mu reduced the clonogenic (p<0.05) as well as tumor-forming ability of LLC CD133+ treated with Pa and Cis (p<0.05). We suggest that 4Mu improves the efficacy of Qx to inhibit tumor progression and could be implicated in preventing tumor recurrence.

486. (370) **THE GALECTIN-1-GLYCAN AXIS PROMOTES DISSEMINATION AND METASTASIS OF BREAST CANCER**

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The Galectin-1 (Gal1)/glycan axis controls several hallmarks of cancer. Here we investigated the role of Gal1 in breast cancer metastasis. We found at single cell level (scRNAseq) that Gal1 is synthesized by basal cell lineages and mammary stem cells (SCs) in normal mammary gland, where it promotes epithelial branching (**). Moreover, in the MMTV-NeuHER2/transgenic model, Gal1 was induced in early lesions (EL) compared to primary tumors (PT) (RNA-seq). Addition of rGal1 to EL 3D-cultures promoted invasiveness (**) and increased epithelial-to- mesenchymal transition (EMT) markers (RT-PCR). This effect was confirmed in the aggressive Her2+ human cell line JIMT-1, which showed high levels of Gal1 (**, Western) and low levels of α2,6 sialyltransferase-1 (ST6Gal1), an enzyme that incorporates α2,6-linked sialic acid and blocks Gal1 binding (***, RT-PCR), compared with the HER2+ poorly metastatic cell line BT-474. Accordingly, UPLC-HILIC/WAX revealed a Gal1-permissive glycan signature in JIMT1 (***). Treatment of JIMT-1 cells with rGal1, induced a CD44hi/CD24low cancer stem cell phenotype (***, flow cytometry) and enhanced migration (*), mamosphere formation (**) and EMT markers (RT-PCR). *In vivo*, treatment of HER2+PDX with rGal1 revealed increased lung metastasis (*). Bioinformatics analysis (TCGA) showed that tumors displaying a Gal- 1hi/ST6GAL1low phenotype had the poorest prognosis. Remarkably, these tumors upregulated transcripts associated with EMT and downregulated those linked to antitumor immunity (GSEA), as validated by the immunosuppressive infiltrate (Mixture). Our findings highlight the rele-

vance of the Gal1/glycan axis in controlling normal mammary gland branching and emphasize its critical role in metastatic spreading of breast cancer. We propose that the Gal1/ST6Gal1 pair might serve as a possible biomarker capable of predicting the outcome of breast cancer patients and as a therapeutic target of novel anti-metastatic therapies ($p<0.05$*;$p<0.01$**;$p<0.001$***).

487. (373) **EXTRACELLULAR VESICLES IN BREAST CANCER MICROENVIRONMENT: THE MECHANISMS UNDERLYING ENDOCRINE RESISTANCE**

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Antiestrogen adjuvant treatments are first-line therapies in patients with estrogen receptor-positive (ER +) breast cancer. The treatment strategies need to be improved because most patients eventually become endocrine resistant and many others are initially refractory to anti-estrogen treatments. The tumor microenvironment, and mainly macrophages, play an essential role in the development and progress of cancer; however, the molecular mechanisms underlying these effects remain poorly understood. Extracellular vesicles (EVs) secreted by tumor cells or by cells from the microenvironment have been proposed as one of the main forms of cell-cell communication. Many reports involve them in processes that are essential for cancer progression such as proliferation, migration, endocrine resistance, invasion, administration of drugs, among others. We proposed that EV are one of the most important actors in cell communication and could be one of the responsible for the endocrine resistance that we had observed in our previous work. The first steps for the study of these vesicles are the isolation and characterization of EVs from our cells of interest, then we evaluate the effect of EVs from macrophages and activated macrophages on mammary cells (tumor and non-tumor). These results suggest that EVs are involved in increased proliferation of mammary cells, and this increase depends on the amount and type of EVs, as well as the recipient cells. This and other analyzes will allow us to determine whether EVs are involved in communication between tumor-associated macrophages (TAMs) and tumor cells, and whether they are responsible for endocrine resistance in estrogen receptor-positive breast cancers.

488. (375) **A NOVEL IMPLICATION OF NEURONAL PROTEINS IN CANCER: CHARACTERIZING THE ROLE OF SYNUCLEIN PROTEINS IN MELANOMA**

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** Contributed equally to this work*

Synucleins are small proteins expressed primarily in neural tissue and certain tumors. While alpha-synuclein (aS) was recently connected to melanoma development, gamma-synuclein (gS) was associated with a range of tumor types. Our goal was to explore the role aS and gS in melanoma. For that, we worked with mouse (B16F0, B16F10) and human (SKMEL28, A375) melanoma cells. By Western Blot (WB) and immunocytochemistry (ICC), we observed that both proteins were expressed in these cells. Then, we modulated (by shRNA and expression vectors) aS and gS levels ($P<0.01$ for both proteins by qPCR and WB). Growth studies (cells count and MTT) indicated that reduced expression of aS and gS leads to proliferative defects ($P<0.05$; $P<0.01$, respectively), while increased expression was associated with cytoskeletal changes, migration and focal adhesions ($P<0.01$ and $P<0.05$, respectively), observed by ICC. Interestingly, melanoma cells were able to uptake different exogenous aggregation species of aS. These species, although toxic for neuronal cells (SH-SY5Y, $P<0.01$), failed to trigger toxic effects on melanoma cells, promoting instead proliferation ($P<0.05$), clonogenic capacity ($P<0.05$), cytoskeletal rearrangement and migration ($P<0.01$). We confirmed these observations *in vivo* by two approaches injecting subcutaneously B16-F10 cells (control and incubated with aS fibers) in the right flank in 8-week-old female C57BL/6 mice ($7x10^4$ cells; n=5/group and $2x10^5$ cells; n=6/group). By the first method, we observed that animals injected with treated cells developed tumors within 4 weeks post-inoculation (no tumor was observed in control group at this time). By the second, we analysed melanoma growth measuring tumor volume periodically. Growth kinetics indicated that αS treatment significantly promoted tumor growth ($P<0.05$).
Altogether, our results indicate that aS and gS have a role in melanoma growth and development. Further studies should be addressed to confirm and complement our observations.

489. (380) **Rac1-DEPENDENT Vav2 INVOLVEMENT IN MELANOMA**

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Melanoma is the most dangerous form of skin cancer, associated with an increasing incidence in the population.
Vav proteins are guanosine nucleotide exchange factors (GEFs) for the Rho GTPase family (mainly composed by Rac1, RhoA and Cdc42). In previous works we explored the role of Vav2 in events associated to melanoma development. We modulated Vav2 expression in the mouse-derived melanoma cell line B16-F0 observing that Vav2 is involved in cellular processes linked to proliferation, migration and cytoskeletal rearrangement, promoting melanoma development.
In this work, we studied the dependency of these phenotypes on Rac1 introducing by lipotransfection a fast cycling form of Rac1 (Rac1F28L) in Vav2-deficient cells. By MTT based methods we observed that defective proliferation and migration associated to reduced levels of Vav2 ($P<0.01$) were rescued by Rac1 expression. When cells were subcutaneously injected on C57BL/6 animals (n=4), we observed that Rac1 restored the defective tumor growth associated to a decreased in Vav2 ($P<0.05$). Indeed, defective expression of the epithelial markers beta-catenin observed by Western Blot, was increased by Rac1 ($P<0.05$) to similar levels observed in catalytically active Vav2 expressing cells.
As enhanced nuclear plasticity can promote cell migration during invasive processes, we evaluated the impact of Vav2 modulation on nuclear shape by DAPI staining, noting that decreased levels of Vav2 in melanoma cells was associated to an increased percentage of rounded nuclei and a lower amount of elongated ones ($P<0.01$).
Finally, to analyze the impact of Vav2 on melanoma cytokines production, we quantify by RT-PCR IL-6 expression, noting that Vav2-deficient cells expressed reduced levels of IL6 ($P<0.05$). Dependency of these two last phenotypes on Vav2 GEF activity needs to be addressed.
Altogether, our data indicate that Vav2 could participate in different cellular processes in melanoma cells through Rac1 activity, promoting tumor development.

490. (388) **CHARACTERIZATION OF A LUMINAL B AND A HER2 BREAST CANCER PATIENT-DERIVED XENOGRAFT**

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Molecular classification of breast cancer (BC) includes four main subtypes defined as luminal A and B, both of which express estrogen receptor alpha (ERα); HER2+-enriched, which overexpress HER2 and are $ERα^-$, and triple negative (TN) tumors, which are $ERα^-$/$HER2^-$. Patient-derived tumor xenografts (PDX) are generated by implanting tumor fragments directly from patients into immune-deficient mice. This model reflects more accurately the human tumor

biology as compared with cell line xenografts and have potential applications in precision medical treatments. We have recently established and genetically characterized 9 PDX (2 luminal, 1 HER2+, and 6 TN) derived from BC patients from *Hospital Magdalena V. de Martínez*. The aim of this study was to characterize one of the luminal PDX (707) and the HER2+ PDX (474) in terms of biomarker expression and treatment response to be used as models of these BC subtypes. PDX707 and the parental tumor are both $ER^+/PR^-/HER2^-$, while PDX474 and its parental tumor are $ER^-/PR^-/HER2^+$. Androgen receptor (AR) expression was also evaluated being both PDXs AR+. PDX707 was treated with tamoxifen (TAM; 5 mg/kg/5 days a week, sc) or testosterone (TESTO; 20 mg *pellet*, sc) and PDX474 with trastuzumab (TZ) and TZ emtansine (TDM1; 15 mg/kg/3 days a week, sc). Treatments started when tumors were 25 mm^2. TAM and TESTO inhibited PDX707 tumor growth ($p<0.001$) and, TZ and TDM1, inhibited PDX 474 tumor growth ($p<0.001$), being the effect of the latter more effective than the former. In conclusion, we have developed and characterized one luminal PDX suitable to explore the effect of combined endocrine therapies, and one HER2+ breast cancer model sensitive to HER2 inhibitors that may be used to test novel HER2 ligands or combined therapies for personalized medicine. Our results also highlight the role of AR ligands in ER+PR- tumors which have earlier recurrence than ER+PR+ tumors.

491. (403) **BIOLOGICAL RELEVANCE OF GALECTINS IN PATIENT-DERIVED GLIOMA STEM CELLS AND THEIR POTENTIAL APPLICATION IN THE DEVELOPMENT OF PERSONALIZED ANTINEOPLASTIC STRATEGIES**

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High-grade gliomas exhibit a hierarchical organization that relies on a minor subpopulation of gliomas stem cells (GSC). When injected into immunodeficient mice, these highly tumorigenic cells can develop and propagate brain tumors. GSCs are characterized by their self-renewal potential and their differentiation capacity. The use of multiple patient-derived GSCs constitutes a valuable tool to understand the biology of gliomas in greater detail and to develop translational projects that might contribute to personalized therapies. In the past years, many studies have demonstrated that galectins, a family of highly conserved glycan-binding proteins, play key roles in different aspects of cancer biology, including cellular transformation, proliferation, and apoptosis. In addition, these lectins contribute to tumor progression by favoring angiogenesis, tumor invasion and immune escape. In this study, we found that patient-derived GSC lines exhibit high expression of galectins-1 and -3 and an intermediate expression of galectins-2, -8, and -9. Importantly, by siRNA-mediated gene silencing, we found that galectins-1 and -3 participate in the control of different processes associated with GSCs. By Ki-67 immunostaining, we determined that decreased levels of these galectins lead to a reduction in GSC proliferation from 38,8% to 51,6% ($p<0.05$, n=3). Also, propidium iodide staining revealed that downregulation of galectin-1 exacerbates cell death in a cell line-specific manner from 76,7% to 126,6% ($p<0.05$, n=3), and this effect occurs only when galectin-3 expression is unaltered. Finally, as shown by cell spreading migration assays, silencing of these galectins also impairs GSC migration from 17% to 48% ($p<0.05$, n=3). Thus, involvement of these lectins in multiple processes associated with GSCs suggests their role as potential targets of therapeutic strategies in high-grade gliomas.

492. (412) **CHARACTERIZING A TUMOR SUPPRESSOR ROLE FOR Vav3 IN MELANOMA**

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Melanoma is the most deadly form of skin cancer, with globally increasing prevalence and mortality. A better understanding of this pathology at molecular level is a key challenge to improve diagnostic and therapy.

Vav proteins are Rho GTPases guanosine nucleotide exchange factors (GEFs). They modulate processes associated to tumor development and metastasis. As GEFS, these proteins were classically considered as protumoral.

We previously characterized the role of Vav2 in melanoma. Now we describe that Vav3 and Vav2 display antagonistic roles in this tumor type, contrary to what is described in other cancer types (including non-melanoma skin cancers). Through bioinformatic approaches we found that Vav3 expression varies significantly between healthy skin and melanoma ($P\leq0.01$) while this GEF acts like a double agent in other tumor types.

We modulated Vav3 expression in B16-F0 cells. By MTT assays we observed that Vav3 expression affects proliferation ($P\leq0.001$). We also found Vav3 levels affect both cell morphology and migration capacity; decreased Vav3 promotes a star shape associated to greater migratory capacity by wound healing assays ($P\leq0.001$), while increased expression induces elongated shape and poor migration ($P\leq0.001$). Indeed, increased expression of Vav3 promotes apoptosis by starvation.

By *in vivo* assays with 8-weeks old C57BL/6 female mice (n=6/group) subcutaneously injected, we demonstrated that Vav3 downmodulation increases tumor growth while high Vav3 levels drastically impairs tumor kinetics.

Altogether our data suggest a new tumor suppressor role for Vav3 in melanoma.

493. (416) **EFFECT OF TRANSDIFFERENTIATION ON PANCREATIC DUCTAL ADENOCARCINOMA CELLS**

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Pancreatic ductal adenocarcinomas (PDAC) represent the fourth leading cause of cancer-related deaths in the world. The 5-year survival rate of patients is 9%, and this is largely due to the great metastatic potential of this type of tumor.

Different studies have shown that ectopic expression of specific transcription factors can successfully transdifferentiate pancreatic tumor cells from the exocrine to the endocrine lineage. Our aim was to analyze the effect of the exocrine-endocrine transdifferentiation of PDAC in relation to their migratory phenotype, and to develop an *in vivo* model for pancreatic cancer studies.

We compared the gene expression profiles of ductal and endocrine pancreatic cells through the analysis of single cell RNA-seq. We identified 371 genes that are expressed at least twice as much in ductal as in endocrine pancreatic cells and performed a functional analysis of ontologies and signaling pathways (GO and KEGG). 54 genes were identified by both strategies as potentially related to tumor aggressiveness through characteristics such as cell migration and cell adhesion.

Additionally, PANC-1 and SW1990 cells were implanted on the chorioallantoic membrane (CAM) of chick embryos, and tumor growth was analyzed at different stages. We found significant tumor growth 10 days after implantation.

In order to induce transdifferentiation, PANC-1 cells were treated with BRD7552 for either 4 or 9 days, and migration rates were analyzed by wound healing assays. Significant decreases in migration

rates were observed, whereas MTT assays showed no significant differences.
In this work we identified genes involved in transdifferentiated cell phenotype, we revealed the inhibited migratory potential of transdifferentiated cells, and we established an alternative *in vivo* model for the study of PDAC based on cell implantation on the CAM of the chick embryo, well-suited for the *in vivo* evaluation of the effects of drugs on tumor growth, invasiveness, and metastatic potential.

494. (425) **PIN1 DELETION DOWNREGULATES TELOMERE MAINTENANCE AND REDUCES ONCOGENIC FEATURES IN GLIOBLASTOMA CELLS**

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PIN1 is the only isomerase capable to isomerize the phosphorylated Serine/Threonine-Proline motif and regulate diverse cellular functions. Thus, both PIN1 overexpression and its involvement in various oncogenic pathways has been reported in several cancer types, including glioblastoma. This is a lethal disease with limited therapeutic resources so far. Due to the need to develop new therapies, there is an interest in exploring PIN1 role in glioblastoma. For these reasons, in a previous study, our group developed a PIN1 *knock-out* glioblastoma cell model using LN229 cells. Results revealed that PIN1 deletion suppressed their malignant phenotype by reducing migration, cell cycle progression, and increasing doubling time. The current work focuses on deciphering the molecular mechanisms underlying the less aggressive phenotype of PIN *knock-out* glioma cells. First, the effect of PIN1 deletion on NF-κB pathway activation was studied using an NF-κB-LUC system. Additionally, IL-8 and telomerase catalytic subunit (htert) transcription levels, were quantified by qPCR. We also carried out a telomerase activity assay by RQ-TRAP; determination of relative telomere length by qPCR; senescence study by b-Galactosidase staining and apoptosis activation by measuring caspase-3 activity, in both LN PIN1 *knock-out* and LN229 cells.
Results showed that PIN1 deletion decreased NF-κB activity and both IL-8 and htert expression, a decrease of 55,40% in telomerase activity ($p<0,0001$) and a consequent telomere shortening of 45,14% ($p<0,0001$). Telomere shortening in LN PIN1 *knock-out* generated an increase in senescent cells and apoptotic cells, raising a value of 51,48% ($p< 0,001$) and 55.60% ($p< 0,05$) in comparison to LN229 cells, respectively.
These results highlight PIN1 implication in tumor progression and telomere maintenance in glioblastoma, suggesting this mechanism as a new potential therapeutic approach for the treatment of this disease.

495. (426) **SYNERGISTIC APOPTOTIC EFFECT OF 2´NITROFLAVONE COMBINED WITH SAFINGOL IN MURINE MAMMARY TUMOR CELLS**

Juan Manuel Anselmi Relats, Leonor Roguin, Mariel Marder, Julieta Marino, Viviana Blank

In a previous work, we have demonstrated that the synthetic flavonoid 2´-nitroflavone (2NF) is a potent and selective antitumor agent *in vitro* and *in vivo* in a murine LM3 breast cancer model. Sphingosine kinase 1 (SphK1), a lipid kinase overexpressed in some mammary tumor cells, regulates the balance between proapoptotic ceramides and prosurvival sphingosine-1-phosphate (S1P). Furthermore, safingol, a competitive SphK1 inhibitor, prevents catabolism of ceramides, contributing to tumor cell death. It has been reported that certain flavonoids exert antitumor activity through an increment in ceramide levels. Thus, we explored the antiproliferative effect of the simultaneous incubation of 2´NF and safingol in LM3 cells and found that they synergistically inhibited cell proliferation. Based on these results, we studied the apoptotic effect of 2NF in combination with safingol. When we evaluated the expression of bax proapoptotic protein, results obtained by Western blot showed that each compound alone did not modify bax expression, but a fivefold increase was observed after cell incubation with both 2NF and safingol for 24 h ($p<0.001$). Similarly, results obtained by flow cytometry showed a higher increment in the percentage of hypodiploid cells after simultaneous exposure to both compounds ($p<0.001$, 24h and $p<0.05$, 48 h). As recent reports demonstrated that some flavonoids inhibit SphK1 activity interacting directly with the kinase, we performed molecular docking analysis with the docking web server SwissDock. Results obtained showed that safingol and 2NF would bind to different molecular regions of the SphK1.
Based on the synergistic antiproliferative and apoptotic effects of 2NF in combination with safingol, we propose that the interaction of both molecules with different sites of SphK1 would favor the generation of apoptotic ceramides by inhibiting the formation of S1P. Further experimental approaches should be performed to confirm a direct interaction of 2NF and SphK1.

496. (453) **GADD45A AND CDKN1A, AMONG OTHER GENES, ARE EPIGENETICALLY REGULATED IN PROSTATE TUMORS FROM PATIENTS**

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DNA methylation, histone modifications and miRNAs regulation are important epigenetic mechanisms of gene regulation that can be detected before prostate cancer (PCa) becomes invasive, suggesting they are pivotal events in tumor initiation and progression. Metabolic syndrome (MeS) increases PCa's risk and aggressiveness. Previously, using MeS NSG male mice injected *s.c.* with PC3 PCa cells, we demonstrated that *DNMT1* and *SUV39H1* expression levels were repressed, while *RIZ1* and *GADD45A* were increased in prostate tumors from MeS mice compared with control. Also, mRNA levels of *CDH1* and *ZEB1*, two DNMT1 targets, were repressed and induced, respectively, in these tumors. Our hypothesis is that aberrant epigenetic changes in PCa favor tumor development and progression. Here we investigated expression and methylation levels of several epigenetic genes in PCa patients using Xena database: *DNMT1*, *SUV39H1*, *EZH2*, *DNMT3A*, *DNMT3B*, *TET2*, *TET3*, *RIZ1*, *EP300*, *HDAC2, SIRT1*, *HBO1*, *CDH1*, *ZEB1*, *GADD45A* and *CDKN1A*. We found that
GADD45A and *CDKN1A* mRNA levels were significantly diminished in prostate tumors compared to normal adjacent tissue (NAT). Accordingly, methylation levels of *GADD45A* and *CDKN1A* were significantly increased in comparison with NAT and between Gleason Scores. Moreover, expression and methylation levels of other genes involved in epigenetic changes were significantly altered in prostate tumors vs NAT and between Gleason Scores. Additionally, to further investigate epigenetic changes mediated by miRNAs, we assessed the expression levels of a panel of miRNAs involved in PCa development. Altogether these data showed that aberrant expression and methylation of epigenetic genes, particularly the correlation between the increased methylation and decreased expression levels of *GADD45A* and *CDKN1A* in prostate tumors from patients, might be consider as promising mechanisms to further investigate in PCa aggressiveness and even identifying novel biomarkers for patient prognosis.

497. (456) **COUMARIN 4-METHYLUMBELLIFERONE (4MU) REDUCES THE RESISTANCE TO CONVENTIONAL CHEMOTHERAPY AND THE TUMORIGENIC CAPABILITY OF CD133+ LUNG CANCER CELLS**

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Patients with non-small cell lung cancer (NSCLC) will ultimately progress or relapse after treatment with conventional chemotherapy (Qx) with platinum-taxanes. In the tumor microenvironment (TME) cancer stem cells (CSC), which express CD133, CD44 and CD47 among other markers form residual cell niches involved in the recurrence after treatment. Hyaluronan (HA), a component of the TME, regulates, at least in part, the function of CSCs. We previously demonstrated that tissue sections from murine Lewis Lung Carcinoma (LLC) tumors present high levels of HA and it is higher on isolated LLC CSCs (CD133+) compared with the non-CSCs population (CD133-; by FACS). Our analysis of TCGA data from patients with NSLCS showed that HA Synthase (HAS) 3 expression strongly correlates with levels of transcription factors involved in CSC phenotype. We modulated HA with the coumarin 4Mu, detecting an increase in the sensibility of LLC cells to paclitaxel (Pa). We aimed to cross-validate the TCGA findings on whole LLC and isolated CD133+ cells after exposure to Pa or cisplatin (Cis), alone or in combination with 4Mu. We analyzed the expression of HAS and CSCs genes by qPCR. The expression of HA and their clonogenic and tumor-forming capability was also evaluated. Pa increases mRNA levels of HAS3 ($p<0.05$) and HAS2 ($p<0.01$) while LLC treated with Pa+ 0.25 mM 4Mu showed reduced HAS3 levels($p<0.05$). 4Mu also reduced HA levels produced by LLC. CD47 and SOX2 gene expression were enhanced by Pa while they decreased with Pa+4Mu ($p<0.05$). About 8.53 ± 0.35% of LLC are CD133+, and express more HA compared to CD133- ($p <0.05$). Viability of CD133+ cells decreased when treated with 4Mu+ Qx (Pa: $p<0.01$, Cis: $p<0.05$). Remarkably, 4Mu reduced the clonogenic ($p<0.05$) as well as tumor-forming ability of LLC CD133+ treated with Pa and Cis ($p<0.05$). We suggest that 4Mu improves the efficacy of Qx to inhibit tumor progression and could be implicated in preventing tumor recurrence.

498. (457) **A BRIEF REPORT OF COVID-19 CASES IN CANCER PATIENTS FROM AMBA: DESCRIPTION OF HOSPITALIZED POPULATION AND THEIR IMMUNITY AGAINST SARS-COV-2**

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BACKGROUND: Until today there are 229,414,751 registered cases and 4,707,872 deaths attributable to the disease caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) called COVID-19, all over the world. COVID-19 can be asymptomatic or cause a respiratory distress syndrome and death. The risk of COVID-19 and mortality for COVID-19 have been associated with older age and comorbidities, such as cancer. The situation is a great challenge for these patients. Furthermore, in patients with active treatment, the decrease in cell populations involved in protection is very common and immunity against SARS-CoV-2 may be impaired. OBJECTIVES: Here we described the population of oncological (P On) and non-oncological (P NOn) patients with moderate or severe COVID-19 hospitalized at the HUA from December, 2020 to September, 2021. Peripheral blood samples were collected at different times to analyze the duration of the protective response (Elisa; flow cytometry). RESULTS: We incorporated 24 patients: 11 [45%] with oncological disease in treatment, 5 male median age 59 with lung (4) and scalp (1) cancer and 6 female median age 36 with ovarian (1), cervix (2), renal (1) rectum (1) and breast cancer (1) with moderate (9) or severe (2) COVID-19 and 13 patients without oncological disease [54%], 7 male median age 59 years, 6 female median age 40, with moderate (12) or severe (1) COVID-19. The POn group had a longer hospital stay (15.9 vs 8.8 days; p 0.016) and higher oxygen requirement (high flow 36.4% vs 7.7%; p 0.085). The mortality rate in POn was 36% and there were no deaths in PNon. The circulating immune response for SARS-CoV-2 was analyzed in 50 samples at different times from the 24 hospitalized patients. The protective response was significantly lower in the POn population ($p <0.001$) with low detection of IgM and IgG. CONCLUSIONS: in POn the protective response is lower compared to PNon, with probable implications in morbidity and mortality.

499. (458) **ANALYSIS OF RUNX-CBFβ ACTIVITY ON BASAL BREAST CANCER PROGRESSION THROUGH DIRECT CONTROL OF RSPO3 EXPRESSION**

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** Carla M. Felcher and Micaela N Stedile contributed equally to this study.*

We have determined that R-spondin3 (RSPO3), a secreted protein that potentiates Wnt signaling pathway, is a key modulator of tumor progression and stem cell behavior in basal breast cancer. Previous reports indicated the potential role of RUNX1-CBFβ axis on Rspo3 expression in mammary tumor cells. Besides, we found that treating basal breast cancer cells with small molecules that block the interaction between CBFβ and RUNX reduced Rspo3 mRNA and protein levels. These treatments also induced inhibition of cell migration, an ability that was recovered upon addition of recombinant RSPO3. Also, we have determined that those inhibitors enhanced the effects of the chemotherapeutic drug Doxorubicin on MDA-MB231 cell survival and migration. To determine whether there is a direct control of RUNX1-CBFβ on Rspo3 mRNA transcription, we performed an *in silico* analysis of publicly available data from two RUNX1 CHIP-seq reports and an ATAC-seq study from human breast cell lines. We aligned the emerging data with the occurrences of the RUNX1 DNA-recognition-motif in the Rspo3 locus. A few putative RUNX1 binding sites were revealed by this analysis. Among them, a region located on Rspo3 first intronic region that seems to be particularly active in triple negative breast cancer cells. Importantly, we have determined that RUNX1 actually binds to that site in MDA-MB231 cells by ChIP-qPCR assay (3-fold increase compared to control). Then, to further explore the mechanisms underlying the control exerted by RUNX-CBFβ on Rspo3 expression, we proceeded to delete that RUNX1 binding site by introducing, through electroporation, Cas9 protein together with specific sg-RNAs in MDA-MB231 cells. We are currently analyzing the phenotype of the obtained cells to verify the relevance of the discovered RUNX1 binding site. In summary, our results confirm that RUNX-CBFβ axis may contribute to basal breast cancer progression by directly inducing Rspo3 expression in mammary tumor cells.

500. (493) **MECHANISMS ASSOCIATED WITH CYTOSINE DEAMINASE::URACIL PHOSPHORIBOSYL TRANSFERASE/5-FC SUICIDE GENE SYSTEM BYSTANDER EFFECT IN HUMAN MELANOMA**

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We have previously shown the therapeutic potential of yeast cytosine deaminase::uracil phosphoribosyl transferase/5-fluorocyto-

sine (CDU/5-FC) suicide gene (SG) system in 8 human melanoma cell lines. CDU catalyzes 5-FC conversion to 5-fluorouracil (5-FU), which interferes with RNA processing and DNA synthesis. The aim of the present work was to explore CDU lipofected-cells ability to release particulate factors and to analyze its contribution to the cytotoxic effect.
Methods: Five melanoma cell lines (A375, M8, hM1, hM4 and hM9) were used. After lipofection, lipoplex-rich medium was reserved and cells were washed thrice with medium, to remove remaining lipoplexes. After the last wash, cells were cultured in fresh medium. Conditioned media (CM) was obtained after 48 h of incubation from CDU- and HSVtk-lipofected cells with or without 5-FC and from unlipofected cells with or without 5-FU. The supernatant (SN) and pellet (P) fractions of the CM were obtained by centrifugation at 12000 × g for 60 min. These fractions were incubated on receptor cells with or without 5-FC, and cell viability was determined by the APH method after 5 days.
Results: As expected, the SN fraction of 5-FU CM was accountable for bystander cytotoxicity, while the P fraction was not. In most cell lines only the SN fraction of CDU/5-FC treated cells was responsible for cytotoxicity, presumably given by 5-FU presence on the CM. Interestingly, the addition of 5-FC on cells that received both fractions of CDU CM caused significant cytotoxicity ($p<0,05$), indicating transfer of the enzyme or its gene. While CDU lipoplex-rich medium was able to deliver enzyme or its gene ($p<0,05$), the last-wash medium was unable to do it, denoting that there were no remaining lipoplexes on CM.
Conclusion: The CDU/5-FC suicide gene therapy system bystander effect would be mainly attributable to 5-FU and to the CDU fusion enzyme or its coded information (plasmid DNA o mRNA) carried by particulate subcellular fractions.

501. (506) **THE ANDROGEN RECEPTOR REGULATES RUNX1, A NEW POTENTIAL WAY TO TARGET CHEMOTHERAPY RESISTANT TRIPLE NEGATIVE BREAST CANCER**

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Triple negative breast cancer (TNBC) is an aggressive breast cancer (BC) subtype for which no effective targeted therapies are available. Growing evidence suggests that chemotherapy-resistant BC cells with stem-like properties (CSC) may repopulate the tumor. Therefore, therapies that target the CSC in combination with chemotherapy might prevent tumor recurrence. Androgen Receptor (AR) is expressed in at least half of all TNBC. AR inhibition decreases CSC *in vitro* and tumor initiation i*n vivo*. RUNX1 is regulated by AR in prostate cancer. In TNBC patients, RUNX1 protein levels correlate with poor prognosis. Our group has shown that RUNX1 promotes TNBC cell migration and regulates tumor gene expression, such as the oncogene *RSPO3*. Also, by RUNX1 ChIP assays, we found SOX4 as a potential target gene. We hypothesized that RUNX1 is regulated by AR and that both may work together in TNBC CSCs to promote persistence and disease recurrence following chemotherapy. Here we show that, in MDA-MB-453 cells, RUNX1 expression is upregulated by dihydrotestosterone, an AR agonist, and that this effect is blocked in the presence of Enzalutamide (AR antagonist). ChIP-seq experiments revealed AR binding to RUNX1 regulatory regions, suggesting direct regulation. RUNX1 expression is increased in a CSC-like experimental model and responds to AR activity. Inhibition of RUNX1 transcriptional activity by AI-10-104 (a synthetic drug) reduced the expression of the CSC marker SOX4. Interestingly, this inhibition drives a reduction of MDA-MB-453 and BT-549 cell proliferation and enhanced paclitaxel sensitivity. It was reported that AR inhibition combined with chemotherapy results in a more effective outcome than chemotherapy alone *in vitro* and *in vivo*. In sum, RUNX1 inhibition may also be an attractive target to potentiate the anti-tumor effect of AR inhibition, specifically in the slow growing CSC-like populations that resist chemotherapy and lead to metastatic disease.

502. (507) **VITAMIN D RECEPTOR AND PACLITAXEL IN TRIPLE NEGATIVE BREAST CANCER: IS THERE A LINK BETWEEN THEM?**

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Paclitaxel (PTX) is an antitumor agent employed in the treatment of Triple-Negative Breast Cancer (TNBC). TNBC expresses Vitamin D Receptor (VDR), a member of the nuclear receptor superfamily. The aim of this work was to investigate the involvement of VDR in the antitumor action of PTX in TNBC cells. To this end, viability assays by crystal violet staining were performed in murine 4T1 TNBC cells and in 4T1 stably expressing a shRNA against VDR (4T1 shVDR), treated with PTX (10 nM) or vehicle. Also, cell cycle was studied by flow cytometry. Cellular studies were complemented with in silico analyses including molecular docking and molecular dynamics (MD) simulations to describe the pharmacodynamic interaction between PTX and VDR. The results show that PTX reduced the viability of 4T1 wild type cells ($p<0.001$). These viability effects were lost in 4T1 shVDR cells which display approximately 53% of VDR levels with respect to control cells. Cell cycle analysis of 4T1 wild type and 4T1 shVDR cells treated with PTX showed that the chemotherapy causes an increase in the percentage of cells in sub G0/G1 phase compared to vehicle-treated cells. However, this PTX effect was significantly higher in wild type than in VDR-silenced cells ($13.72 \pm 2.37\%$ vs $6.18 \pm 1.07\%$, $p<0.001$). Docking and MD studies showed that PTX was not able to bind to the classical ligand-binding pocket of VDR. However, an exhaustive search of allosteric sites identified its stable binding to a cavity adjacent to the activating factor 2 (AF-2) region. MD studies verified a conformational restraint on AF-2, which triggers transcriptional and antitumor effects. Furthermore, a potential cooperativity in the interaction with VDR between PTX and the natural ligand of the receptor was observed. Altogether, these results suggest that PTX could interact with VDR to display its antitumor effects in TNBC by its binding in an alternative site to that of the classical VDR agonists.

503. (509) **NOVEL HISTAMINE H_3 RECEPTOR ANTAGONISTS WITH POTENT ANTINEOPLASTIC PROPERTIES AS TARGETED DRUG THERAPY FOR BREAST CANCER**

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We have reported the expression of the histamine H_3 receptor (H_3R) in human benign and malignant lesions, and cell lines derived from human mammary glands. Its expression is highly correlated with proliferation in breast cancer specimens.
In this work, we aimed at investigating the potential antitumoral activity of 4 novel H_3R antagonists, 1-(2,3-dihydro-1-benzofuran-2-yl) methylpiperazines (LINS01 compounds), which showed excellent

selectivity and high affinity for the human H_3R.[1,2] Cell viability and proliferation were assessed by cell titer blue assay and colony formation in human MDA-MB-231 and murine 4T1 triple negative breast cancer cells. Cell apoptosis was assessed by Annexin V staining and flow cytometry, while cell migration was evaluated by wound-healing assay and transwell system. The lipid accumulation was assayed by flow cytometry using Nile-red staining.
Results indicate that compounds LINS01022, LINS01023, LINS01009, LINS01010 (0.1-100 μM) produced a concentration-dependent inhibition on cell growth. The highest responses were observed for LINS01022 and LINS01023, showing an IC_{50} in the cell viability assay of 82.7 and 78.2 μM for MDA-MB-231 cells, and 87.0 and 59.2 μM for 4T1 cells. LINS01022 and LINS01023 (25-50 μM) induced cell apoptosis (4 to 7 fold-increase) and differentiation (2 to 3 fold-increase), while suppressed cell migration in both cell lines ($P<0.01$).
The allylpiperazines LINS01022 and LINS01023 exhibited better antiproliferative and proapoptotic effects together with a higher affinity constant for the H_3R than their corresponding methylpiperazine analogues LINS01009 and LINS1010, respectively.
These effects were not observed with the selective H_3R agonist, (*R*)-alpha-methylhistamine.
In conclusion, this study demonstrates that the H_3R is involved in the regulation of cell growth and progression, offering novel therapeutic potentials for H_3R antagonists.
[1]Correa et al. *Front Pharmacol* **2017**,*8*,825
[2]Correa et al. *Bioorg Med Chem* **2021**,*30*,115924

504. (510) **HIF-1α REGULATES TUMOR PROGRESSION IN A HUMAN EPITHELIAL OVARIAN CANCER MODEL**
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Ovarian cancer is the seventh most common cancer in women and the eighth cause of cancer death. The treatment of this disease has been the same for the past decades, and the development of new drugs is needed. Hypoxia is a common characteristic of solid tumors, usually associated with a more aggressive phenotype. The main transcriptional factor involved in this process is Hypoxia Inducible Factor 1 alpha (HIF-1α).
The present work aimed to study the effect of Acriflavine (ACR), a specific HIF-1α inhibitor, on a human epithelial ovarian cancer model (SKOV3), both *in vivo* and *in vitro.* For the *in vitro* experiments, we performed cell proliferation and wound healing assays to assess cell migration with different ACR concentrations. Cell proliferation was significantly diminished with ACR 1uM after 72 hours of treatment as compared with control cells (1 μM ACR: 22.69 % ± 0.66 vs Control 100,00% ± 10,40 , $p<0.0001$), and migration was reduced with ACR 1uM after 18 h of incubation (Control, 85,75% wound closure ± 5,12 vs ACR 1uM, 52,40 % wound closure ± 3,90, $p<0.0001$).
For the *in vivo* experiments, 5 x 106 cells were s.c. injected into the flank of immunosuppressed NSG mice. The treatment with ACR daily injections (5 mg/kg, 15 days) started when tumors reached 25 mm2. ACR-treated tumors were significantly smaller than control tumors ($p<0.001$), showed a lower proliferation index (*Ki67)* and a lower VEGF and GLUT1 expression through immunohistochemistry as compared with control tumor samples. VEGF and GLUT1 expression are used to evaluate the transcriptional activity of HIF-1α, as both proteins are HIF-1α downstream targets.
Our results show that HIF-1α plays an important role in the proliferation, migration, and tumor growth of the SKOV3 ovarian cancer model. We conclude that ACR could be a potential drug for the treatment of ovarian cancer, alone or in combination with standard therapy.

505. (514) **CLINICAL FEATURES IN PATIENTS WITH CANCER AND COVID-19 IN SANTA FE AND BUENOS AIRES**
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Patients with cancer are high-risk population in the COVID-19 pandemic. We aimed to describe clinical characteristics of patients with cancer and COVID-19 in city of Santa Fe and Buenos Aires. We did a cross-sectional study of 80 patients with laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and with a pathological diagnosis of a malignant tumor from hospitals of city of Santa Fe and from Hospital Italiano (Buenos Aires). Clinical characteristics and cancer histories were investigated and analyzed.
During the study, 80 patients with cancer and laboratory-confirmed SARS-CoV-2 infection were included (median age 65 years). 45 (56,2%) patients were women and 34 (42,5%) were men. 72 patients had solid tumours and 3 patients had haematological malignancies. The most frequently symptoms were fever (29 [40,28%] patients) and cough (28 [38,89%] patients); then dyspnoea (18 [25%] patients) and fatigue (19 [26,36%] patients). The most common solid tumor types were breast (20 [26,7%] patients) and lung cancer (8 [10,7%] patients). 33 (41,25%) of 80 patients had comorbidities: 11 (20,37%) had coronary disease, 10 (18,52%) had diabetes, 7 (12,96%) had chronic kidney disease and 5 (9,26%) chronic obstructive pulmonary disease. The rate of mortility was of 18.75%. In addition, if we analyze how many patients did not survive among the cases with severe pneumonia (41,86%) and compare them with non-severe pneumonia (16,28%), it gives an OR of 20.25 (2.32, 176.6, $p = 0.0028$), indicating that severe pneumonia could be a risk factor of mortality. Current statistics data from Argentina indicate that the rate of mortility of COVID-19 patients was 2% (114000 of 5240000). The data from our analysis indicates that cancer and COVID-19 patients have an 8 times higher risk of death than patients with only COVID-19.
Cancer patients have deteriorating conditions and together with a high frequency of comorbidities, these patients become a vulnerable population. It would be a priority that patients with cancer and COVID-19 infection receiving regular screening and preventives therapies.

506. (516) **DENDRITIC CELL PROFILE IN TUMORAL MICROENVIRONMENT OF HUMAN BREAST CARCINOMAS**
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Argentina has the second highest mortality rate for breast cancer (BC) in South America. The immune cells present in the tumor microenvironment (TME) performs dual functions, being able to eliminate or promote malignancy according to the signals present. We believe that the dendritic cells (DCs) found in the TME play a fundamental role in the development of the mammary tumor. Our hypothesis is that there are different subpopulations and maturation profiles of DCs in the TME, and these profiles are associated with tumor traits of mammary carcinomas. Initially, we propose as a general **objective** to study the different types of DCs present in the TME and to determine the profile of these different subtypes in human

breast carcinomas. **Methodology**: Once the tumors were obtained by surgery, breast carcinoma-derived fractions were mechanically and enzymatically disaggregated. Tumoral (EpCAM+) and non-tumoral (EpCAM-) populations of each fraction were isolated using cell sorting flow cytometry. DC populations were characterized by flow cytometry using the HLA-DR, CD14, CD11c, CD1c cell surface markers to perform the gating strategy. **Results**: We obtained tumoral and non-tumoral populations derived from eight human breast carcinomas fractions. We defined four different DC subpopulations present in the TME: pDCs, inflammatory DCs, cCD1 and cCD2 DCs. Interestingly, we observed that each tumoral fraction has a unique DC profile, according to the high heterogeneity already described for this type of tumor. Based on the tumor cohort analysis, we evidenced a negative correlation between tumor cell and cDC2 DC populations (r= -0.76, p<0.027). **Conclusion**: Breast TME contains different DC profiles associated with tumoral cell proportions in human breast carcinoma fractions. Ongoing and future experiments will allow us to determine the maturation profiles of these DCs and analyze their relationship with genomic/epigenomic and clinicopathological tumor characteristics.

507. (525) **ENZALUTAMIDE AND NOTCH PATHWAY INHIBITION RESTRAIN PROSTATE CANCER CELL GROWTH AND MIGRATION**

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Prostate cancer (PCa) remains among the leading causes of cancer-related deaths in men. Standard therapies for castration resistant prostate cancer (CRPC) include second-generation anti-androgens, such as Enzalutamide (Enz), which prolong patient lifespan. Emerging evidence indicates a regulatory role of Notch signaling in prostate development and growth.
In this work, we aimed to study the Notch and AR pathway involvement and their interaction in prostate cancer.
We first determined the expression of Notch receptors (1/2/4) and the proliferation marker PCNA by IHQ in prostate tumor samples obtained by surgery and its association with Gleasson score.
In prostate cancer PC3 cells, we demonstrated AR expression by RT-qPRC. Instead, PSA expression was absent when evaluated culture cell supernantants by chemiluminescence. Moreover, under Notch pathway inhibition with DAPT, the expression of *TMPRSS2*, an androgen dependent gene, showed lower levels after 24 h of treatment (10 and 30 μM) by RT-qPCR (p=0.02; n=2). In turn, Enz treatment (30 and 50 μM) reduced the levels of *HES1,* a target gene of the Notch pathway, determined by RT-qPCR (n=3).
We observed significantly reduced viability of PC3 cells both with DAPT (p=0.0027; n=3) and Enz isolated treatments (p=0.0018; n=3) using MTS assay. Importantly, the combined treatment with DAPT and Enz significantly reduced PC3 viability at 48 and 72 h (p=0.0043; n=3). We observed reduced migratory abilities both with DAPT (p=0,0022; n=3) and Enzalutamide isolated treatment (p=ns; n=1.2), and also with the combined treatment (p=0,0526; n=3) using wound healing assay.
Our study suggests an interconnection between Notch and AR pathways in PCa. That would restrain in an alone or combined manner the viability and migratory abilities of PC3 cells. Therefore, a combined approach consisting of Notch and AR inhibitors could be more effective than isolated antiandrogen treatment, especially in patients with advanced and metastatic CRPC.

508. (537) **INVOLVEMENT OF RUNX1 IN DRUG RESISTANCE IN TNBC SUBTYPE**

Sofía María Sosa[1], Natalia Fernández[1], Facundo Couto[1], Ana Raimondi[2] & Natalia Rubinstein[1]
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Triple negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT) and an enrichment in cancer stem cell (CSC) population, which according to growing evidence, are involved in tumor chemoresistance. Our group has shown that RUNX1 could be involved in the aggressiveness of this breast cancer subtype. We reported that RUNX1 is able to promote cell migration and regulate tumor gene expression, such as the oncogene RSPO3 and the metastasis marker gene GJA1. ChIP assays done in our lab revealed that RUNX1 can regulate transcription factors involved in EMT. Moreover, RUNX1 protein expression in TNBC correlates with poor patient prognosis. Our aim was to evaluate RUNX1 relevance in drug treated human TNBC cell lines. Here we show that RUNX1, KLF4 (stemness marker) and GJA1 gene expression are significantly upregulated in doxorubicin-or paclitaxel-treated TNBC cell lines (all *p* values were at least <0,02). Interestingly, we observe that loss of RUNX1 transcriptional activity significantly enhances doxorubicin and paclitaxel toxicity in TNBC cell lines (all *p* values were <0,0001). In addition, we found a potential DNA binding site for glucocorticoid receptor (GR) in RUNX1 gene. TNBC cell lines show that *RUNX1* mRNA is significantly upregulated with dexamethasone (GR agonist) and downregulated with mifepristone (GR antagonist) (p=0,0037 on MDA-MB-453 and p<0,0001 on MDA-MB-468). Therefore, our data suggests that RUNX1 may be involved in TNBC chemoresistance and its expression could be externally regulated by GR activity modulation.

509. (573) **INVOLVEMENT OF RUNX1 IN DRUG RESISTANCE IN TNBC SUBTYPE**

Sofía María Sosa[1], Natalia Fernández[1], Facundo Couto[1], Ana Raimondi[2] & Natalia Rubinstein[1]
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Triple negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT) and an enrichment in cancer stem cell (CSC) population, which according to growing evidence, are involved in tumor chemoresistance. Our group has shown that RUNX1 could be involved in the aggressiveness of this breast cancer subtype. We reported that RUNX1 is able to promote cell migration and regulate tumor gene expression, such as the oncogene RSPO3 and the metastasis marker gene GJA1. ChIP assays done in our lab revealed that RUNX1 can regulate transcription factors involved in EMT. Moreover, RUNX1 protein expression in TNBC correlates with poor patient prognosis. Our aim was to evaluate RUNX1 relevance in drug treated human TNBC cell lines. Here we show that RUNX1, KLF4 (stemness marker) and GJA1 gene expression are significantly upregulated in doxorubicin-or paclitaxel-treated TNBC cell lines (all *p* values were at least <0,02). Interestingly, we observe that loss of RUNX1 transcriptional activity significantly enhances doxorubicin and paclitaxel toxicity in TNBC cell lines (all *p* values were <0,0001). In addition, we found a potential DNA binding site for glucocorticoid receptor (GR) in RUNX1 gene. TNBC cell lines show that *RUNX1* mRNA is significantly upregulated with dexamethasone (GR agonist) and downregulated with mifepristone (GR antagonist) (p=0,0037 on MDA-MB-453 and p<0,0001 on MDA-MB-468). Therefore, our data suggests that RUNX1 may be involved in TNBC chemoresistance and its expression could be externally regulated by GR activity modulation.

510. (588) **RAC-FUNCTION MODULATES IN A DIFFERENT WAY PROLIFERATION OF NORMAL RENAL PROXIMAL AND CLEAR RENAL CARCINOMA CELLS: EFFECT OF ALKALOSIS AND ISOFORM 1 OF NA^+/H^+ EXCHANGER**

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One of the hallmarks of cancer is tumor extracellular acidosis. Then, we hypothesize that extracellular pH (pHe) affects differently cancer or normal cells. Our previous studies showed that, in cells derived from renal cell carcinoma (RCC), the isoform 1 of the Na^+/H^+ exchanger (NHE1) function is antiproliferative in media at pH 7.4. Alkalosis reverts this effect. On the other hand, in normal proximal cells, while at pH 7.4 NHE1 favors cells proliferation, NHE1 is antiproliferative alkaline media. The aim of this study was to further investigate this process. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with 9.6 mM NaOH for 72h. Then, we estimated cell proliferation by BrdU experiments in the presence of IA116, a Rho GTPases Rac1 inhibitor and NHE1 expression by immunoblot studies. In normal HK2 cells at media 7.4 Rac inhibition enhanced proliferation (%BrdU + cells: +Rac: 36±3 vs -Rac= 71±4, $p<0.001$ n=11). This effect was reverted when NHE1 function was inhibited with 1µM HOE. Thus, Rac function potentiated NHE1-related proliferation. In 786-O RCC cells, at media 7.4 Rac inhibition inhibited NHE1-related antiproliferative effect (NHE1-induced proliferation: +Rac: 14±2 vs -Rac= -8±2, $p<0.001$ n=16). We also investigated if NHE1 expression could change with alkaline exposure. Our immunoblot studies showed that normal renal cells had higher NHE1 expression than RCC cells (NHE1 expression HK2 5.4±0.5 vs 786-O= 2.7±0.2, $p<0.01$ n=8). Exposure to alkaline media rose NHE1 expression only in RCC cells (NHE1 expression in 786-O pH7.4= 2.7±0.2 vs pH=7.5= 4.5±0.7, $p<0.05$ n=8). In summary, Rac function would reduce NHE1-related proliferation in normal renal proximal cells. In RCC cells, however, NHE-1 related antiproliferative effect would depend on Rac function. Finally, these effects would not be related to NHE1 expression.

511. (604) **TUMOR ASSOCIATED FIBROBLAST: IMPACT ON OSTEOSARCOMA PRIMARY AND METASTATIC TUMORAL MICROENVIRONMENT AND TREATMENT RESPONSE**

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Tumor associated fibroblast (TAF) have been implicated in almost every aspect of tumoral biology. Of relevance TAF could be modulating response to treatment and overall microenvironment development. Given that Osteosarcoma (OS) have the same 5-year survival rate for metastatic and treatment resistant patients since 1970´s, we dediced to investigate the role of TAF in OS primary and metastatic niches.
Aim: Evaluate TAF and human OS cell lines interaction in primary and pulmonary metastatic environments. To analyze if metastatic OS cell line has a higher inducing power than non-metastatic OS cell line analyzing the expression of different ABC transporters implicated in chemoresistance and the ability to exclude doxorubicin and rhodamine.
Methods: The expression of ABC transporters was analyzed by RT-qPCR on conditioned fibroblast. Rhodamine 123 exclution assay was used to determine the activity of P-glycoprotein (P-gp) mediated transport and doxorubicin (DOX) exclution was performed to analysis the overall ABC-related chemoresistant capacity. To evaluate the interaction of fibroblast with metastatic (LM7) and non-metastatic (SAOS2) OS human cells hetero – spheroid formation assays were performed.
Results: LM7 conditioned medium (CM) induced an overall upregulation of ABC transporters in comparison with SAOS2 CM. Conditioned fibroblast with LM7 CM showed lower levels of intracellular DOX and Rhodomine in comparison with SAOS2 CM fibroblast. Mixed spheroid compose of fibroblast ans OS cell lines display a lower area and more compact than single type aggregates.
OS has not changed the 5-year rate survival for metastatic patients since the 70', so the need to understand aspects of OS metastatic biology and chemoresistance could be helpful to develop new treatments to this group. Knowing aspects of the assocaited stroma and in particular TAF, could allow the development of new therapeutic possibilities targeting the tumoral associated stroma.

QUÍMICA MEDICINAL

512. (137) **A NEW MIXED LIGAND COPPER(II) COMPLEX OF NARINGENIN AS A PROSPECTIVE ANTICANCER AGENT**

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Objective: The flavonoids can be chemically modified by complexation with metals to be applied for adjuvant therapy. In this context, we evaluated the anticancer activity on the lung cancer cell line A549 of CuNarBatho. Nar: naringenin, Batho: bathophenanthroline. Their bovine serum albumin (BSA) binding properties have also been evaluated.
Methods: The effects of the compounds (CuNarBatho, CuNar, ligands and metal) on the A549 cell viability were measured by MTT assay. To evaluate the probable mechanism of action, morphological changes, intracellular reactive oxygen species ROS content (using CM-H_2DCFDA probe), mitochondrial membrane potential (MMP) (using $DIOC_6$ probe), and GSH depletion and cell viability in the presence of NAC were examined. The interaction between CuNarBatho and BSA was studied using tryptophan fluorescence quenching.
Results: CuNarBatho was more efficient than Batho, and Nar in inhibiting A549 cell viability (IC_{50} values 0.3, 12.1 and > 100 μM respectively at 24 h incubation). CuNar slightly inhibited cell viability. The probable mechanism of action of the ternary complex implies increased ROS levels, reduced GSH and GSH/GSSG ratio levels, and decreased MMP. Also, increment of cytoplasm condensation and presence of pycnotic nuclei were observed. Upon addition of NAC, the anticancer effect has been reverted. The binding constant values (K_b) for the CuNarBatho-BSA system are 36.2, 14.9 and 2.3 × 10^5 M^{-1} at 298, 303 and 310 K suggesting that the compound can be stored and carried by the protein. The number of binding sites was *ca.* 1.0 corresponding to the binding sites with high affinity. The negative ΔH and ΔS values (-177.03 and -0.47 KJ/mol) obtained for the interaction of CuNarBatho with BSA indicated that hydrogen bonding and Van der Waals forces played major roles during the interaction.
Discussion: The results suggest that the CuNarBatho complex could serve as a pharmacologically active compound for the treatment of lung cancer.

513. (501) **ENZYMES INVOLVED IN EXTRACELLULAR MATRIX PROTEOGLYCAN´S SYNTHESIS AS A POTENTIAL THERAPEUTIC TARGET FOR COLORECTAL CANCER STEM CELLS**

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Cancer Stem Cells (CSC) are characterized by self-renewal, differentiation, chemoresistance and phenotypic reversibility, which is associated with worse prognosis in tumors. Interaction with the micro-

environment is one of the factors related with stemness. The aim of the present work is to analyze the expression of extracellular matrix proteoglycans in CSC derived from cell lines. CSC were enriched by scaffold free 3D culture (colonospheres) of human colorectal cancer cell line HCT116 in the presence of bFGF and EGF, employing ultra-low attachment plates in serum-free culture conditions. After 14 days of culture, microscopy studies were performed to assess colonosphere formation. Stemness was addressed by the expression of master genes SOX2, Nanog and CD44 by RT-PCR. Decorin and biglycan proteoglycan´s core protein expression was also analyzed by RT-PCR. Moreover, glycosaminoglycan and protein quantification was addressed by ionic exchange chromatography of the culture medium followed by colorimetric determination. RT-PCR was performed for the study of glycosaminoglycan synthesis enzyme expression. Bright light microscopy showed colonospheres around 50-100um. The expression of master genes was heterogeneous among cultures correlated with an heterogeneous expression of decorin (number of experiments, n =3). On the other hand, no biglycan expression was detected among different colonospheres (n=3). No differences were registered in glycosaminoglycan/protein ratio among spheres (0,274 ± 0.127). Nevertheless, Chondroitin-4-O-Sulfotransferase (C4ST) expression was detected in colonospheres while no expression was observed for Dermatan-4-O-Sulfotransferase (D4ST). The heterogeneity presented by 3D cultures represents the heterogeneity reported for CSC within tumors and C4ST and D4ST pattern suggests differences in GAG chain´s quality. Therefore, colonospheres are a suitable model for the study of GAG enzymes as potential therapeutic targets.

REPRODUCCIÓN

514. (008) **EXPRESSION OF TRANSGLUTAMINASE 2 AND HISTOMORPHOLOGICAL CHARACTERIZATION OF THE OVIDUCT OF ADVANCED PREGNANT COWS**

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The oviduct suffers changes during the estrous cycle in relation to its function in reproduction that are attributed in part to estrogen (E2) variations. Raise of E2 and E2 receptors expression occurs along pregnancy, thus we hypothesized that effects on the oviduct should be observed in advanced pregnancy (AP). In this work, we studied changes in the oviductal epithelium and fluid through the estrous cycle and AP in cows. In AP oviducts higher leaf-like folds were observed and the width of the mucosa and height of the epithelium were lower. Also, PAS-positive apical protrusions and TUNEL-positive extruded cytoplasmic material were observed in oviducts from cows in AP. A particular protein was detected only in oviductal fluid of pregnant cows. This protein was identified by LC/MS-MS as Transglutaminase 2 (TGM2) and its identity was confirmed by Western blot. TGM2 was detected in pregnant cows oviductal fluid but not in cells from any stage and its mRNA was present in differential amounts in cells from every stage, indicating possible translation and/or secretion regulation during the estrous cycle and in pregnancy. TGM2 is related to apoptosis, protein modification, modification of extracellular matrix and cell growth/differentiation. Our results lead to the presumption that TGM2 may have a role in oviductal epithelium growth/differentiation. In conclusion, important morphological changes occur in the oviductal epithelium in advanced pregnancy and secretion of TGM2 to the oviductal fluid is exclusive of this period. These last may be regulated at several levels of expression and of secretion, indicating an important role for the enzyme in pregnant oviductal tissue.

515. (014) **EFFECT OF A MATERNAL DIET ENRICHED IN EXTRA VIRGIN OLIVE OIL ON PROINFLAMMATORY MARKERS AND LIPID PROFILE IN CORD BLOOD FROM GDM PREGNANCIES**

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Objectives: Gestational diabetes mellitus (GDM) is a prevalent disease that increases the risk of maternal and fetal complications. Previously, we found that a maternal diet enriched in extra virgin olive oil (EVOO) reduced the levels of proinflammatory markers and triglycerides (TG) in maternal blood from GDM pregnancies. Here, we aim to determine whether proinflammatory markers (interleukin 6 (IL-6) and inducible nitric oxide synthase (iNOS)), lipid profile and the lipid chaperone Fatty Acid Binding Protein 4 (FABP4) levels are altered in umbilical cord blood from pregnancies with GDM treated or not with a diet enriched in EVOO.
Methods: Fifty healthy (Control) and GDM patients were enrolled (protocol approved by Hospital Pirovano Ethics Committee) and advised to follow the WHO diet for pregnancy. GDM patients were randomized to receive or not 36 g/day of EVOO from week 24-28 of gestation until delivery. At delivery, cord blood was obtained to evaluate iNOS, IL-6 and FABP4 levels (Western blot), and TG, Total and HDL-Cholesterol (TC, HDL-C; colorimetric assay).
Results: Neither IL-6 nor iNOS were altered in the three evaluated groups compared to the controls. TG and HDL-C levels were decreased in umbilical cord blood from the GDM group compared to controls (25% and 7% respectively, $p<0.05$), alterations prevented by the diet enriched in EVOO. TC and FABP4 were unchanged in the GDM groups treated or not with the EVOO diet compared to controls.
Conclusion: Despite the proinflammatory and metabolic alterations previously observed in maternal blood, no alterations in proinflammatory markers, TC and FABP4 levels were observed in umbilical cord blood from GDM patients. The observed prevention of reduced HDL-C suggests an improvement in lipid metabolism in the EVOO group. Besides, the observed reduction in TG levels in cord blood from GDM patients may reflect lipid accretion in fetal tissues prevented in the EVOO group.

516. (020) **UTERINE ANORMALITIES AND PTEN EXPRESSION IN PCOS RAT MODEL**

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Women with Polycystic ovary syndrome (PCOS) have an increased risk for developing endometrial hyperplasia and cancer. Previously, we demonstrated in the PCOS rat uterus an increase in epithelial height, gland density and thickness of subepithelial stroma and myometrium. Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN) is a tumor suppressor gene. Mutation or changes in PTEN expression is a frequent event in endometrial cancers. The aims of this study were to investigate the presence of uterine abnormalities and to evaluate whether uterine PTEN expression was altered in PCOS rat model. Female rats were injected subcutaneously with sesame oil (Control group) or dehydroepiandrosterone (6mg/100g body weight, PCOS group) from 21 to 40 days of age. At day 41 the uterine horns were collected. Uterine abnormalities were studied on histological sections counterstained with hematoxylin-eosin or picrosirius-hematoxylin. PTEN expression was evaluated by immunohistochemistry. Uterine morphology showed stratification of luminal epithelium (more than 3 layers of cells), intraepithelial lumens, intraepithelial glands and polyps in treated rats. Also, different morphological types of uterine glands were identified: cystic, dilated and/or tortuous, with squamous metaplasia, with cellular atypia and conglomerates of glands. The incidence of epithelial and glandular abnormalities increased in PCOS rats (Control: 28.6 % vs PCOS: 100%, Control: 0 % vs PCOS: 90.9%, respectively). In this group of rats, PTEN expression was not modified in the glandular epithelium, whereas decreased in the luminal epithelium, subepithelial

stroma and myometrium (p<0.05). These results suggest that the decreased PTEN expression in the uterus could be associated with the greater development of endometrial abnormalities observed in PCOS rat.

517. (021) **LACTOFERRIN AFFECTS FERTILIZATION AND EMBRYO IMPLANTATION IN RATS**

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Previously we found that lactoferrin (LF) is present in the human oviduct secretion and it can bind to both gametes and reduce the gamete interaction. The aim of this work was to investigate the effect of LF on the acrosome reaction (AR), *in vitro* fertilization (IVF) and implantation in Wistar rats. Rat cauda epididymal sperm (SP) were incubated under capacitating conditions in the absence or presence of LF (10 and 100 μg/ml) at 37°C and 5% PCO_2. SP were then incubated with or without 20 μM progesterone to determine the induced and spontaneous AR, respectively, by Coomassie blue staining. For IVF, oocytes were recovered from the oviduct of rats (80 days) after ovarian stimulation and placed in HTF medium, inseminated with SP and incubated in the absence (controls) or presence of LF (1, 10 and 100 μg/ml) at 37ºC and 5% PCO_2 for 24 h. To estimate the IVF rate, pronucleus formation was detected with Hoechst 33258. For the *in vivo* study, female rats (80 days, n=23) were placed with a male on the day prior to proestrus. From the following diestrus, females were randomly assigned to one of four treatment groups and received a daily i.p. injection of 0.9%w/v NaCl (controls), 50 mg, 100 mg, or 200 mg LF/kg, for 8 days. Then, they were sacrificed and the implantation sites in uterus were assessed, considering the average number in controls as 100%. The presence of 10 μg/ml LF (51.43±3.95%; p<0.05) and 100 μg/ml LF (49.96±3.2%, p<0.05) increased the spontaneous AR respect to controls (38.5±2.3%), but did not affect the induced AR. LF presence decreased the IVF rate (control: 68.7±5.3% vs. LF 10: 41.03±6.6%, p<0.05; LF 100: 31.43±6.4%, p<0.001). The implantation decreased in rats treated *with* 100 or 200 mg LF/kg (LF 100: 0%, p<0.001; LF 200: 10.7±8,9%, p<0.001). The results suggest a dose-dependent effect of LF on fertilization and implantation in rats. Likewise, the presence of LF stimulated the spontaneous AR, which could contribute to the reduction observed in the IVF rate.

518. (031) **TRANSGENERATIONAL CHANGES CAUSED BY DIET-INDUCED MATERNAL OBESITY AT REPRODUCTIVE LEVEL**

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Previously, we demonstrated that female offspring from overfed rats exhibit higher body and ovarian weight, early puberty, lower number of immature follicles, higher ovulatory rate and lower number of healthy oocytes than control animals. In the present work, we continued evaluating the effects of maternal obesity caused by the diet on the reproductive capacity and outcome in the female offspring (F1) and some parameters of their offspring (F2).
To this end, female offspring from rats fed standard (F1OSD) or cafeteria (F1OCD) diet were used. At 60 days of age, both groups were mated with control males on the day of proestrus and euthanized at different times. Fertility, gestation, viability and lactation indices were evaluated in these offspring.
Between 45% and 80% of the F1OCD rats exhibited significant changes compared to the F1OSD group: i) a longer estrous cycle (5.5±0.4 vs 4.1±0.1, p<0.05); ii) a decrease in the fertility index (12.7±0.3 vs 15.5±0.5, p<0.05) and litter size (11.7±0.4 vs 14.7±0.3, p<0.01); and iii) a lower male to female ratio (0.77±0.08 vs 1.5±0.2, p<0.01). No differences were found in the gestation, viability or lactation indices.
Comparison of some parameters of the offspring from F1 (F2OCD) with controls (F2OSD) showed that i) both the female and male F2OCD groups exhibited lower body weight at birth (7.9±0.2 vs 9.6±0.2; 8.2±0.3 vs 10.3±0.1; respectively, p<0.001) and an increase in the weight gain at 21 days of age (570±20 vs 460±30, 550±20 vs 440±10, respectively, p<0.05). Also, these F2OCD rats showed a decrease in the gonadal indices (1.3±0.05 vs 1.6±0.09, $x10^4$; 2.12±0.05 vs 2.53±0.07, $x10^3$; respectively, p< 0.05).
These results suggest that maternal obesity, induced by the diet, may severely affect the reproductive ability of their offspring, likely as a result of altering organogenesis, which may also cause transgenerational systemic and reproductive disorders.

519. (038) **ECS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS IS MODULATED IN AN LPS-INDUCED PRETERM MURINE MODEL**

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Preterm birth (PTB) is the leading cause of mortality and morbidity in neonates. The endocannabinoid system (ECs) is one of several signaling pathways involved in different aspects of the physiopathology of reproduction, comprising the enzyme that synthesizes AEA, (N-acylphosphatidylethanolamine-specific phospholipase D, NAPE-PLD), the enzyme that hydrolase AEA (fatty acid amide hydrolase, FAAH), and the receptors CB1, CB2 and TRPV1. Previous works from our lab have demonstrated that LPS altered FAAH activity in deciduas and PBMC in an embryo resorption model. Using a murine model of preterm labor, induced by two injections of LPS on day 15 of pregnancy, we demonstrated that ECs of the decidua is involved in the triggering of PTB. Due to the decidua is highly infiltrated by immune cells, in this study we investigated if the ECs of peripheral blood mononuclear cells (PBMC) is modulated in our LPS-induced preterm labor model.
We observed that CB1 receptor protein levels increased in PBMC after LPS treatment (p<0.05). However, CB2 and TRPV1 receptors protein levels were not modified by LPS.
Regarding the enzyme that synthesizes AEA, (N-acylphosphatidylethanolamine-specific phospholipase D, NAPE-PLD), we observed that its protein levels were diminished in PBMC from LPS treated mice (p<0.05).
On the other hand, the enzyme that degrades AEA (fatty acid amide hydrolase, FAAH) protein levels were diminished in PBMC after LPS treatment (p<0.05). Also, we evaluated FAAH activity by radioconvertion and we observed that FAAH activity is decreased in PBMC from LPS-treated mice.
In summary, these data show that endocannabinoid system from peripheral blood mononuclear cells could be implicated in the pro-inflammatory response associated with LPS-induced preterm birth.

520. (042) **INVOLVEMENT OF GLUTAMINOLYSIS IN THE REGULATION OF SERTOLI CELL PROLIFERATION**

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The final number of Sertoli cells (SC) reached during the proliferative periods determines sperm production capacity in adulthood. FSH is the major SC mitogen and mTORC1/p70S6K pathway is involved in FSH-stimulated SC proliferation. On the other hand, glutaminase (GLS), which converts glutamine into glutamate, plays a vital role in up-regulating cell metabolism for cell growth, and even, the glutamine catabolism might be required for the fully activation of mTORC1/p70S6K pathway. Previously, we have demonstrated that FSH increases the expression of GLS isoform 2 in proliferating SC, however, the role of GLS activity in the regulation of SC proliferation remains unknown. The aim of this work was to analyze whether SC depends on glutaminolysis to proliferate. SC obtained from 8-day old rats were maintained in the absence or presence of a pharmacological inhibitor of GLS -6-diazo-5-oxo-L-norleucine (DON) 500 µM- under basal conditions (B) or stimulated with FSH 100ng/ml. BrdU

incorporation, c-Myc transcriptional activity by luciferase reporter assay after cell transfection and phosphorylated p70S6K (P-p70S6K) levels by western blot were evaluated. Results are expressed as mean±SD of three independent experiments (Different letters indicate statistically significant differences, P<0.05). DON prevented FSH from stimulating BrdU incorporation (B:5.6±0.7[a]; DON:1.6±0.6[b]; FSH:9.7±1.5[c]; FSH+DON:1.4±0.7[b] %BrdU-positive cells) and c-Myc transcriptional activity. In addition, FSH was unable to activate p70S6K in the presence of DON (DON:0.60±0.25[a]; FSH:2.30±0.52[b]; FSH+DON:0.74±0.28[a] fold variation P-p70S6K/β-tubulin vs. B). These results suggest that glutaminolysis might be necessary for the regulation of SC proliferation by modulating mTORC1/p70S6K pathway. PICT: 2015-228; 2018-1291.

521. (060) **ASSESSMENT OF THE MECHANISMS UNDERLYING ACTIVIN A-INDUCED SERTOLI CELL PROLIFERATION**

Gustavo Marcelo Rindone, Cecilia Lucía Centola, Agostina Gorga, Marina Ercilia Dasso, Eliana Herminia Pellizzari, María del Carmen Camberos, María Noel Galardo, Silvina Beatriz Meroni, María Fernanda Riera.

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Immature Sertoli cells (SC) proliferate to reach a steady population size that will ultimately correlate with sperm output in adulthood. Activin A (ActA), a member of the TGF-β superfamily, acts as a paracrine factor on fetal and postnatal proliferation of SC. However, besides an increase in Cyclin D2 expression, little is known about the mechanisms that explain ActA-induced proliferation of SC. The aim of this study was to assess the signaling pathways and gene expression changes involved in ActA-induced proliferation of SC. SC were isolated from 8-day-old Sprague-Dawley rats and incubated under basal conditions (B) or stimulated with ActA (50 ng/mL) during different periods of time. Results are expressed as X±SD, n=3 (*p<0.05; **p<0.01 vs B). In agreement with previous reports, BrdU incorporation assays showed that ActA increased SC proliferation (B:5.0±0.8; ActA:8.8± 0.7** % BrdU positive cells). In western blot analysis, ActA induced higher levels of phosphorylated AKT and p70S6K (2.9±0.6*and 1.8±0.5* fold change vs. B) after 1-h treatment suggesting the activation of classical signaling pathways linked to cell proliferation. Regarding cell cycle regulated proteins, ActA significantly increased both c-Myc mRNA levels measured by RT-qPCR and c-Myc transcriptional activity in a luciferase reporter assay. Cyclin D1 mRNA levels remained unchanged whereas Cyclin D2 mRNA levels showed a maximal increase (5.5±0.7** fold change vs. B) after 4 h. Cyclins E1 and E2 mRNA levels showed an increase (1.9±0.3** and 1.8±0.3** fold change vs. B) after 24 h of treatment. Overall, these results suggest that ActA stimulates SC proliferation increasing c-Myc activity and cyclins expression as well as activating signaling pathways classically linked to cell cycle progression. (PICT:2015-228; 2018-1291).

522. (075) **PREVALENCE AND ASSOCIATION OF *CHLAMYDIA TRACHOMATIS, UREAPLASMA* SPP. AND *MYCOPLASMA HOMINIS* UROGENITAL INFECTIONS IN PATIENTS WITH PRIMARY INFERTILITY**

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Currently, infertility affects 15-20% of couples of reproductive age worldwide, with women and men equally contributing to infertility cases. Among others, urogenital infections are known causes of infertility. In fact, infertility have been associated to *Chlamydia trachomatis* (Ct), *Ureaplasma* spp. (Uu) and *Mycoplasma hominis* (Mh) urogenital infections. However, evidence from large studies assessing their prevalence and putative associations in patients with infertility is still scarce. Herein, we aimed to evaluate the prevalence and associations of Ct, Uu and Mh infection in women and men seeking care for infertility. A cohort of 5464 patients with a diagnosis of couple's primary infertility and 404 control individuals were enrolled. Cervical-swab and semen samples were collected from female and male individuals, respectively, and infections assessed by PCR or culture. Overall, the prevalence of Ct, Uu and Mh urogenital infection was significantly higher in patients than in control individuals (5.3%, 22.8% and 7.4% versus 2.0%, 17.8% and 1.7%, respectively). Ct infection was more prevalent in male than in female patients (OR: 1.36, *p=0.034*), being males younger than 25 years at the highest risk (OR: 2.51, *p=0.002*). Conversely, Uu and Mh infections were more prevalent in female patients, since males were less likely at risk of Uu (OR: 0.52, *p<0.001*) and Mh (0.41, *p<0.001*) infection. In addition, Uu infection was more prevalent in patients younger than 25 years, either in women (OR: 2.27, *p=0.003)* or men (OR: 1.66, *p=0.034*). Finally, a significant association between Mh and Uu infections was found in either female (OR: 33.84, *p<0.0001*) or male (OR: 71.83, *p<0.0001*) patients. Our data revealed that Ct, Uu and Mh are prevalent uropathogens in patients with couple's primary infertility. Taken together, our results show the importance of including the screening of urogenital infections in the diagnostic work up of male infertility.

523. (077) **INTERFERON γ, IL-17, AND IL-1B IMPAIR SPERM MOTILITY AND VIABILITY AND INDUCE SPERM APOPTOSIS**

Maria Sol Martinez[1], Daniela Andrea Paira[1], Silene Silvera-Ruiz[1], Andrea Daniela Tissera[2], Rosa Ines Molina[2], José Javier Olmedo[3], Virginia E. Rivero[1], Rubén Darío Motrich[1].

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Urogenital inflammation is a known cause of male infertility. Increased levels of inflammatory cytokines, leukocyte counts and oxidative stress are highly detrimental for sperm quality thus compromising male fertility. Although cytokines affect sperm by recruiting and activating leukocytes consequently inducing oxidative stress, scarce to absent data have been reported about the putative direct effects of inflammatory cytokines on spermatozoa. Herein, we analyzed whether IFNγ, IL-17, IL-1β, and IL-8 can directly impair human sperm motility and viability. Fractions of viable and motile spermatozoa from normospermic healthy donors were in vitro incubated with recombinant human IFNγ, IL-17, IL-1β or IL-8 and sperm motility, viability and apoptosis were analyzed. Sperm exposed to different concentrations of IFNγ, IL-17 and IL-1β, or a combination of them, for either 1 or 3 h showed significantly reduced motility and viability with respect to sperm incubated with vehicle. Moreover, the exposure to IFNγ, IL-17 and IL-1β resulted in significantly higher levels of early and/or late apoptotic and/or necrotic spermatozoa. Interestingly, no significant differences in sperm motility, viability and apoptosis were observed in sperm incubated with different concentrations of IL-8, for either 1 or 3 h, with respect to sperm incubated with vehicle. In conclusion, our results indicate that IFNγ, IL-17 and IL-1β directly impair sperm motility and decreases viability by inducing sperm apoptosis. Our results suggest that examining inflammatory cytokines in semen would be an additional helpful tool for the diagnostic workup of male infertility.

524. (083) **NEW ADVANCES IN THE USE OF MELATONIN AS A FERTOPROTECTIVE AGENT DURING CHEMOTHERAPY**

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Melatonin (MEL) is a neurohormone with a potent antioxidant activity. Premature ovarian failure (POF) is a pathology defined as the depletion of the ovarian reserve; one of its causes is chemotherapy. Currently treatments for POF have proven to be ineffective. The aim of the present study is to assess whether MEL can protect ovaries from chemotherapy-induced gonadotoxicity using a mice model of POF induced by cyclophosphamide (CTX). Previously, we have reported that MEL enhances the % of antral follicles, diminishes the % of atretic follicles and increases SOD1 expression in POF model. To induce POF, CTX was administered (75 mg/kg, i.p.) to F1 mice (C57XBalbC, 6-8 weeks old) on day 1. CTX+MEL group also received MEL (15 mg/kg, i.p.) on days 1, 6 and 11. Animals were sacrificed on day 15 and their ovaries processed for histological analysis. Data was analysed by ANOVA followed by Tukey's test.
Histopathological analysis of ovarian sections showed that CTX caused fibrotic foci and blood vessel hyalinization; MEL decreased these parameters. IHC for AMH (ovarian reserve marker) showed that CTX diminished the % of follicles expressing AMH compared to control ($p<0.05$), whereas MEL increased this parameter compared to CTX ($p<0.05$). IHC for DDX4 (oocyte marker) revealed that CTX diminished the number of primordial follicles compared to control ($p<0.05$), whereas MEL increased it ($p<0.05$). IHC for CD31 (endothelium marker) revealed that CTX reduced the blood vessel density compared to control ($p<0.05$), while IHC for α-SMA (vascular stability marker) showed that CTX reduced mature vessel density ($p<0.05$). MEL increased these parameters compared to CTX ($p<0.05$).
In conclusion, these results, combined with our previous report, suggest that MEL could protect ovarian function from gonadotoxicity, preventing primordial follicle loss and restoring vascular function. MEL might represent a non-invasive treatment to preserve female fertility in patients undergoing chemotherapy.

525. (084) **NITRIC OXIDE (NO) BUT NOT TNF ALPHA INHIBITS GERM CELL CYCLE PROGRESSION AND IMPAIRS RAT SPERMATOGENESIS**

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Low spermatogenic efficiency in infertile men is not only due to post meiotic events, but also to decreased meiotic activity and spermatogonia (Spg) number. We demonstrated that Spg decreased number negatively correlates with the number of immune cells in testicular biopsies of azoospermic patients. Proinflammatory agents nitric oxide (NO) and TNFα produced by the immune cells that infiltrate the testis might impair spermatogenesis. Our objective was to evaluate the effect of NO and TNFα on Spg and preleptotene spermatocyte (PLs) proliferation and spermatogenesis progression in adult *Wistar* rats. DETA-NOnoate (DETA-NO), a NO donor, or TNFα were injected in one testis, saline was injected in the contralateral testis. On day 5, a group of animals received BrdU injection (ip) and were euthanized 2h later in order to evaluate proliferation, by immunofluorescense. Another group was sacrificed on day 60 to evaluate the effect on spermatogenesis. DETA-NO (2 and 10mM) significantly reduced the number of BrdU+Spg/seminiferous tubule (ST) ($p<0.05$, Student paired t test, n=3) and the number of BrdU+PLs/ST ($p<0.01$; Student paired t test, n=3) vs saline. These events slowed seminiferous epithelial cycle, demonstrated by the significant reduction in the number of ST at VII-VIII stage ($p<0.01$; Student t test, n=3). Variations in STs' area reflect the magnitude of spermatogenesis damage; after 60d DETA-NO (2 and 10mM) significantly increased the frequency of STs of small and reduced area respectively vs. saline ($p<0.05$; Student t test, n=3). TNFα (0.1 and 1μg) exposure affects neither Spg nor PLs proliferation or spermatogenesis.
We demonstrated that NO arrests the cell cycle of premeiotic GCs, limiting Spg mitotic amplification division and the entrance of PLs in meiosis. These events might generate time gaps in the spermatogenic wave lastly affecting sperm production.

526. (086) **COMPARATIVE ANALYSIS OF SPERMATOGENESIS AND HORMONAL PROFILE OF INFERTILE PATIENTS WITH IDIOPATHIC ORCHITIS VERSUS RATS WITH AUTOIMMUNE ORCHITIS**

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Experimental autoimmune orchitis (EAO) is a well-established rodent model of organ specific autoimmunity associated to infertility. Testis immunopathology is similar in rats and humans undergoing a chronic testicular inflammation. A comparative analysis of other aspects of the disease like the quantification of spermatogonia (Spg) and Sertoli cells (SCs), by immunohistochemistry, as well as the hormonal serum profile of infertile patients with idiopathic orchitis vs rats with EAO was undertaken (RIA). We evaluated testicular biopsies from patients with idiopathic non-obstructive azoospermia, diagnosed with hypospermatogenesis (mild: n=8, severe: n=10) (HypE) and Sertoli cell only syndrome (SCOS, n=9). All groups displayed twice the number of immune cells ($CD45^+$) vs patients with obstructive azoospermia and complete spermatogenesis (control group, C, n=8). The number of undifferentiated and differentiated SPg/seminiferous tubule (ST) decreases in mild and severe HypE while the number of SCs/STs increases in severe HypE and SCOS vs. control ($p<0.01$). In EAO undifferentiated Spg (CD9+) increased in focal and decreased in severe EAO vs. normal (N) rats. Differentiated SPg (c-Kit+)/ST decreases (mean±SEM, N:10.5±0.3, focal EAO:4.4±0.1, severe EAO:3.1±0.3, $p<0.05$, n=3) and the SCs/STs number increases vs. N (mean±SEM, N:10.14±1.13, focal EAO:17.32±2.24, severe EAO:19.5±3.5, $p<0.05$, n=3). FSH, was higher in severe HypE vs C and also in severe EAO vs N. Testosterone and LH were similar to C in severe HypoE and also in severe EAO vs N. Prolactin in mild and severe HypoE was similar to C and in focal and severe EAO was similar to N (mean±SEM ng/ml, N:13.1±2.0, focal EAO:10.2±1.8, severe EAO:14.9±2.8, n=11).
We showed that particularly the late stages of EAO closely reflect Spg and SCs behavior, and hormonal profile observed patients with severe HypE. These results validate EAO as a valuable model for studying the impact of inflammatory processes on spermatogenesis.

527. (106) **EVOO RESTORES THE STEROL REGULATORY ELEMENT-BINDING PROTEIN 2 CHOLESTEROL PATHWAY OVER-STIMULATED BY A HFD IN RABBIT TESTIS**

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Male fertility depends on cholesterol (chol) homeostasis. Chol is essential for testosterone synthesis and spermatogenesis, and must be maintained in an optimal range for proper functioning of the testes. Rabbits on a high-fat diet (HFD) exhibit hypercholesterolemia associated with poor seminal quality, related to cholesterol overload in seminiferous tubule cells. Sterol regulatory element-binding protein (SREBP)-2 governs the cholesterol pathway in testis and it is sensitive to dietary lipids. We have previously seen that Extra Virgin Olive Oil (EVOO) supplementation improved semen parameters affected by high fat diet. The aim of this study was to explore the effects of EVOO supplementation to HFD on rabbit testes at the molecular level, analyzing the SREBP-2 pathway. Male New Zealand White rabbits were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), a high-fat diet (plus 14% bovine grease, hypercholesterolemic rabbits, HCR), or 7% bovine grease plus 7% EVOO (HCR + EVOO). Serum lipid levels, body weight and seminal

parameters were measured, and mRNA and protein levels of the SREBP-2 pathway were assessed by PCR, Western blotting and immunofluorescence. At 12 months of diet, HCR rabbits show an increase in the expression of SREBP 2 and downstream molecules of the pathway: HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) and LDLR (low-density lipoprotein receptor). Interestingly, the addition of EVOO showed a recovery in the expression of the mentioned proteins. In addition, preliminary studies of SREBP-2 regulatory molecule, INSIG1 (Insulin induced gene 1), and the molecule responsible for the esterification of cholesterol, SOAT2 (Sterol O-Acyltransferase 2), showed no significant changes between diets so far. The data showed that dietary supplementation with EVOO promoted testicular improvements by modifying the expression of cholesterol pathway regulated by SREBP2.

528. (112) **THE ENDOMETRIAL EXPRESSION OF INTERLEUKIN-1 FAMILY: THEIR INVOLVEMENT IN DELAYED CONCEPTION OF DAIRY COWS**

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The cytokines of the interleukin-1 family are closely involved in processes such as resolution of uterine inflammation and are locally produced by macrophages and endometrial cells under stimuli. However, little is known about the role of these cytokines in the absence of disease during postpartum period where conception and pregnancy occur in cattle. The aim of this study was to analyse the gene and protein expression levels of the members of IL-1 family: IL-1α, IL-1β, IL-1RI, IL-1RII and IL-1RA during postpartum period, and their possible association with delayed conception.

Endometrial biopsies were obtained from multiparous Holstein cows (n=16) at 45 and 60 days in milk (DIM). The voluntary waiting period of cows was 70 days. All procedures were approved by the Ethics Committee (FCV-UNL). The gene expression of IL-1α, IL-1β, IL-1RI, IL-1RII and IL-1RA was analyzed by real-time PCR. The immunolocalization of the cytokines was assessed by indirect immunohistochemistry.

Kaplan–Meier test was used to evaluate the possible association between the gene and protein levels of each cytokine and delayed conception. Then, when the results from Kaplan-Meier showed significant association, we grouped the animals like 'fewer number of days to conception' (FDC) and 'greater number of days to conception' (GDC) for comparison. To analyze the differential expression levels over time in FDC and GDC groups, a Generalized Linear Model was used considering DIM as a fixed factor. The gene expression of all members of IL-1 family showed no significant association with delayed conception. However, the association between the protein expression of IL-1β and IL-1RA in glandular epithelium (GE) and delayed conception was significant. Then, the GLM showed no significant differences in GE between 45 and 60 DIM, both in the FDC and GDC group. These results suggest a potential role of some members from IL-1 family, as IL-1β and IL-1RA, in the mechanisms involved to early conception.

529. (121) **LEPTIN REGULATES THE EXPRESSION OF GENES RELATED TO LIPID STORAGE IN SERTOLI CELLS**

Marina Ercilia Dasso, Gustavo Marcelo Rindone, Agostina Gorga, Cecilia Lucía Centola, Eliana Herminia Pellizzari, María del Carmen Camberos, María Noel Galardo, Silvina Beatriz Meroni, María Fernanda Riera.

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Sertoli cells (SC) are necessary to provide an adequate environment for germ cell development. SC actively metabolize glucose but most of it is converted to lactate, an energy source for germ cells. SC also oxidize fatty acids (FA) to sustain their energy status. SC have numerous lipid droplets (LD), which are thought to be the site of storage of FA. In this context, we have demonstrated that leptin (Lep), an adipokine that is present in the testis, increases triacylglycerols (TAGs) and LD content in SC. Several proteins such as the FA transporter FAT/CD36, the enzymes involved in the synthesis of TAGs (glycerol-phosphate-acyl-transferases (GPATs) and diacylglycerol-acyl-transferase 1 (DGAT)), and the proteins associated with LD formation (PLINs) have a role in lipid storage. The aim of this study was to analyze the effects of Lep on the expression of proteins involved in FA storage. SC isolated from 20-day-old rats were cultured and maintained under basal conditions (B) or stimulated with Lep (100 ng/ml). mRNA levels of FAT/CD36, GPAT1-4, DGAT1 and PLIN1-3 were analyzed by RT-qPCR. Results are expressed as mean±SD, n=3, (* $p<0.05$ vs B). Lep increased FAT/CD36, GPAT3, and PLIN1 mRNA levels at 48 h (1.8±0.2*; 1.5±0.2* and 1.8±0.1* fold variation vs. B). To elucidate which signaling pathways are involved in Lep regulation of FA storage, SC were treated with Lep in the presence of 1 μM Static (STAT3 inhibitor), 1 nM Rapamicyn (mTORC1 inhibitor) or 50 μM T0070907 (PPARγ antagonist). Then, LD content was analyzed by Oil Red O staining. Neither of the inhibitors tested modified leptin-stimulated LD number. Altogether, the results suggest that Lep regulates the expression of genes involved in FA transport, TAG synthesis, and LD formation as a mechanism to increase lipid storage. Further studies will be necessary to clarify the signaling pathways participating in the above-mentioned regulation. (PICT2015-228; PICT2018-1291).

530. (140) **INTERFERON γ, IL-17, AND IL-1β IMPAIR SPERM MOTILITY AND VIABILITY AND INDUCE SPERM APOPTOSIS**

Maria Sol Martinez[1], Daniela Andrea Paira[1], Silene Silvera-Ruiz[1], Andrea Daniela Tissera[2], Rosa Ines Molina[2], José Javier Olmedo[3], Virginia E. Rivero[1], Rubén Darío Motrich[1].

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Urogenital inflammation is a known cause of male infertility. Increased levels of inflammatory cytokines, leukocyte counts and oxidative stress are highly detrimental for sperm quality thus compromising male fertility. Although cytokines affect sperm by recruiting and activating leukocytes consequently inducing oxidative stress, scarce to absent data have been reported about the putative direct effects of inflammatory cytokines on spermatozoa. Herein, we analyzed whether IFNγ, IL-17, IL-1β, and IL-8 can directly impair human sperm motility and viability. Fractions of viable and motile spermatozoa from normospermic healthy donors were in vitro incubated with recombinant human IFNγ, IL-17, IL-1β or IL-8 and sperm motility, viability and apoptosis were analyzed. Sperm exposed to different concentrations of IFNγ, IL-17 and IL-1β, or a combination of them, for either 1 or 3 h showed significantly reduced motility and viability with respect to sperm incubated with vehicle. Moreover, the exposure to IFNγ, IL-17 and IL-1β resulted in significantly higher levels of early and/or late apoptotic and/or necrotic spermatozoa. Interestingly, no significant differences in sperm motility, viability and apoptosis were observed in sperm incubated with different concentrations of IL-8, for either 1 or 3 h, with respect to sperm incubated with vehicle. In conclusion, our results indicate that IFNγ, IL-17 and IL-1β directly impair sperm motility and decreases viability by inducing sperm apoptosis. Our results suggest that examining inflammatory cytokines in semen would be an additional helpful tool for the diagnostic workup of male infertility.

531. (141) **SEMINAL LEVELS OF INFLAMMATORY CYTOKINES AND SPERM QUALITY IN PATIENTS RECOVERED FROM COVID-19**

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The novel coronavirus disease (COVID-19) pandemic is an emerging global health threat that can cause multiorgan damage and shows a higher risk for men than women. Despite the considerable knowledge gained about the underlying pathophysiology, little is known about the putative andrological consequences of COVID-19. Thus, we herein prospectively evaluated sperm quality parameters and levels of inflammatory cytokines in semen in a cohort of 293 reproductive-aged male patients who had recovered from COVID-19 and in 63 control individuals. Semen specimens were collected by masturbation, semen analysis performed according to the WHO guidelines, and inflammatory cytokines quantitated by ELISA.
Significantly higher levels of IL-1β, TNFα and IFNγ were found in semen from patients recovered from mild and/or severe COVID-19 with respect to control individuals ($p<0.02$, $p<0.02$ and $p<0.001$, respectively). Moreover, patients recovered from mild and/or severe COVID-19 showed significantly reduced semen volume ($p<0.001$), lower total sperm counts ($p<0.03$), and impaired sperm motility ($p<0.02$) and viability ($p<0.01$). Remarkably, no significant differences were found in semen leukocyte counts from patients and controls ($p>0.001$).
We provide experimental evidence indicating that COVID-19 associates with increased levels of inflammatory cytokines in semen and significant alterations in sperm quality. Although it should be interpreted carefully, these findings indicate an adverse but potentially reversible consequence of COVID-19 on sperm quality. Although beyond our current understanding of the disease, our data suggest that the reproductive function of patients recovering from COVID-19 should be precisely followed and evaluated to detect and avoid more serious reproductive problems in the future.

532. (146) **EFFECT OF MATERNAL DIETS ENRICHED IN PUFAS ADMINISTRATED TO DIABETIC RATS DURING EARLY POSTIMPLANTATION ON STRUCTURES INVOLVED IN DECIDUAL HISTOTROPHIC FUNCTION AND FETO-PLACENTAL GROWTH**

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Maternal diabetes induces alterations in decidualization, which may impact on decidual histotrophic function that include key roles of the uterine glands (UG) and the glycogenic area (GA). uNK cells, involved in angiogenesis and vessels remodeling, are needed for an adequate decidualization and feto-placental development. We previously found that maternal diets enriched in polyunsaturated fatty acids (PUFAs) prevent increased resorption rates in diabetic pregnancies. Aim: To evaluate the effect of diets enriched in sunflower and chia oil (rich in n-6 and n-3 PUFAs respectively) administrated during early postimplantation to diabetic rats on PAS staining of the GA and UG and on the number of uNK cells at day 9 of pregnancy and on decidual, fetal and placental growth at day 14 of pregnancy. Methods: Pregestational diabetes was induced in Wistar rats by streptozotocin (50 mg/kg). On days 7 to 9 of pregnancy diabetic rats received a standard diet or diets enriched in 6% of sunflower or chia oil. On day 9 of pregnancy GA, UG and uNK cells in the decidua were evaluated by PAS staining. On day 14 of pregnancy the fetal cephalic length was measured and the decidua and placenta were weighted. Results: At day 9 of pregnancy PAS staining of GA ($p<0.001$;74%) and UG ($p<0.05$;64%) and the number of uNK cells ($p<0.001$;62%) were reduced in diabetic rats, alterations prevented by the PUFAs enriched diets. At day 14 of pregnancy a decreased fetal cephalic length in diabetic rats (7.5%; $p<0.05$) was prevented by the sunflower oil supplementation. Both decidual (18.3%;$p<0.05$) and placental (12.4%;$p<0.05$) weights were decreased in the diabetic group. The diet enriched in sunflower oil prevented the reduced decidual weight while the diet enriched in chia oil prevented the reduced placental weight. Conclusion: The early postimplantation is a key period for decidual and feto-placental development, affected by maternal diabetes, and in which dietary treatments can exert beneficial effects.

533. (150) **LEUKOCYTES INFILTRATION CHAPERONES HYPERTHYROIDISM-INDUCED INCREASE OF FETAL GROWTH, PLACENTAL CHANGES, AND IMPAIRED OFFSPRING DEVELOPMENT**

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Hyperthyroidism (HP) induces reproductive disorders like preterm birth and lactation failures and could influence immune cells homeostasis. Therefore, our work aimed to evaluate the role of thyroid hormones in leukocytes in milk and placenta. To this end, 12 weeks old *Wistar* rats were injected with T4 (hyper) and euthanized on day 19, 20 of gestation (G19 and G20), and day 2 of lactation (L2). Placenta and milk immune cells (CD45+, CD3+, CD11b/c+) were analyzed by flow cytometry, and mRNA hormone receptors and cytokines by qPCR. Histological analysis of the mammary gland was performed. We observed that fetuses of hyper group (hyper) weighed more in G19 and G20 (*$p<0.01$ $p<0,001$*) compared to controls /Co). In placenta of hyper we showed a decrease in β2 thyroid receptor expression (*$p<0.05$*) in G19 with an increase in prolactin receptor expression on G20 (*$p<0.05$*). Furthermore, in hyper prolactin receptor, progesterone receptor, glucocorticoid receptor, and VEGF expression were higher in G20 vs G19 (*$p<0,05$ $p<0,01$ $p<0,05$*). On G19, the percentage and absolute count of placental leukocytes were higher in hyper (*$p<0.05$*) vs Co. In Co, on G20, we showed an increase in leukocyte infiltration compared with G19 (*$p<0.01$*), however, we did not observe this in hyper. On lactation, the hyper offspring presented lower weight on days 1 and 2 (*$p<0,001$*). In L2, milk had an increase in the percentage of CD45+ cells in hyper (*$p<0,05$*). In addition, CD3+ cells/ μl increased respect to the Co while the number of CD11 b/c+ cells/ μl diminished (*$p<0,05$*). In the hyper, the alveolar area and mammary adipose tissue were lower while mammary connective tissue was higher than Co. These results suggest that immunity may accompany alterations in fetal growth and in placental hormonal receptors expression at the end of pregnancy induced by HP. However, their relationship with preterm birth and early lactation impairment needs to be addressed. Placenta and milk leukocytes would be impacted by HP.

534. (158) **EVOO RESTORES THE STEROL REGULATORY ELEMENT-BINDING PROTEIN 2 CHOLESTEROL PATHWAY OVER-STIMULATED BY A HFD IN RABBIT TESTIS**

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Male fertility depends on cholesterol (chol) homeostasis. Chol is essential for testosterone synthesis and spermatogenesis, and must be maintained in an optimal range for proper functioning of the testes. Rabbits on a high-fat diet (HFD) exhibit hypercholesterolemia associated with poor seminal quality, related to cholesterol overload in seminiferous tubule cells. Sterol regulatory element-binding protein (SREBP)-2 governs the cholesterol pathway in testis and it is sensitive to dietary lipids. We have previously seen that Extra Virgin Olive Oil (EVOO) supplementation improved semen parameters affected by high fat diet. The aim of this study was to explore the effects of EVOO supplementation to HFD on rabbit testes at the molecular level, analyzing the SREBP-2 pathway. Male New Zealand White rabbits were fed commercial rabbit pellet (normocholes-

terolemic rabbits: NCR), a high-fat diet (plus 14% bovine grease, hypercholesterolemic rabbits, HCR), or 7% bovine grease plus 7% EVOO (HCR + EVOO). Serum lipid levels, body weight and seminal parameters were measured, and mRNA and protein levels of the SREBP-2 pathway were assessed by PCR, Western blotting and immunofluorescence. At 12 months of diet, HCR rabbits show unexpected increase in the expression of SREBP 2 and downstream molecules of the pathway: HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) and LDLR (low-density lipoprotein receptor). Interestingly, the addition of EVOO showed a recovery in the expression of the mentioned proteins. In addition, preliminary studies of SREBP-2 regulatory molecule, INSIG1 (Insulin induced gene 1), and the molecule responsible for the esterification of cholesterol, SOAT2 (Sterol O-Acyltransferase 2), showed no significant changes between diets so far. The data showed that dietary supplementation with EVOO promoted testicular improvements by modifying the expression of cholesterol pathway regulated by SREBP2.

535. (160) **PREVALENCE AND ASSOCIATION OF *CHLAMYDIA TRACHOMATIS*, *UREAPLASMA* SPP. AND *MYCOPLASMA HOMINIS* UROGENITAL INFECTIONS IN PATIENTS WITH PRIMARY INFERTILITY**

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Currently, infertility affects 15-20% of couples of reproductive ages worldwide, with women and men equally contributing to infertility cases. Urogenital infections are known causes of infertility. In fact, infertility have been associated to *Chlamydia trachomatis* (Ct), *Ureaplasma* spp. (Uu) and *Mycoplasma hominis* (Mh) urogenital infections. However, evidence from large studies assessing their prevalence and putative associations in patients with infertility is still scarce. Herein, we aimed to evaluate the prevalence and associations of Ct, Uu and Mh infection in women and men seeking care for infertility. A cohort of 5464 patients with a diagnosis of couple's primary infertility and 404 control individuals were enrolled. Cervical-swab and semen samples were collected from female and male individuals, respectively, and infections assessed by PCR or culture. Association between infections and demographic were analysed by Chi-square test. The prevalence of Ct, Uu and Mh urogenital infection was significantly higher in patients than in control individuals (5.3%, 22.8% and 7.4% versus 2.0%, 17.8% and 1.7%, respectively). Ct infection was more prevalent in male than in female patients (OR=1.36, *p=0.034*), being males younger than 25 years at the highest risk (OR=2.51, *p=0.002*). Conversely, Uu and Mh infections were more prevalent in female patients, since males were less likely at risk of Uu (OR=0.52, *p<0.001*) and Mh (OR=0.41, *p<0.001*) infection. In addition, Uu infection was more prevalent in patients younger than 25 years, either in women (OR=2.27, *p=0.003)* or men (OR=1.66, *p=0.034*). Finally, a significant association between Mh and Uu infections was found in either female (OR=33.84, *p<0.0001*) or male (OR=71.83, *p<0.0001*) patients. Our data revealed that Ct, Uu and Mh are prevalent uropathogens in patients with couple's primary infertility and the importance of including the screening of urogenital infections in the diagnostic work up of infertility.

536. (161) **DO SEXUALLY TRANSMITTED PATHOGENS PLAY A ROLE IN MALE INFERTILITY?**

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Male urogenital infection/inflammation has been proposed as responsible for up to 15% of male infertility cases. In fact, several microorganisms including *Ureaplasma spp.* (UU)*, Mycoplasma hominis* (MH) and *Chlamydia trachomatis* (CT) have been associated to infertility. However, compelling evidence from large studies about the role of these infections on male infertility is currently lacking. Herein, we studied the prevalence of UU, MH and CT infections and their impact on semen quality in a cohort of 3484 male partners of infertile couples. Semen specimens were collected by masturbation and semen analysis assessed according to the WHO guidelines. Infections were analysed by PCR or culture. Chi-square and Kruskal-Wallis tests were used to test infection associations. A prevalence of UU, MH and CT infection of 18.8%, 4.34%, and 4.05% was found, respectively. A single infection was detected in 18.5% of patients, whereas the simultaneous presence of two and the three uropathogens was detected in 4.6% of patients. A significant association between MH and UU infections was found (OR=39.64, *p<0.0001*). On the other hand, MH or CT infected patients showed reduced sperm concentration (*p<0.05*), lower sperm viability (*p<0.05*), and decreased counts of morphologically normal sperm (*p<0.001*), with respect to non-infected patients. Moreover, CT and UU infected patients revealed significantly increased leukocyte counts in semen (*p<0.05*). Our results describe for the first time the prevalence of three common urogenital infections in male partners of infertile couples from Argentina, which are similar to those already reported worldwide. Moreover, our data indicate that MH and UU infections are mutual risk factors of their co-infection. In addition, MH and CT infections were associated with alterations in some sperm quality parameters. Altogether, our results point out the relevance of including the screening of urogenital infections in the diagnostic workup of male infertility.

537. (163) ***CHLAMYDIA TRACHOMATIS* UROGENITAL INFECTION DOES NOT APPEAR TO DETERIORATE SEMINAL QUALITY IN SEMEN IN YOUNG PATIENTS**

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Chlamydia trachomatis (CT) is the most prevalent sexually transmitted bacterial infection. Although CT urogenital infection and associated pathology has been widely described in females, the study of male urogenital infection has been neglected. Herein, we analyzed the prevalence of urogenital infection and its impact on semen quality parameters and in inflammatory markers in patients. A cohort of 140 male patients, aged 20-49 y.o., who attend a reproduction and andrology clinic by control, symptoms of infection or possible. Semen samples were collected by masturbation and semen analysis performed according to the WHO manual. Infections by CT; Human Papiloma Virus; *Mycoplasma hominis* (MH); Herpes Simplex Virus type 1 and type 2 (HSV2); *Ureaplasma urealyticum* (UU); *Trichomona vaginalis*; *Mycoplasma genitalium* (MG); *Treponema pallidum* and *Neisseria gonorrhoeae* were assessed by PCR. Semen quality parameters, ROS, inflammatory cytokines, subpopulations of leukocytes were analyzed. Statistical analysis was performed by Kruskal-Wallis test. A prevalence of CT, UU, MH and MG infection of 33.6%, 18.0%, 10.0% and 7.2% was found, respectively. Other pathogens showed to be much less prevalence. Patients infected with CT alone or co-infected with HSV2 showed neither significant alterations in most of the sperm quality parameters analyzed nor increased inflammatory biomarkers in semen ($p>0.05$). Noteworthy, CT-infection was associated with significantly reduced levels of ROS in semen ($p<0.05$). On the contrary, patients co-infected with CT and MG showed a significant reduced levels of sperm viability ($p<0.05$) and increased frequencies of necrotic sperm ($p<0.001$), not associ-

ated to leukocytospermia. Our results revealed a high prevalence of CT-infection in young men from our region. Although, CT infection does not significantly impair sperm quality, men would provide a reservoir for continuous transmission of the infection.

538. (199) **OXIDANT-ANTIOXIDANT MARKERS BEHAVIOR IN FOLLICULAR FLUID OF PATIENTS UNDERGOING FERTILITY TREATMENT**

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Biochemical characteristics of the follicular fluid (FF) surrounding the oocyte may play a critical role in determining oocyte quality and their subsequent potential to achieve fertilization and embryo development. The aim was to study the modifications in redox markers in human follicular fluid and to investigate their behavior according to the number of oocytes recovered. Sixty-four infertile women aged between 23-44 years were categorized in terms of the number of oocytes retrieved as (A)lower: 0–4 oocytes, (B)intermediate: 5–8 oocytes and (C)higher: ≥9 oocytes. We determined, by spectrophotometric methods, a) oxidative stress markers: concentrations of malondialdehyde (MDA) and nitrite (NO2-); b) antioxidant defenses: glutathione (GSH) concentration and antioxidant enzyme: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx). Statistical analyses were performed by InfoStat software and were considered significant at $p<0,05$. MDA and NO_2^- levels were similar between groups studied. However, GSH and GPx values were significantly lower in the higher oocyte retrieval group compared to the other groups [GPx μmol/mg prot: A=25,7(14,8-39,5); B=27,3(15,6-38,5); C=9,9(5,8-16,4)-GSH μmol/mg prot: A=19,5(11,4-45,3); B=15,6(12,9-19,6); C=9,5(7,5-13,2)]. Positive IVF outcomes were highest when oocyte retrieval was in the range of 5–8 (with pregnancy rate 23%) compared to 0–4 and >9 oocytes groups (with pregnancy rate 10%). These findings show the behavior of the redox state in the follicular environment according to the number of oocytes recovered, evidencing a marked imbalance in the group with the greatest recovery of oocytes which could affect the IVF outcome.

539. (213) **CAPACITATION-INDUCED MITOCHONDRIAL ACTIVITY IS REQUIRED FOR SPERM FERTILIZING ABILITY IN MICE BY MODULATING HYPERACTIVATION**

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To become fully competent to fertilize an egg, mammalian sperm undergo a series of functional changes within the female tract, known as capacitation, that require an adequate supply and management of energy. However, the contribution of each ATP generating pathway to sustain the capacitation-associated changes remains unclear. Based on this, we investigated the role of mitochondrial activity in the acquisition of sperm fertilizing ability during capacitation in mice. Previously we had shown, that mitochondrial membrane potential (MMP) increases during mouse sperm capacitation and that mitochondrial activity could be associated with the maintenance of sperm motility during this process. Also, we had demonstrated that the MMP rise was prevented when sperm were exposed during capacitation to the mitochondrial uncoupler Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) or the protein kinase A (PKA) inhibitor H89. In the present study we demonstrated by western blot that treatment with CCCP during capacitation did not affect the PKA substrate and tyrosine phosphorylations (n=3; $p>0.05$) but produced a decrease in hyperactivation measured by CASA, similar to that observed after H89 exposure (n>3; $p<0.01$). In addition, CCCP inhibited the in vitro sperm fertilization capacity when sperm were incubated with cumulus-oocyte complexes (n=4; $p<0.05$) or with *zona pellucida* (ZP) intact eggs (n=5; $p<0.05$) without affecting gamete fusion (n= 4; $p>0.05$) and cumulus penetration (n=6; $p>0.05$), indicating that hyperactivation supported by mitochondrial function is necessary for penetration of the ZP. Finally, complementary in vivo fertilization experiments further demonstrated the fundamental role of mitochondrial activity for sperm function (n>4; $p<0.05$). Altogether, our results show the physiological relevance of mitochondrial functionality for sperm fertilization competence.

540. (254) **METABOLIC REPROGRAMMING OF MONOCYTES/MACROPHAGES BY FIRST TRIMESTER TROPHOBLAST DERIVED FACTORS**

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Immune regulation during placentation is crucial for fetal growth. Loss of immune homeostasis at the maternal-fetal interface is associated with preeclampsia and fetal growth restriction. A tight interaction between trophoblast cells (Tb) and recruited monocytes and macrophages from early stages of pregnancy maintains an anti-inflammatory microenvironment. We have previously reported on soluble factors present in the conditioned media (CM) of Tb that contribute to CD14+ cell expression of an anti-inflammatory profile. Tb cells present high glycolysis rate with high lactate production which is known to induce tolerogenic and anti-inflammatory profiles in tumor-associated macrophages.
Our aim is to evaluate the effect of Tb derived factors on CD14+ cell metabolic reprogramming, focusing on glucose and fatty acid metabolism.
For CD14+ isolation, peripheral blood of healthy donors was processed by Ficoll-Paque/Percoll. Cells were cultured or not for 5 days with M-CSF. CM was collected from human first trimester Trophoblast-derived cell line Swan-71. Phenotypic marker expression, glucose uptake with D-glucose fluorescent analog (2-NBDG) and lipid droplets with Bodipy 493/503 were analyzed by flow cytometry. Lactate production was quantified by Accutrend Plus system.
Glucose uptake by CD14+ cells increased upon 20 min LPS (100 ng/ml) stimulation (% of CD14+ 2-NBDG (Mean±SEM): Basal 32.9 ± 4.1%; LPS 51.8 ± 8.5%; n=11). Tb CM prevented LPS-induced glucose uptake: CM-LPS 23.7 ± 4.5% ($p<0.01$, ANOVA). Tb CM also inhibited the expression of proinflammatory markers such as CD86 after 18h of stimulation with LPS. 18 h stimulation with LPS induced lipid droplet accumulation in CD14+ cells, while Tb CM reduced lipid storage. Moreover, LPS induced the release of lactate and CM partially inhibited this effect.
Our preliminary findings indicate that Tb-derived factors induce metabolic reprogramming of CD14+ cells associated to inhibition of a proinflammatory phenotype.

541. (258) **OVARIAN MONONUCLEAR CELLS DISTRIBUTION AND ITS INFLUENCE ON PATHOGENESIS OF BOVINE CYSTIC OVARIAN DISEASE (COD)**

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COD results from failure in the ovulation and the study of the processes that lead to this failure and persistence of the dominant follicle in the ovary is the key to understand the pathogenesis of COD in cattle. Ovulation has been characterized as an inflammatory process and mononuclear cells (MC) participate in this process, therefore, the aim of this study was to evaluate the populations of macrophages (CD14+), T (CD2+) and B (CD79+) lymphocytes in ovaries

of animals with induced follicular persistence and spontaneous COD (sCOD). Ovariectomy was performed to obtain ovaries with sCOD (n = 5). Also, an experimental model of follicular persistence was performed, with an intravaginal progesterone (P4) device to get subluteal concentrations of P4, obtaining dominant follicles around ovulation (n = 5; P0) and follicles that persist for 5 (n = 5; P 5), 10 (n = 5; P10) or 15 days (n = 5; P15) after the expected time of ovulation. Controls cows were ovariectomized in proestrus (n = 5; C). MC populations were evaluated through immunohistochemistry in ovarian cortex, medulla, theca interna and externa of persistent follicles, cysts and dominant. The specificity of the antibodies was corroborated by western blot. The data were analyzed through ANOVA with Duncan post-test to contrast the evaluated structures of the experimental model and C group, and T-student test for sCOD and C group. The number of CD14+, CD2+ and CD79+ cells was higher in ovarian cortex, medulla, theca interna and externa of C group than in sCOD, P0, P10 and P15 groups ($p<0.05$). Exceptionally, CD79+ cells in the ovarian cortex of the P0 group were similar respect to the C group ($p>0.05$). Because these populations constitute potential *in situ* modulators of ovarian function, acting through the secretion of regulatory factors of the inflammatory process related to ovulation, we propose that this low proportion of these cells could be partly responsible for the anovulation observed in COD.

542. (291) **SIMPLE, EFFICIENT AND LOW-COST METHODOLOGY FOR CELL FREE RNAs EVALUATION IN HUMAN PLASMA AND URINE SAMPLES**

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Cell-free ribonucleic acids (cfRNAs) analysis could be used as biomarkers for monitoring different pathological conditions such as high-risk pregnancies. Most studies use commercial columns for high-quality isolation, ready-to-use RNA. However, it is important to have an efficient, simple, reliable and low-cost method. We optimized an alternative method for cfRNAs isolation using TRIzol reagent. Plasma and urine samples from 18 to 40-year-old women were used. Some of them were obtained from pregnant women (during the first, second and third trimester) and others from non-pregnant women. Plasma samples were obtained by venipuncture in ethylene diamine tetraacetic acid (EDTA) tubes. All samples were centrifuged at high revolutions to remove cell debris and cfRNAs were isolated from supernatants using TRIzol reagent (Invitrogen). Several volumes were assayed: 150, 250, 400 and 600 μL. Then, cfRNAs were converted to cDNA by retrotranscription (RT) using Moloney murine leukemia virus reverse transcriptase (Promega). The ribosomal protein L19 (L19) cfRNA was analyzed by real time quantitative PCR. Several cDNA dilutions were assayed: 1/2, 1/4 and 1/8. Amplification products were analyzed by 1.5% agarose gel electrophoresis. cfRNAs were purified in all volumes assayed, except in 150 μL plasma samples due to aqueous phase absence. cfRNAs concentrations were from 10.9 to 158.5 ng/μL. RT was carried out using 0.2-1 μg RNA. The L19 target was amplified in all samples using 5 μL cDNA. Furthermore, Cts values obtained demonstrated amplification progression in cDNA successive dilutions assayed, with low standard deviation between duplicates (difference <0.5). Similar results were obtained using pregnant and non-pregnant women samples. Our results suggest that cfRNAs isolation using TRIzol reagent could be a suitable method compared to commercial kits for specific analysis of these molecules on plasma and urine samples.

543. (292) **OPTIMIZATION OF CELL-FREE DNA ISOLATION AND DETECTION BY REAL TIME QUANTITATIVE PCR IN PLASMA SAMPLES**

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Cell-free DNAs (cfDNA) are short DNA fragments derived from cell death and NETosis. cfDNA is emerging as a promising biomarker for pregnancy disorders. We optimized a method to isolate and detect cfDNA from plasma. Plasma samples from 18 to 40-year-old pregnant (during first, second and third trimesters) and non-pregnant were obtained by venipuncture in EDTA tubes. They were centrifuged at high revolutions to remove cell debris and cfDNA was isolated from 400 μL supernatants using QIAmp DNA Blood Mini Kit (QIAGEN). Several elution volumes (Ve) were assayed: 25, 40, 50 and 60 μL. cfDNA was detected by real time quantitative PCR of total cfDNA representative genes: *B-Actin* and *Glyceraldehyde-3-phosphate Dehydrogenase* (*GAPDH*). Different cfDNA volumes (5 and 10 μL) and dilutions (1/2, 1/4 and 1/8) in a 20 μL final volume were assessed. In addition, different amplification conditions were analyzed, i.e.: primer concentration (0.25 and 0.5 pmol/μL), and annealing temperature (Ta) (*B-Actin*: 50, 51, 52.5 °C; *GAPDH*: 58.5, 60 °C). Similar results were obtained in pregnant and non-pregnant women. For *GAPDH* gene, non-specific amplification products were detected in all assays, while for *B-Actin* gene, it depends on the conditions assayed. Among all Ve assayed, 50 μL yielded a specific *B-Actin* amplification. Using a Ta=51 °C and 0.5 pmol/μL sense-antisense-primer both specific and no-specific amplification products were detected. However, using 0.25 pmol/μL sense-antisense-primer and 5 μL cfDNA eluate, only specific amplification products were detected, with Ct values above 28 and low standard deviation between duplicates (difference <0.5). We optimized a method for cfDNA isolation and detection from plasma samples. The optimal methodological conditions were: *B-Actin* as gene target, Ve=50 μL, 5 μL cfDNA eluate, Ta=51 °C and 0.25 pmol/μL sense-antisense-primer. It may be a suitable method for biomarker evaluation in the obstetric field.

544. (293) **CELL FREE DNA ISOLATION AND DETECTION IN HUMAN URINE**

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Cell free DNA (cfDNA) exists in different biological fluids and is pro-

posed as a potential diagnostic indicator for different clinical conditions. Urine CfDNA detection would provide a tool for a less invasive prospective diagnosis. Limited information about urinary cfDNA detection is available, and cfDNA stability is a challenge due to urine variable pH. We optimized a reliable method for cfDNA isolation and detection from urine. Urine samples from 18 to 40-year-old women were centrifuged at high revolutions to remove cell debris. To assess whether urine acidity influences isolation efficiency, acidic (pH5) and neutralized (pH7) urine samples were assayed. cfDNA was isolated from 800 μL urine samples aliquots using QIAamp DNA Blood Mini Kit (QIAGEN) and eluted with 50 μL elution buffer. cfDNA concentration was measured by Nanodrop spectrophotometer. A real time quantitative PCR was performed using an optimized protocol for B-Actin amplification. Different cfDNA volumes (5 and 10 μL) and dilutions (1/2, 1/4 and 1/8) in a 20 μL final volume were assayed. Amplification products were analyzed by 1.5% agarose gel electrophoresis. cfDNA concentrations in neutralized and non-neutralized samples were 4.7 and 5.3 ng/μL, respectively. Specific B-Actin amplification product, in both neutralized and non-neutralized samples, was detected at Tm=80.1 °C; but at Tm=75.4 °C a non-specific amplification product was also detected. Sample neutralization prior to isolation considerably decreased non-specific amplification products. Furthermore, Cts values obtained demonstrated amplification progression in cfDNA successive dilutions assayed, with low standard deviation between duplicates (difference <0.5). We isolated and detected cfDNA from urine, and demonstrated the importance of performing sample neutralization prior to isolation. Although limited information about urine-cfDNA is available, it may be a promising biological biomarker.

545. (295) **THE OTHER SIDE OF COVID-19 PANDEMIC: EFFECTS ON FEMALE FERTILITY**

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SARS-CoV-2 invades the target cell by binding to angiotensin converting enzyme 2 (ACE-2). In the human ovary, ACE-2 is expressed in stromal and granulosa cells.
Our objective was to evaluate the effect of SARS-CoV-2 infection on female gonad.
FF (follicular fluid) from patients undergoing ART (n= 80; 21–41 years old; November 2020-April 2021) were divided in two groups: FF from control patients and FF from recovered COVID-19 patients (asymptomatic and with mild symptoms).
The levels of IgG antibodies against SARS-CoV-2, IL-1β, IL-10 and VEGF were measured in FF by ELISA.
Using a granulosa cell line (COV434) and an endothelial cell line (EA.hy926), we studied the effect of FF from control and recovered COVID-19 patients. The expression of StAR, ERα and ERβ, 3β-HSD, VEGF, ANGPTs (angiogenesis-related proteins) and γH2AX (DNA damage marker) was evaluated by WB. Proliferation was evaluated by a WST-1 assay. Endothelial cell migration was evaluated by a wound healing assay. We performed Student's t test or one-way ANOVA.
The results showed that 91.3% of post-COVID-19 FF was positive for IgG against SARS-CoV-2. Patients with higher levels of SARS-CoV-2 IgG showed a decrease in the number of retrieved oocytes ($p<0.05$). The levels of VEGF and IL-1β were lower ($p<0.05$) in post-COVID-19 FF, while IL-10 did not differ.
In COV434 cells with post-COVID-19 FF, the expression of StAR, Erβ and VEGF was decreased ($p<0.05$), while ERα and 3β-HSD did not change.
In EA.hy926 cells with post-COVID-19 FF, a decrease in cell migration was observed ($p<0.0001$) without changes in the expression of ANGPTs. Both cell types showed higher expression of γH2AX with post-COVID-19 FF ($p<0.05$). No differences were found in COV434 and EA.hy926 cell proliferation rates between the groups.
In conclusion, these results describe that SARS-CoV-2 infection alters the follicular microenvironment, damaging ovarian function, and affecting reproductive performance in recovered COVID-19 patients. This project that involves the use of human samples from assisted fertilization techniques has been approved by the IByME Ethics Committee in 2020 (REGISTRATION CODE 2850, October 2020). This project was carried out between January and June 2021.

546. (342) **COPPER CHELATION INHIBITS ANGIOGENESIS AND MODULATES THE OXIDATIVE IMBALANCE IN A MODEL OF ENDOMETRIOSIS**

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Endometriosis (EDT) is an estrogen-dependent disease that affects 5-15% of reproductive-aged women. It is characterized by the growth of endometrial-like tissue outside the uterine cavity and often causes chronic pelvic pain and subfertility. Currently, EDT has no cure, and there is an unmet need for new treatment options. Angiogenesis is essential for the growth of endometriotic implants because it ensures an adequate supply of oxygen and nutrients and the removal of waste products. Elevated copper (Cu) levels have been linked to EDT. Cu is required by many enzymes, some involved in the antioxidant system. In cancer, this metal promotes angiogenesis, tumor progression, and oxidative stress. Therefore, our objective was to evaluate the effect of Cu chelation with ammonium tetrathiomolybdate (TM) on angiogenesis and oxidative stress in endometriotic-like lesions. Sixteen female C57BL/6 mice were divided into two experimental groups: EDT and EDT+TM. The EDT induction was performed by autologous uterine tissue transplantation to the intestinal mesentery. The EDT+TM group received 0.30 mg of TM/day in their drinking water for two weeks from the postoperative 15th day. Bodyweight and hematocrit were periodically monitored. Endometriotic-like lesions were collected one month after the pathology was induced to analyze the expression of angiogenic markers (RTqPCR), the presence of endothelial cells (immunofluorescence), and oxidative stress (spectrophotometric methods). Treatment with TM induced anti-angiogenic effects by decreasing the number of blood vessels ($p<0.001$), the mRNA expression of *Fgf2* and *Pdgfb* ($p<0.05$), and the presence of endothelial cells ($p<0.001$). Besides, it decreased antioxidant activity (SOD and CAT, $p<0.05$) and increased lipid peroxidation (TBARS, $p<0.05$). In conclusion, TM acts as an effective anti-angiogenic agent and modulates the oxidative imbalance in EDT. These observations support the study of TM as a possible non-hormonal treatment for EDT.

547. (350) **ORAL INFECTION AND ADVERSE PREGNANCY OUTCOME: *PORPHYROMONAS GINGIVALIS* OUTER MEMBRANE VESICLES ENTER TROPHOBLAST CELLS**

AND ALTER TROPHOBLAST-MONOCYTE INTERACTION

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An association between periodontitis and deep placentation disorders has been reported although the mechanisms remain unclear. *Porphyromonas gingivalis (Pg)* is among the most relevant pathogens in periodontal disease. Pg produces and releases outer membrane vesicles (OMVs). The production of OMVs is involved in bacterial functions and has a role in pathogenesis. During placentation, trophoblast cells secrete cytokines and chemokines in order to interact with immune cells and maintain immune homeostasis. Alterations in this interaction associate with pregnancy complications. In this work, we analyzed the effect of Pg-OMVs on trophoblast cell function.

OMV were isolated by ultracentrifugation and labelled with PKH26 dye for uptake assays. OMVs were characterized using dynamic light scattering and transmission electron microscopy and were on the nanometric size range (108 ±39 nm). Swan-71 and HTR-8 trophoblast cells were treated with 0.1-10 ug/ml Pg-OMV for 2-24h depending on the assay. MTT assay was used to evaluate cell viability. To test trophoblast–monocyte interaction, monocytes were isolated from healthy volunteers using Percoll gradient and incubated with trophoblast conditioned media (Tb-CM).

We found that Pg-OMVs were internalized by HTR-8 and Swan-71 trophoblast cells in a time and concentration dependent manner without affecting cell viability. Inhibition of actin polymerization by cytochalasin D reduced the uptake of Pg-OMVs. Regarding immune homeostasis maintenance, increased mRNA expression of IL-1beta, MCP-1, RANTES was found in Pg-OMV treated trophoblast cells. In addition, Tb-CM of Pg-OMV treated cells decreased the frequency of IL-10 + CD14+ monocytes ($p<0,05$).

Results are consistent with a mechanism of Pg-OMVs to affect trophoblast cell cytokine profile and trophoblast-immune interaction that might impair placentation and contribute to adverse pregnancy outcome.

548. (359) **EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION IS MODULATED BY KLF6 TRANSCRIPTION FACTOR THROUGH REACTIVE OXYGEN SPECIES**

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During placental development, extravillous trophoblasts (EVT) acquire an invasive and migratory phenotype to anchor the placenta to the uterus. Alterations in these processes are associated with pathologies such as preeclampsia and placenta accreta spectrum. KLF6 is a transcription factor highly expressed in placenta and required for proper placental development. KLF6 immunoreactivity is higher in the placental bed of preeclamptic than in that of uncomplicated pregnancies. Its expression is regulated by hypoxia and inducers of oxidative stress. Reactive Oxygen Species (ROS) modulate essential physiological processes but can lead to oxidative or reductive damage. We have demonstrated that KLF6 silencing increases ROS level and EVT cell migration. We hypothesize that KLF6 modulates EVT differentiation through ROS. Herein, we demonstrate that ROS induced upon KLF6 downregulation is not associated with cell damage, based on Anexin V/7-AAD and MTT assays. Cell proliferation, measured by BrdU uptake, is reduced and correlates with an increase in p21 expression. In addition, the decrease in KLF6 levels increase metalloproteinase 9 activity and the expression of molecules involved in cell migration, promotes the translocation of β-catenin to the nucleus, measured by confocal microscopy, and increases polarized cell migration evaluated by orientation of the microtubule organizing center. Furthermore, KLF6-silenced cells acquire a more fibroblastic phenotype and treatment with two antioxidants, NAC and tempol, partially recovers cell morphology. In line with the data obtained in vitro, placenta accretas, characterized by being abnormally invasive, have lower KLF6 immunostaining levels. Altogether these results suggest that KLF6 modulates EVT differentiation through the regulation of ROS levels and suggest that its dysregulation may contribute to the development of pregnancy pathologies like placenta accreta spectrum and preeclampsia.

549. (383) **ALTERED CHEMOTAXIS AND MONONUCLEAR CELL INFILTRATION IN BOVINE OVARY AFTER ACTH STIMULATION**

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Before ovulation the ovary exhibits signs of local inflammation: chemokine and cytokine release, vasodilation, leukocyte infiltration; and altered immune responses in ovary often results in reproductive failures. However, the effects of adrenocorticotropin (ACTH) on the complexity of this inflammatory response are not yet well described. The aim of this study was to evaluate the effect of ACTH during preovulatory period on the number of mononuclear cells (MCs) infiltrated in the ovary and on the mechanisms associated with cell infiltration. ACTH (100 IU) was administered to Holstein cows (n=11) during proestrus every 12 h for four days before ovulation when ovariectomy was performed (day 18). Daily ovarian Doppler ultrasonography was used to evaluate the percentage of irrigated area (IA), the pulsatility and resistance index on the dominant follicles. Also, blood sample was taken on day 18 to analyze alterations on circulating MCs using an hematology analyzer. Macrophages (CD14), T (CD2) and B (CD79) lymphocytes distribution in ovary was analyzed by immunohistochemistry. In follicular wall samples, gene expression of chemokines MCP1, IL8, CXCL1, CCL25 were evaluated. And, IL17a expression was analyzed by western blot. A Generalized Linear Model was used to analyze the results. The number of systemic monocytes and lymphocytes were similar between groups ($p>0.05$). However, the total number of CD14, CD79 and CD2 infiltrated cells were lower in ACTH group than in control group ($p<0.05$). The IA showed to be higher on day 18 than in day 16 and 17 ($p<0.05$) but no effects of treatment (T) or interaction (T x time) was found ($p>0.05$). Chemokine's gene expression showed a lower mRNA of CCL25 on ACTH group ($p<0.05$). IL17a protein expression was similar between groups ($p>0.05$). Our results suggest that ACTH, released as consequence of stress in preovulatory period, could impair MCs infiltration in bovine ovary and this could be due to a lower chemotaxis capacity of the ovary.

550. (398) **MECHANISMS INVOLVED IN LEPTIN ANTIAPOPTOTIC EFFECT AFTER HIF-1α STABILIZATION**

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Leptin acts as a regulatory hormone in the maternal fetal interface. We demonstrated that leptin promotes proliferation and survival of trophoblastic cells. Moreover, leptin prevents cellular stress under hypoxic condition in trophoblastic cells. In this sense, Leptin is incremented in different pregnancy pathologies such as preeclampsia. In this work we aimed to elucidate the signaling pathway involved in Leptin antiapoptotic effect on placental apoptosis induced by cobalt chloride (CoCl2). This agent stabilizes HIF-1α transcription factor. All procedures were approved by ethical review committee at the Alejandro Posadas National Hospital. We used Swan-71 cells, a cytotrophoblast human cell line and human term placental explants cultured under normoxia and hypoxia conditions. Both cell models were treated with CoCl2 (50,100 or 250 μM) in presence or absence of leptin (100 ng/ml). We studied the MAPK signaling pathway after hypoxia treatment, using pharmacological inhibitors. The expression of Bid, Bad, Bax, Caspase 9 and Caspase 3 was determined by Western blot and qRT-PCR. Leptin diminishes Bax/Bcl-2 ratio (0.2 ± 0.1) in Swan-71 cells, under hypoxia condition; determinate by qRT-PCR and WB. Leptin treatment in hypoxia condition, regulates anti and proapoptotic proteins, decreasing t-Bid (0.5 ± 0.4); Bax (0.8 ±0.1), Bid (1.3 ± 0.09) and enhance Bcl-2
(2.0± 0.3), Bcl-XL (2.3 ± 0.3). Apoptosis was determined analyzed by DNA ladder assay in placental explants. All these results suggest that Leptin is capable to protect, placental apoptosis after HIF-1α stabilization.

551. (415) **THE IMPACT OF OVARIAN FOAMY MACROPHAGES ON THE REPRODUCTIVE AGING OF VIP KNOCK OUT MICE**

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Background: Reproductive aging is proposed to be associated with a chronic low-grade systemic inflammation (inflammaging), a dysfunction of immune tolerance with a detrimental impact on pregnancy. Since, VIP is an immunopeptide found in the reproductive tract with antiinflammatory effects inducing a tolerogenic response, we aim to understand VIP contribution to control inflammaging in order to prevent premature aging, particularly in the ovary.
Methods: Animals used: VIP Knockout KO (-/-), deficient HT (+/-) and wild type WT (+/+) 3 or 8 months old in estrus. The serum hormones were measured with immulite Xpi Siemens platform. The ovaries were examined after histological staining and IL-1β secretion measured by ELISA. Phagocytosis assays of apoptotic bodies were monitored using light microscopy analysis to obtain foamy macrophages. Lipid droplets were stained with BODIPY and quantified by FACS analysis.
Results: We confirmed that during reproductive aging WT females gain weight, show cycling disorders with lighter ovaries accompanied by altered levels of ovarian hormones in serum. These parameters were found to be exacerbated in young VIP KO mice behaving as WT animals of advanced reproductive age. Moreover, young VIP KO mice displayed an inflammatory ovarian microenvironment with increased IL-1β production ($p<0.05$ Two Way ANOVA) and the presence foamy macrophages, linked to premature aging. We also found histological differences between the ovaries with more secondary follicles, less corpora lutea and more atretic follicles ($p<0.05$ Two Way ANOVA) indicating ovarian failure. Next, peritoneal macrophages were obtained from WT mice and cultured with apoptotic thymocytes +/- VIP antagonist during different time points. Lipid droplets in the macrophage cytoplasm were increased in the presence of VIP antagonist after 72 h of phagocytosis.
Conclusion: VIP may contribute to control the inflammatory milieu of the aging ovary preventing foamy macrophage generation.

552. (422) **ENDOPLASMIC RETICULUM STRESS MODULATES CELLULAR SENESCENCE IN ENDOMETRIAL STROMAL CELLS**

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Objectives: Endometrial stromal cells undergo an acute senescence response as well as endoplasmic reticulum stress (ERS) during decidual differentiation. Here, we focus on the link between both processes to support embryo implantation.
Materials and methods: Human endometrial stromal cell line (HESC) was decidualized with MPA and db-cAMP for 8 days (Dec). To induce ERS, non-decidualized (nonDec) cells were stimulated with 1ug/ml of thapsigargin (Tg) for 4h. Public data of genome-wide transcriptome analysis of primary ESCs (GSE160702) was used to evaluate expression of senescence-associated genes Deiodinase 2 (DIO2), Lumican (LUM), Ferritin (FTL) and Forkhead box protein O1 (FOXO1). Gene expression was evaluated by RT-qPCR in HESC cells and in endometrial biopsies from fertile women and recurrent implantation failure (RIF) patients. FOXO1 and β-galactosidase activity were tested by FACS. *$p<0.05$ was considered significant.
Results: β-galactosidase activity was quantified in HESC cells throughout the decidualization, displaying a peak during the first days of the process. Senescence-associated genes expression showed a downregulation of DIO2 and an upregulation of FTL, LUM* and FOXO1* in Dec cells (vs nonDec). Results were in accordance with *in silico* transcriptome analysis of primary ESC. FOXO1 increased was also confirmed at protein level by FACS. Then, HESC cells were stimulated with Tg, a strong ERS inducer, showing a similar expression pattern. Finally, RIF biopsies showed a downregulation of DIO2* while FTL* was upregulated (vs fertile controls).
Conclusion: HESC cells *in vitro* model depicted a similar senescence response that in primary cultures. Additionally, this was modulated by ERS induction, indicating a link between both processes. This was also observed in RIF patients, which we have previously reported to show an altered ERS response in addition to the altered senescence response observed here, and that might be related to endometrial receptivity failure.

553. (440) **GESTATIONAL TREATMENT OF BUTYRATE MODULATES MATERNAL AND FETAL LIPID METABOLISM IN A RAT MODEL OF MATERNAL OVERWEIGHT**

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In a rat model of maternal overweight we described in mothers, hypertriglyceridemia and in fetuses, overgrowth and liver lipid overaccumulation. Fetal alterations persisted in the offspring that develop fatty liver disease. Butyrate (B), a product of fiber metabolism from intestinal microbiota, improves lipid metabolism and prevents inflammation. Maternal oral administration of B prevented maternal hypertriglyceridemia, fetal overgrowth and liver lipid overaccumulation.
Our aim was to clarify the effects of B on lipid metabolism that helped to prevent maternal and fetal alterations.
Methods: Female Wistar rats were fed standard (CT rats) or saturated fat-rich-diet (FD rats) for 8 weeks and mated with control males. Vehicle or B (3%) was orally delivered daily during gestation (FDB rats). At gestational day 21, all rats were euthanized. Fetuses, maternal and fetal liver were explanted and weighed. Maternal liver levels of triglycerides (TG) were assessed by TLC, mRNA levels of enzymes involved in lipid metabolism by RT-qPCR and alanine aminotransferase (ALT) circulating activity by EIA.

Results: Maternal livers showed TG overaccumulation in FD rats (300% p>0.01 vs CT) which persisted in FDB rats. Maternal hepatic mRNA levels of *Aco* and *Cpt-1* were decreased (30%p>0.05 vs CT) in FD and FDB rats, while *Srebp-1c* mRNA levels were increased in FDB rats (40% p>0.05 vs CT). Fetal hepatic mRNA levels of *Lpl* and *Srebp-1c* were increased (70% p>0.05 vs CT) in FD, while B prevented the increase in *Srebp-1c* in female fetuses (60% p>0.05 vs FD). FD fetuses showed an increase in ALT activity (30% p>0.05 vs CT), which was prevented by B (20% p>0.05 vs FD).
Conclusions: Butyrate increased the already FD-induced maternal liver lipid overaccumulation. A decrease in lipid oxidation enzymes and an increase in Srebp-1c expression may be involved in maternal effects, while in fetuses, prevention of overgrowth, liver lipid overaccumulation and damage may involve sex dependent pathways.

554. (445) **VIP DECREASES ZIKA VIRUS PROPAGATION IN FIRST-TRIMESTER CYTOTROPHOBLAST CELLS AND RESTORES CELL MIGRATION**

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Objective: Zika virus (ZIKV) infection during pregnancy is associated to an increased risk of fetal growth impairment and altered central nervous system development. We have previously demonstrated that ZIKV impaired trophoblast cell (Tb) migration, increased glucose uptake and decreased the brain derived neurotrophic factor (BDNF) expression. Up to date there is no treatment or vaccines to ameliorate the observed fetal growth defects. We previously demonstrated that the vasoactive intestinal peptide (VIP) not only favors immune homeostasis maintenance but modulates trophoblast cell invasion and metabolism at early pregnancy. Our aim is to elucidate the metabolism and signaling pathways altered by ZIKV in first-trimester human Tb cells and explore the potential antiviral effect of the endogenous polypeptide VIP. Material and Methods: We infected first-trimester Tb-derived cell line Swan-71 with an isolated local ZIKV strain in the presence/absence of VIP. Tb migration was assessed in wound healing assays, RNA expression by RT- qPCR and viral production by the lysis plaque assay. Results: ZIKV induces an increase of NFKB and the proapoptotic factor BAK mRNA expression. However, a significantly higher increment in the anti-apoptotic factor BCL-2 was detected (2.5-fold increase of BCL-2 vs BAK p<0.01). Tb cells infected in the presence of VIP showed significantly lower levels of viral particles production (n=4; p<0.01) accompanied by a decrease in viral RNA detection in Tb cells. Interestingly, VIP induced higher levels of BST-2 expression, an IFN-induced cell membrane protein involved in the impairment of virion release and viral cell-to-cell transmission (Z vs. VIP-Z; p<0.05). Moreover, VIP ameliorated the impairment of cell migration induced by ZIKV (n=4; p<0.05).
Conclusion: We propose VIP as a potential antiviral endogenous factor since it reduces the permissively of the cells to Zika infection and ameliorates Tb migration impairment.

555. (452) **IMPACT OF PLACENTAL ZIKA VIRUS INFECTION DURING EARLY PREGNANCY: EFFECT ON TROPHOBLAST FUNCTION, METABOLISM AND IMMUNE-TROPHOBLAST INTERACTION. POTENTIAL ANTIVIRAL EFFECT OF VIP**

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Objective: Zika virus (ZIKV) infection during pregnancy is associated to fetal growth impairment and altered central nervous system development. Up to date, there is no treatment or vaccines to ameliorate fetal growth defects. We previously demonstrated that the vasoactive intestinal peptide (VIP) modulates trophoblast cell (Tb) function and metabolism at early pregnancy. Our aim is to determine the metabolism and signaling pathways altered by ZIKV in first-trimester human Tb cells, the impact on the Tb-immune cell interaction and the potential antiviral effect of VIP. **Material and Methods:** First-trimester Tb-derived cell line Swan-71 was infected with an isolated local ZIKV strain with/without VIP. Tb migration was assessed in wound healing assays and RNA expression by RT- qPCR. PBMC from healthy volunteers were conditioned with media (CM) from Tb-infected cells to analyze migration and functional profile. **Results:** ZIKV impaired Tb migration and decreased the expression of the neurotrophic factor BDNF. CM of Tb infected cells increased the recruitment of monocytes, CD4+ and NK cells and modified the activation profile of CD14+ cells favoring immune homeostasis maintenance. ZIKV infection increased Tb glucose uptake and modulated the signaling pathway of retinol inducing RIG-1 and RAR-alpha expression while a decrease of RAR-beta was detected. Interestingly, Tb cells infected in the presence of VIP produced lower infectious viral particles (p<0.01) along with a decrease of viral RNA in Tb cells. Moreover, VIP ameliorated ZIKV effect on Tb migration (p<0.05). **Conclusion:** Zika viral infection might impact early pregnancy by affecting Tb function, altering Tb metabolism and modulating Tb-leukocyte interaction thus sustaining Tb survival and virus persistence in the placenta. VIP emerges as a potential antiviral candidate to reduce the impact of ZIKV placental infection at early pregnancy since it decreases ZIKV propagation and restores Tb cell migration.

556. (472) **SERPIN F1 (PEDF) EXPRESSION IN MURINE MALE TRACT SPERM AND ROLE AS CAPACITATION FACTOR**

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Introduction: PEDF is a serin protease inhibitor recently described by our group in male reproductive tract of adult Wistar rat with androgen dependency. It is known that sperm reaches their maturity in epididymis and then are capable of fecundation in female tract after a series of changes such like capacitation, hypermotility and acrosomal reaction.
Objectives: analyse the PEDF expression in male tract and its effect over mouse sperm capacitation by the addition of recombinant PEDF (rPEDF) to the media.
Material and methods: mice C57/bl6 were sacrificed, testis and epididymis were removed and cauda sperms and tissues were obtained. We detected the presence of PEDF in mice reproductive tract by immunocytochemistry and over the sperm cells by immune fluorescence. Capacitation assays were performed. Briefly, sperm were pre incubated with rPEDF in two different concentrations: 50 and 100 ng/ml for 20 minutes in capacitating and non-capacitating media (HMB/HM). After 80 minutes, the acrosomal reaction was evaluated by Coomasie blue stain and was observed in microscope Nikon 80i. We counted 200 sperm by condition and the results were compare respect the non-capacitated state and the incubation in presence or absence of rPEDF.
Results: PEDF was expressed in the mice reproductive tract from testis to epididymis. Positive stain was observed mostly in seminiferous epithelium and peritubular cells. In epididymis it was detected in cytoplasm of caput epididymal cells and in cauda the sign was mainly nuclear. The capacitation assays shown an increase in acrosomal reaction (10 and 20%) due to the addition of 50 and 100 ng/ml of rPEDF respectably (Chi square test).
Conclusion: The presence of PEDF in the murine model suggests that could be involved in sperm biology in the male and inside female tract and contribute with the fertilization process. Further studies are needed in order to clarify PEDF role in sperm capacitation and or acrosomal reaction.

557. (492) **DYNAMIC CHANGES IN MOUSE SPERM MIDPIECE DURING ACROSOMAL EXOCYTOSIS**

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As the mammalian sperm are ejaculated from the male reproductive tract, they are not capable of fertilizing oocytes. In order to gain fertilization competence, they must undergo a series of biochemical and physiological events as they travel across the female tract that are collectively known as capacitation. One of the outcomes of capacitation is the ability to undergo acrosomal exocytosis, a unique secretory event. It occurs as the result of fusion events between the plasma membrane and a specialized vesicle called acrosome. A commonly employed method to assess acrosome exocytosis in single cell imaging is the use of FM dyes, which stain plasma membrane and it allows to follow the dynamics of this unique process. By using this dye, we observed that the mouse sperm midpiece encounters a decrease in diameter during the acrosome reaction induced either with progesterone, ionomycin or spontaneously. The contraction is initiated in any segment of the midpiece but preferentially begins near the neck. In single-cell super resolution experiments, we also employed Fluo4-AM and SiR-actin to monitor how intracellular calcium and actin dynamics, respectively, are involved in this process. We observed that this contraction is accompanied by an increase in intracellular calcium and a significant change in the F-actin structure located in the midpiece. Taken together, these results demonstrate that during acrosome reaction the mouse sperm midpiece goes through dynamic and structural changes that could affect the motility pattern of fertilizing sperm.

558. (504) **HIGH FAT DIET FEEDING AND ITS ADVERSE EFFECTS ON FEMALE MICE REPRODUCTION**

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Fertility depends on a correct energy balance. Metabolic disturbances due to western diets in women of reproductive age lead to menstrual dysfunction, subfertility and pregnancy complications. However, it is not clear how high fat diets (HFD) affect reproductive function.
Hypothesis: HFD causes metabolic and reproductive alterations in female mice. Metformin improves these effects.
Objective: To analyze the effects of HFD and metformin on metabolism and reproductive function in female mice.
Methodology: 21d.o. female C57BL/6 mice were fed with HFD or control diet for 16 weeks. One HFD group received metformin the last four weeks. Mice were weighed once a week. Serum, gonadal and visceral fat and the ovaries were extracted. Estrus cycle, glycemia and ovarian angiogenesis were evaluated. Another set of animals were mated with males. Offspring number, weight and the time until pregnancy were recorded. One-way- ANOVA was used.
Results: The HFD-mice had higher body weight, glycemia, GTT, total cholesterol, visceral and gonadal adipose tissue. Metformin improved GTT and decreased adipose tissues. The estrus cycle was shorter in HFD mice and the number of cycles/14d was increased in the HFD group. However, anovulatory stages were longer in HFD mice. Metformin had no effect on estrous cycle. The days until pregnancy were higher in HFD group and metformin reversed this effect. We found less pups per litter but no differences in pups'weight. However, total litter weight was lower in HFD group. Metformin improved this parameter. Ovarian periendothelial area was increased in HFD and PDGFB was decreased. Metformin decreased periendothelial area with no effect on PDGFB.
Conclusion: HFD affects metabolism, ovarian function, estrous cycle and the litter size. Metformin improves some of these alterations. HFD also increases the time to pregnancy and metformin reverses this delay. Changes in ovarian vasculature and PDGFB may be some of the possible causes of the observed alterations.

559. (512) **THE ABSENCE OF CATSPER CHANNELS AFFECTS THE DISTRIBUTION OF TUBULIN IN MOUSE SPERM**

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In several cellular systems, tubulin plays an important role in the regulation of ion channels. Protein extraction experiments with non-ionic detergents such as Triton X-100 showed that this tubulin is not a structural part of the cytoskeleton, but is close to the plasma membrane and accomplish its regulatory function through its association/dissociation to ion channels. In mammalian sperm, the presence of tubulin outside the axoneme has not been reported. Our hypothesis is that there is a fraction of tubulin associated with CatSper channels, fulfilling regulatory functions for the development of hyperactivated motility and consequently fertilizing ability.
In the present work, we carried out extracts of sperm proteins in the presence of Triton X-100. We observed by immunoblotting that a small portion of tubulin remains in the soluble fraction, indicating that it is not part of the cytoskeleton. When we performed these experiments on sperm derived from CatSper knockout mice (CatSper KO), the amount of tubulin in the soluble fraction was significantly lower than in wild-type (WT) sperm. As the opening of CatSper occurs in response to capacitation, we proceeded to study if the partition of the soluble tubulin follows a specific dynamic during this process in WT sperm. The levels of tubulin in the soluble fraction increase throughout the 60-min of incubation under capacitating conditions. In order to investigate the localization of this soluble tubulin, we performed super-resolution microscopy. Sperm from WT mice were incubated in the presence of the permeable tubulin probe SPY555-tubulin and analyzed using SRRF (Super-Resolution Radial Fluctuations). Novel structures were observed in the most distal region of the flagellum and in the head.
Taken together, our results suggest that there is tubulin beyond the axoneme that presents a specific partition dynamic during sperm capacitation and could play a regulatory role on CatSper channels.

560. (513) **GROWTH HORMONE AND ITS RECEPTOR: IMMUNOLOCALIZATION IN PREOVULATORY FOLLICLES AND ITS RELATIONSHIP WITH THE RESUMPTION OF OVARIAN CYCLICITY IN DAIRY COWS**

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Growth hormone (GH) acts as a key endocrine regulator for adequate metabolic adaption and optimal reproductive function in dairy cows. Moreover, the postpartum negative energy balance is negatively associated with the return of postpartum ovarian activity. The aims of this study were therefore i) to analyze the GH gene expression, its receptor (GHR) and metabolic sensors such as β-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), glucose and insulin in the resumption of postpartum ovarian cyclicity; and ii) to evaluate the localization and protein expression of GH and GHR

in preovulatory follicle (PF). The gene and protein expression were evaluated by real time PCR and immunohistochemistry, respectively. For the aim i), lactating cows (n=37) were selected. Blood and follicular fluid (FF) were sampled at the time of first dominant postpartum follicle (1ºDPF; day 9±2) and PF (day 45±2). Cows with fewer postpartum days (FPPD, n=15) or more postpartum days (MPPD, n=22) were analyzed by a generalized linear mixed model. For aim ii), complete ovaries of non-lactating cows (n=6) were obtained by ovariectomy. Mann-Whitney test was applied. A weak GH gene expression was found in GC thus could not be quantified. The GHR gene expression was higher in the FPPD group than in the MPPD on the 1ºDPF ($P<0.05$). NEFA and BHBA levels (in FF and serum) were higher in the MPPD group than in the FPPD ($P<0.05$). Glucose and insulin concentrations (in FF) were higher in the FPPD group than in the MPPD ($P<0.05$). The protein expression of GH was higher in GC than in theca interna (TC) whereas for GHR was higher in TC than GC ($P<0.05$). These results show a local production of the GH-GHR and an earlier return to postpartum cyclicity in cows with higher GHR gene expression. High levels of BHBA and NEFA and low (in FF) glucose and insulin levels in the MPPD group could generate an adverse microenvironment within follicle and delay the resumption of postpartum ovarian activity.

561. (545) **HYPERPOLARIZATION OF THE PLASMA MEMBRANE IS NECESSARY FOR MOUSE ACROSOMAL EXOCYTOSIS AND SPERM MIGRATION *IN* VIVO**

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To acquire fertilizing capacity mammalian sperm must undergo a complex process called capacitation (CAP). Among other biochemical and physiological processes, hyperpolarization of the plasma membrane (Em), an early event that occurs during CAP, leads up to acrosomal exocytosis (AE). It has been shown in mouse sperm that hyperpolarization of the Em is necessary and sufficient to prepare non-capacitated (NC) sperm for the AE. Furthermore, in mice where the sperm-specific potassium channel Slo3 has been knocked-out (KO), hyperpolarization of the Em doesn't take place and mice are infertile. Recently we have shown in human sperm that an oscillatory intracellular Ca^{2+} ($[Ca^{2+}]_i$) pattern prevents the occurrence of AE in NC sperm. Moreover, pharmacological hyperpolarization of the plasma membrane modulates the $[Ca^{2+}]_i$ pattern, reducing the oscillatory $[Ca^{2+}]_i$ increase enabling sperm to induce AE. The aim of this study was to evaluate the physiological role of Em hyperpolarization during CAP *in vivo*. We generated a new transgenic mouse by mating female Slo3 KO mice, with male transgenic mice with EGFP in their acrosome and DsRed2 in the mitochondria. This new Slo3 KO transgenic mice allowed us to observe the acrosomal status while sperm are migrating through the female reproductive tract. We performed mattings between KO or Wild Type (WT) males and WT female mice. First, we found that KO males mated less than the WT. More interestingly, although the number of sperm in the uterus was similar when mated with WT or KO males, the number of sperm reaching the oviductal *isthmus* was lower when the male were KO. Surprisingly, WT and KO sperm reached the *ampulla,* the site of fertilization. However, unlike the WT sperm, KO sperm were acrosome intact. Based on these results we conclude that 1) sperm Em contributes to the selection of sperm that enter and colonize the oviduct and is essential for AE in vivo; 2) the AE is not necessary for sperm migration to the ampulla.

562. (553) **SEMINAL TRANSFERRIN LEVELS AND DNA DAMAGE**

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Spermatogenesis is the process of proliferation and maturation of male germ cells. Damage to sperm DNA can occur at any stage of this process, and this damage is a multifactorial phenomenon. Transferrin is the major transporter of iron (Fe) in the body. In seminal plasma (SP) is found an isoform, testicular transferrin (TfT), which is essential for spermatogenesis. The objective of this work was to evaluate the relationship between TfT levels in SP and sperm DNA damage. Semen samples from 49 patients were studied. A basic semen analysis was performed according to WHO guidelines (WHO 2010).In order to evaluate DNA damage, sperm DNA denaturation was assessed using the acridine orange test (AOT) technique (Tejada et al., 1984) and sperm DNA fragmentation index was determined by the TUNEL test (Terminal Transferase dUTP Nick End Labeling). The TfT concentration was quantified by radial immunodiffusion (RID), whith an adapted technique. Statistical analysis was performed by the Spearman ordered rank correlation coefficient (r) for TfT versus sperm DNA fragmentation index (TUNEL) and DNA integrity (AOT). For the index of DNA fragmentation with TfT levels, the results were: $r = -0.7328$ $p < 0.01$. The percentage of sperm with denatured DNA (AOT) also showed a negative relationship with TfT concentration ($r = -0.4398$; p-value = 0.0055, $p < 0.01$). Statistical data suggest that high levels of TfT correlate with less damage DNA in spermatozoa. Future studies should include a larger cohort of samples to get closer to more accurate conclusions about the relationship between spermatogenesis, TfT, a promising biomarker of male infertility, and the integrity of sperm DNA, a factor that is becoming increasingly important on reproductive outcomes such as fertilization, implantation, miscarriage, pregnancy, and live birth rates.

563. (559) **OCCURRENCE OF MODIFIED HISTONES IN CHROMATIN OF HUMAN SPERMATOZOA AND THE SUCCESS IN ASSISTED REPRODUCTION TREATMENTS (ART)**

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Assisted reproductive treatments (ART) bypass male infertility, removing most barriers to fertilization. However, there are cases in which these techniques fail due to idiopathic causes. In search of possible causes of infertility in couples, we studied the presence of post-translational marks on the remaining histones in human spermatozoa, as it has been found that they are involved in the functionality of the post-fertilization zygote and may contribute to embryonic development. The aim of the study was to evaluate the relationship between the remaining sperm histones and the success of ART. In this study, the presence of modified histones was analyzed by immunocytochemistry using antibodies against H3K4me3, H3K27me3, H3K9me2/3, H3K4me2, H3K9me and H4K12ac. The presence of these marks was studied in sperm samples from normozoospermic donors ($n = 5$) and from infertile patients undergoing assisted reproductive techniques ($n = 13$) and then, we correlated them with sperm parameters and ART success. A negative correlation was found between H3K4me3 and H3K27me3 modifications with motility in donor sperm ($p < 0.05$ and $p < 0.01$, respectively) and a positive correlation was found between H3K4me3 and motility in sperm from patients ($p < 0.05$). When we analyzed the correlation of the modified histones with the fertilization rate, we observed a negative correlation between the presence of H3K4me3 and H3K27me3 ($p < 0.05$ in both cases) and a negative correlation was observed between the presence of H3K4me2 and the embryonic score ($p < 0.05$). Based on these results, our study provides critical information on the implications of the presence of certain modified histones in sperm chromatin, on sperm functionality and the success of ART.

TOXICOLOGÍA

564. (028) **PERINATAL EXPOSURE TO A MIXTURE OF GLYPHOSATE AND PROPICONAZOLE ALTERS THE DEVELOPMENT OF THE VENTRAL PROSTATE IN POSTPUBERTAL RATS**

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Glyphosate (GLY) exposure induces adverse effects on the male reproductive system in rats, whereas propiconazole (PRO) exposure alters the steroidogenesis and induces a hepatotoxic action, which could increase or decrease the effects produced by GLY when both agrochemicals are used together. Our aim was to evaluate whether perinatal exposure to a mixture of PRO and GLY (PROGLY) alters the ventral prostate (VP) development in postpubertal rats. Pregnant rats were orally exposed to vehicle (saline solution) or a mixture of PRO and GLY (each 4 mg/kg/day), from gestation day 9 until weaning. On postnatal day 60, male offspring were euthanized, and the prostate and serum samples were collected. Body weight and wet weight of the prostate complex (anterior, dorsolateral and ventral lobes) plus seminal vesicles were recorded. The testosterone (T) serum level was measured. In the VP, height of the prostatic epithelium (HPE), acinar luminal area (ALA) and acinar area (AA) were evaluated, as well as the presence of lesions in histological sections. Also, the proliferation index (Ki67) was assessed by immunohistochemistry. Both body weight and relative prostate weight were similar between the experimental groups. Although a decreased serum T level was observed in rats exposed to PROGLY, no significant differences were found compared to controls. Histologically, the HPE was increased (Control: 20.3±1.1 μm vs PROGLY: 26.0±1.2 μm; $p<0.05$) and the ALA was decreased (Control: 28578±2241 μm^2 vs PROGLY: 22321±915 μm^2; $p<0.05$) in the VP of PROGLY rats compared to controls. However, no significant differences were observed in the AA between groups. In PROGLY rats, foci of epithelial hyperplasias were found in the VP, whereas the epithelium proliferation index was similar compared to controls. In conclusion, perinatal exposure to a mixture of PROGLY alters the development of the VP, inducing hypertrophy of the prostatic epithelium and prostate lesions in postpubertal male rats.

565. (047) **LOW DOSES OF GLYPHOSATE-BASED HERBICIDE IN EWE LAMBS ALTER GENES INVOLVED IN FOLLICULAR DEVELOPMENT**

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The exposure to endocrine disruptor compounds (EDC) during critical periods of development may alter ovarian development. In previous studies, we demonstrated that glyphosate-based herbicides (GBH), acting as EDC, altered ovarian follicular development, in the ewe. In present study, we tested if the exposure of neonatal ewe lambs with the recently defined reference dose of GBH (US-EPA) alters the expression of genes involved in ovarian follicular development. Ewe lambs received oral treatment from postnatal day (PND) 1 to PND14 and the ovarian genes were evaluated at PND45. Ewe lambs were randomly assigned to the groups, GBH (n=5) received the reference dose (glyphosate at 1 mg/Kg/day) and controls (n=6) received saline solution. On PND45, the ovaries were collected and immediately frozen at -80 °C for further RNA extraction. Levels of mRNA of genes involved in follicular development such as steroid receptors (ESR1, ESR2, and PR), follicle-stimulating hormone receptor (FSHr), bone morphogenetic protein 15 (BMP15), Follistatin (FST), growth and differentiation factor 9 (GDF9), insulin-like growth factor 1 (IGF-1) and Activin A Receptor Type 2A (ACVR2) were determined. The mRNA expression of β-Actin was used as housekeeping gene. The mRNA expression levels in GBH-treated lambs were indicated as values relative to those of the control group. Treated ewe lambs showed a significant reduction ($p<0.05$) of mRNA of ESR1 (57%), ESR2 (63%), PR (73%), FSHr (33%) IGF1 (64%), ACVR2 (86%) and FST (57%). No variation was found in mRNA expression of BMP15 or GDF9. Our results demonstrate that neonatal exposure to low doses of GBH altered the expression of genes involved in follicular development and differentiation. These results provide evidence supporting previous studies showing that GBH alter follicular development in prepubertal lambs and raise concern about ovarian function in adults.

566. (094) **THE PESTICIDE HEXACHLOROBENZENE DISRUPTS HORMONAL SIGNALING AND INDUCES CELL MIGRATION AND INVASION IN HUMAN ENDOMETRIAL STROMAL CELLS**

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Endometriosis is a chronic illness defined by the presence and growth of endometrial tissue outside of the uterus. Progesterone and estrogen signaling are disrupted, commonly resulting in progesterone resistance and estrogen dominance. Several studies suggested that endocrine disrupting chemicals such as organochlorine pesticides could be a risk factor for endometriosis. Previously, we reported that Hexachlorobenzene (HCB) acts as an endocrine disruptor in uterus and mammary gland, modulating the hormonal signaling. Is a weak ligand of the aryl hydrocarbon receptor (AhR) and promotes metalloproteinase and cyclooxygenase-2 (COX-2) expression, as well as, c-Src kinase activation in human endometrial stromal cells (T-HESC) and in rat endometriosis model. Our aim was to evaluate the HCB effect on hormonal pathways, studying Estrogen and Progesterone Receptors (ERα; PR) expression and activation (WB), aromatase levels (WB), and on cell migration (scratch motility assay) and invasion (transwell assay) in endometrial stromal cells (T-HESC). Moreover, we examine the dependence of AhR, c-Src, COX-2 and ER on these events. T-HESC cells were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) at different lapse of time. Results show that HCB increases ERα levels at 0.5 and 5 μM ($p<0.05$) and reduces PR content at 5 μM ($p<0.05$), in concordance with endometriosis estrogen-dependent and progesterone-resistance. In addition, aromatase expression is also raised by HCB at 0.005-0.5 μM ($p<0.01$; $p<0.05$). Moreover, cell migration (0.005–5 μM) and invasion (0.05 and 5 μM) is promoted by the pesticide exposure involving the AhR, c-Src, COX-2 and ER pathways. HCB also triggers ERα activation via phosphorylation in Y537 (HCB 0.5 μM) through the AhR/c-Src pathway. Our results provide experimental evidence that HCB induces alterations associated with endometriosis. The pesticide could act as a xenoestrogen inducing an invasive profile contributing to the development and progression of the disease.

567. (109) **ESTIMATION OF MYCOTOXIN INTAKE IN BEER IN THE POPULATION OF BUENOS AIRES, ARGENTINA**

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Mycotoxins are secondary metabolites of toxic fungi that are frequently found as contaminants in food and present toxic effects to animals and human. Beer is widely consumed by the population. Cereals used in beer production, particularly barley, wheat and corn, can be contaminated by mycotoxins. **Objective**: was to estimate the exposure to different mycotoxins (FB, trichothecenes, OTA, ZEA, AFB) in beer in the province of Buenos Aires, Argentina. **Materials and Methods**: A survey was carried out by google form (n = 314) to know the consumption of beer and to determine the exposure to different mycotoxins. **Results**: From the surveyed population over 18 years old, 79.8% consume beer. The highest daily beer consumption (284.4 ml/day) was observed in the range of 36-50 years old; 64.5% of the population consumes both craft and industrial beer and 23.4% only consumes craft beer. From the daily beer intake surveyed in this study and the occurrence of mycotoxins in beer of previous publications, the estimated daily intake (EDI) of mycotoxins was obtained. It was observed that beer consumption contributed to 8.5, 5.1, 18.5 and 0.38% of the tolerable daily intake (TDI) of ochratoxin A (OTA), fumonisin B (FB), toxin T-2 (T-2) and zearalenone (ZEA) respectively. In the case of DON, it was estimated that beer consumption contributes with 67.6% of TDI. Previous publications informed that the occurrence of DON in 60 % of beers were less than 10 ng/ml (% of DON TDI= 4.47 %) and craft beer could reach 63 ng/ml (% of DON TDI= 63 %). **Conclusion**: The EDIs were below the tolerable daily intakes (TDI). The beer consumption contributed between 0.38% to 18.5% to each mycotoxins TDI, except DON, where the EDI of DON could be close to the TDI. Therefore, the analysis of mycotoxins in beer (industrial and artisanal) in our environment, especially DON, is necessary.

568. (115) **AIR PARTICULATE MATTER INDUCES VASCULATURE DYSFUNCTION IN A NUTRITIONAL GROWTH RETARDATION RAT MODEL**

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Around 2 billon children (almost 90% of the world's child population) are exposed to air pollution levels over 10 μg/m^3. Furthermore, child malnutrition is recognized as a major problem with devastating effects on children's health. Due to their physical and physiological characteristics, children conform a subpopulation highly susceptible to the adverse effect of environmental pollutants (gases and particulate matter -PM) and vulnerable to malnutrition. PM exposure increases the risk for cardiovascular disease progression, through vascular oxidative stress and inflammation, two main processes involved in the initiation of endothelial dysfunction.
Therefore, we investigate the effects of ROFA (Residual Oil Fly Ash) exposure on the vasculature, in a nutritional retardation (NGR) rat model. In order to achieve NGR animals, male weanling rats were fed a diet restricted 20% compared to *ad libitum* intake (control-C) for 4 weeks. NGR and C rats were intranasally instilled with either 1mg/kg BW of ROFA or its vehicle. 24h after exposure, the thoracic aorta was isolated and biochemical parameters were evaluated: CYP1A1, eNOS and Calcium channel type L by RT-PCR, TGFβ1 by Westen blot and cytokines (IL-6, IL-10) by ELISA.
Our data showed that ROFA exposure induced IL-6 and IL-10 augmentation vs. C rats (35% and 30%, p<0.01). No changes were observed in NGR cytokines levels.
Likewise, ROFA increased CYP1A1 levels vs. C (183%, p<0.001) and TGF-β1 levels vs. C (216%, p<0.001) and vs. NGR (133%, p<0.01). On the other hand, exposure to ROFA decreased eNOs levels vs. C (75%, p<0.001) and vs. NGR (38%, p<0.01), and calcium channels vs C (34%, p<0.01) and vs. NGR (20%, p<0.05).
These results showed that NGR animals failed to activate inflammatory and detoxifying response to ROFA. In relation to the contractile capacity of the vascular system, a frank decrease is observed, as well as a phenotypic change towards a more undifferentiated cell type.

569. (182) **THE UV FILTER BP-3 INDUCES INTRAUTERINE GROWTH RESTRICTION BY AFFECTING THE REMODELING OF SPIRAL ARTERIES**

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We are continuously exposed to personal care products containing chemicals that may affect our health. In previous works we have shown that dermal exposure to a commonly used UV-filter, benzophenone-3 (BP3) affected fetal growth of the progeny in mice. The aim of the present study was to evaluate the underlying mechanism of the effects of BP3 on fetal weight. For that purpose, C57BL6 mice were dermally exposed either to BP3 (50 mg/kg/day) or to vehicle (olive oil) on a daily basis, from gestational day 0 (gd0) until their sacrifice on gd10 or gd14. In the groups of animals sacrificed on gd10, there was a significant difference in the size of the whole implantation sites (WIS), being the WIS of the BP-3 treated animals smaller than those of the control group (p<0,01). When analyzing the wall-to-lumen diameter of the spiral arteries of paraffin-embedded WIS samples, those WIS of smaller size in the BP-3 group presented a bigger wall-to-lumen ratio whereas WIS of normal size from the BP-3 group (p<0,01) or from the control group (p<0,001) presented normal wall-to-lumen ratio, showing that only WIS of abnormal size had impaired spiral artery remodeling. When analyzing the animals at gd14, fetuses from the BP-3 treated animals were significantly smaller than fetuses from the control group (p<0,05), confirming the observations from gd10. Serum samples from all females were analyzed for their BP-3 content by HPLC. In BP3 treated animals, BP3 was found in 4 of 5 serum samples from gd10 and in 3 of 6 serum samples from gd14. BP3 was also found in amniotic fluid at gd14. These results indicate that BP3 is incorporated to the systemic circulation once applied dermally and it is able to reach the fetus. Taken all together, these results confirm that during pregnancy, BP3 is able to reach the maternal-fetal interface and affect the remodeling of spiral arteries, leading to an intrauterine growth restriction phenotype.

570. (189) **GLYPHOSATE BASED HERBICIDE EXPOSURE MAY EXACERBATE ENDOMETRIAL HYPERPLASIA INDUCED BY CAFETERIA DIET IN ADULT RATS**

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Many lifestyle factors can predispose to the development of endometrial carcinoma (EC). Previously, in rats, we detected that: a) cafeteria diet (CAF) induced endometrial hyperplasia (EH), a precursor lesion of EC; b) exposure to glyphosate-based herbicide (GBH) altered uterine development causing EH and increased cell proliferation. In the present work we sought to evaluate whether the exposure to a low-dose of a GBH plus CAF diet might enhance the uterine morphological alterations induced by CAF diet alone. Female Wistar rats were fed from postnatal day 21 (PND21) until PND240 with: chow diet (CON) or CAF. At PND140 one group of rats feed with CAF also received GBH through food, yielding 3 ex-

perimental groups: CON; CAF; CAF+GBH. Rats were sacrificed at PND240 and serum, fat depots, and uterine samples were obtained. Data were analyzed by Kruskal–Wallis followed by Mann-Whitney post-test ($p<0.05$). CAF and CAF+GBH increased the fat depots relative to body weight compared to CON group. At morphological level, CAF and CAF+GBH increased glandular volume fraction and stromal nuclei density. At protein level, we detected higher expression of estrogen receptor alpha (ESR1) and cell proliferation in both treated-groups respect to CON. Moreover, both CAF and CAF+GBH treatments increased volume fraction of abnormal glands (glands with cellular anomalies plus glands with daughter glands). This alteration was more pronounced in CAF+GBH group, suggesting that GBH could enhance CAF effects. Also, only CAF-GBH reduced the expression of PTEN (a tumor suppressor gene) in the subepithelial stroma respect to CON. Progesterone serum levels were higher in CAF+GBH compared to CON. In conclusion, although CAF induces several changes associated with EH, the co-exposure with GBH increases the risk of developing EC. It is important to highlight that the study of lifestyle factors in combination could have a better contribution to understand a complex pathology like the EC.

571. (193) **IN VITRO CYTOTOXIC ASSESSMENTS OF *NECTANDRA ANGUSTIFOLIA* AND *CISSAMPELOS PAREIRA* EXTRACTS AGAINST HUMAN CANCER CELL LINES**

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Secondary metabolites represent an established source of bioactive compounds with different chemical structures. Among the polyphenols, the subgroup of flavonoids arises as promising anticarcinogenic drugs. *Nectandra angustifolia* (Schrad.) Nees & Mart. and *Cissampelos pareira* L. are native plants of the Northeast region of Argentina. Previous studies conducted by our research group showed the presence of flavonoids in the ethanolic extracts and their bioactivities in diverse models. Therefore, the objective of this work was to test the cytotoxic effect of both ethanolic extracts (NaE and CpE) against several human cancer cell lines. The extracts were tested on the following cell lines Caki-2 (kidney clear cell carcinoma), A549 (lung carcinoma), HT-29 (colon adenocarcinoma) and THP-1 (acute monocytic leukemia). Assays were extended to non-cancerous cells lines, the embryonic HEK-293 (kidney) and L-929 (fibroblast). Cells were seeded according to ATCC guidelines and incubated 24 h in humidified atmosphere with 5% CO_2 at 37ºC. Then were treated with NaE or CpE ranging from 10 to 200 mg/mL for 24 h and 48 h. Proliferative rates were assessed with XTT assay compared to untreated cells for determining the IC50. Values were analyzed following the cytotoxicity of the National Cancer Institute, IC50 ≤ 20 μg/mL: high, IC50 ranged from 21 to 200 μg/mL: moderate, IC50 from 201 to 500 μg/mL : weak and IC50 > 501 μg/mL : no cytotoxicity. Both extracts showed a similar bioactivity at 24 h, with a low dose increase in proliferation. However, when analyzed at 48 h, NaE exhibited an IC50 33.37 mg/mL ± 5.4 mg/mL for THP-1, and for non-cancer cell lines an IC50 of 90.10 ± 19.58 μg/mL for L929 fibroblast and 81.11 ± 15.45 μg/mL for HEK-293 embryo cell line. These preliminary results of cytotoxic activity in TPH-1 cell line by NaE encourage us to develop further studies in the identification of its bioactive compounds, as well as, on the underlying anticancer mechanism of action.

572. (269) **THE UV FILTER BENZOPHENONE 3 (BP3) ALTERS THE MIGRATION OF THE EXTRAVILLOUS TROPHOBLAST CELL LINE SWAN 71 VIA ANDROGEN RECEPTOR PATHWAY**

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BP3 is one of the most commonly substances used in sunscreens and personal care products due to its UV blocking efficacy. Several *in vitro* and *in vivo* studies evidenced the ability of BP3 to act like an endocrine disrupting chemical. The present study focuses on the effect of BP3 on the migration capacity of human trophoblast cells (Swan 71 cell line) and the potential involvement of the androgen receptor (AR) pathway. We analyzed three different BP3 concentrations: a) BP3-2: the predicted no-effect concentration (2 µg/L), b) BP3-20: the concentration detected in the amniotic fluid (20 µg/L) in our previous studies and c) BP3-200: the plasma concentrations reported in humans (200 µg/L). We examined cell migration activity by scratch-wound healing assay, as well as mRNA relative expression levels of molecules of interest such as, AR, matrix metalloproteinase 2 (MMP2), inhibitor of MMP-2 (TIMP2) and laminin a4 (LAMA4). The three doses of BP3 reduced the area of wound closure after 24 h of exposure, evidencing reduced migration of Swan 71 cells when compared to the vehicle. Interestingly, BP3 induced an augmented expression of AR mRNA levels in all concentrations assayed, and of TIMP2 and LAMA4 only in BP3-2. MMP-2 did not show significant changes. In order to confirm whether BP3 acts via an AR-dependent pathway, we then analyzed BP3 effects with and without an AR inhibitor (Flutamide, 1 μM). When the cells were treated with BP3 in the presence of flutamide, the area of wound closure did not change after 24 h, clearly indicating that BP3 acts through a AR-dependent pathway. This was confirmed by the AR mRNA expression restoration in cells exposed to BP3 + flutamide. In conclusion, exposure to relevant doses of BP3 is enough to perturb the migration capability and the expression of AR mRNA levels of the trophoblast cell line Swan 71. These effects were reversed in the presence of an AR inhibitor indicating that BP3 could act via AR-dependent pathway.

573. (338) **AIRBORNE PARTICULATE MATTER EXPOSURE IMPAIRS LUNG REDOX METABOLISM INVOLVED IN TISSUE DAMAGE REPAIR MECHANISMS**

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Is estimated that 91% of the world's population breathes polluted air leading to more than 7 million premature deaths per year. Airborne pollutants such as particulate matter (PM) are associated with enhanced health risk as they can trigger or aggravate several pulmonary diseases. Our aim was to assess if alterations in the lung oxidative metabolism initiated by toxicological mechanisms triggered after PM inhalation were associated with a delayed tissue injury repair. To characterize our model, BALB/c mice were exposed to filtered air (FA) or urban air (UA) from Buenos Aires City, in whole-body exposure chambers. Results showed that after 8 weeks of UA exposure, mice developed lung redox alterations and local inflammation without histological damage, therefore that was the time point selected to further evaluate the oxidative metabolism after a moderate lung injury induced by intratracheal instillation of 0.1 N hydrochloric acid (HCl). Pulmonary tissue was evaluated 5 days after HCl treatment. Tissue oxygen consumption was assessed as a whole lung metabolism marker, and the increase observed in mice breathing FA by HCl treatment, was not detected in HCl-mice exposed to UA ($p<0.05$). Interestingly, SOD activity showed the same trend ($p<0.05$), even though transcription factor Nrf2 expression was higher after the in-

jury in the UA group ($p<0.05$). While no edema was observed in any group, local inflammation measured as total cell count in bronchoalveolar lavage (BAL), was significantly increased only after UA exposure and HCl instillation ($p<0.05$) compared to control values. Hence, mice breathing UA might not be able to modulate the redox metabolism involved in lung damage repair mechanisms. Our results highlight the importance of tissue healing mechanisms evaluation as valuable knowledge for developing adequate therapeutic approaches aiming to ensure restoration of normal alveolar architecture required for proper lung function.

574. (352) **CHRONIC EXPOSURE TO URBAN AIR POLLUTION IN BUENOS AIRES CITY INDUCES OXIDATIVE STRESS AND INFLAMMATION IN MICE OLFACTORY BULB**

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Previous reports indicate that central nervous system (CNS) is a target of air pollution, causing tissue damage and functional alterations. Oxidative stress and neuroinflammation have been pointed out as possible mechanisms mediating these effects. The aim of this work was to study the chronic effects of urban air pollution on mice olfactory bulb (OB), focusing on oxidative stress and inflammation markers. Male 8-week-old BALB/c mice were exposed to filtered air (FA, control) or urban air (UA) inside whole-body chambers, located in a highly polluted area of Buenos Aires city, for up to 4 weeks. Reduced glutathione levels (GSH) were decreased by 75% after 4 w of exposure to UA ($p<0.05$). In accordance with these results, an increase in glutathione reductase activity was found at the same time point ($p<0.05$ vs. FA). Total superoxide dismutase (SOD) activity, including a differential analysis of its cytosolic and the mitochondrial isoforms, Cu/Zn-SOD and Mn-SOD respectively, were determined. Cu/Zn-SOD activity showed an initial decrease after 1 w of UA exposure compared to FA, and a subsequent increase of 50% at week 4 ($p<0.05$), while no changes were observed for Mn-SOD activity. Also, protein expression of NOX4 and NOX2 ($p47^{phox}$ subunit), both NOX isoforms commonly found increased in inflammatory processes, were augmented in UA group, after 1 and 4 w, respectively ($p<0.05$). Moreover, inducible nitric oxide synthase (iNOS) protein expression levels were found augmented for all exposure times evaluated, in UA compared to FA ($p<0.05$), suggesting an inflammatory process in OB, which is in accordance with previous results, where astrocyte activation was found in this tissue. Taken together, UA exposure showed an increase in oxidants production and inflammation markers in OB that might lead to tissue oxidative stress and an inflammatory response in this tissue. These data indicate that oxidative stress may play a key role in CNS damage mechanisms triggered by air pollution.

575. (353) **NEONATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE AND ITS EFFECTS ON THE OVIDUCT OF EWE LAMBS**

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Glyphosate-based herbicides (GBH) are the most widely used agrochemicals raising concern about its effects on animal and human health. We have previously reported that neonatal exposure of ewe lambs to a low dose of GBH induced permanent changes in the uterus and ovaries. Moreover, neonatal exposure of female rats to GBH decreased the fertility rate. Here, our aim was to assess the long-term effects of neonatal oral exposure to GBH in the oviduct of ewe lambs. To achieve this, ewe lambs were orally exposed from postnatal day (PND) 1 to PND14 to vehicle (n=6) or the reference dose of a GBH (1 mg glyphosate/Kg/day) (n=4). Since oviduct is target of estrogen, we also tested the effect of a gonadotrophic stimulus (pFSH, Follitropin 50 mg/day from PND41 to PND43) in GBH treated ewe lambs: vehicle+pFSH (n=6) and GBH+pFSH (n=4). At PND45, the isthmus and ampulla of oviducts were collected, paraffin embedded or stored at -80°C until mRNA extraction. The thickness of the myosalpinx was determined by digital analysis of picrosirius-hematoxylin-stained oviduct in transversal sections using FIJI software. The expression of Ki67 (as cell proliferation marker), and of α-alpha smooth muscle actin (α-SMA) were evaluated by immunohistochemistry. The gene expression of steroid receptors (ERα and PR) was evaluated by RT-PCR. The thickness of the myosalpinx and cell proliferation showed no differences between the experimental groups. Moreover, no alterations in the expression of α-SMA or steroid receptors mRNA was found. These results demonstrate that neonatal exposure to a low dose of GBH alone or with a gonadotrophic treatment does not alter the development of the oviduct myosalpinx, which allows us to assume that function of oviduct (gametes transport and fertilization of the oocyte) might not be affected following GBH exposure. Nevertheless, more studies are needed to conclude that the decrease in female fertility due to GBH treatment is not due to oviduct alterations.

576. (361) **EFFECT OF HEXACHLOROBENZENE (HCB) AND PROTEIN X OF THE HEPATITIS B VIRUS (HBV) ON LIVER CELL GROWTH DYSREGULATION**

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Chronic hepatitis B and exposure to persistent organic pollutants (POPs) can lead to cellular hepatocarcinoma (HCC), the most common liver tumor. HBV DNA encodes transactivator X (HBx). HCB is a COP promoter of hepatic preneoplastic foci. We have shown that HCB deregulates cell growth in rat liver and HepG2 cells, involving TGF-β1, a reversed effect with an Rβ1 inhibitor.

Objectives: To analyze in vitro 2 models of HCC generation -associated to HCB or to the expression of HBx- and in vivo the hepatic and angiogenic promoter HCB effect in Balb/c nude mice inoculated with HepG2. M&M: the HCB effect on PCNA (Western blot)/TGF-β1 (RT-PCR) was studied in vitro in: 1.1) Huh-7: HCB at various doses (0.005, 0.05, 0.5 and 5 μM) and times (15, 30, 60, 90 and 120 min); 1.2) Huh-7 transiently transfected with HBx; 2) HepG2.2.15 (with stable expression of HBV) and 3) EA-hy926 (endothelial). In 1.2, 2 and 3 5 μM HCB, 24h was used. Mice: HCB i.p. (0.3 and 3 mg/kg), and were inoculated with HepG2. Were evaluated: a) PCNA, b) TGF-β1, c) Nº. of tumor areas, d) histology (H&E) and e) Nº. of vessels/mm^2. Results: In Huh-7, TGF-β1 increased (20%, $p<0.05$; 69% and 78%, $p<0.01$, with 0.05, 0.5 and 5 μM HCB, respectively) and PCNA (45% and 60%, $p<0.01$, with 0.5 and 5 μM HCB, respectively). In Huh-7 / HBx, PCNA and TGF-β1 increased 66% and 71% ($p<0.01$). In Huh-7 / HBx and 5 μM HCB, PCNA increased 120% ($p<0.001$). In HepG2.2.15 PCNA was overexpressed 76% ($p<0.001$). In EA-hy926, PCNA (29%, $p<0.05$) and TGF-β1 (43%, $p<0.01$) increased. In mice, PCNA (39%; $p<0.01$), TGF-β1 (48%; $p<0.01$), preneoplastic areas (320%; $p<0.001$) and vascularization (35%; $p<0.01$).

Conclusions: HCB promotes preneoplastic cell proliferation and angiogenesis in nude mice inoculated with HepG2, while HCB and HBx induce in vitro cell proliferation associated with the increase of TGF-β1 in the examined lines, a proliferative effect increased to

that promoted by the HCB being able to participate in the induction of HCC.

577. (368) **EXPOSURE TO THE ENDOCRINE DISRUPTOR HEXACHLOROBENZENE PROMOTES BREAST TUMOR GROWTH AND METASTASIS IN A HER2-POSITIVE BREAST CANCER MODEL**

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The death rate from breast cancer has increased significantly in the last 25 years. Exposure to endocrine-disrupting chemicals, such as pesticides, has been postulated as a risk factor in this disease. We have previously demonstrated that the organochlorine Hexachlorobenzene (HCB) has stimulated breast tumor progression, favoring cell migration, invasion and angiogenesis. HCB is a weak ligand of the Aromatic Hydrocarbon Receptor (AhR), a transcription factor related to tumor and vascular development. Furthermore, we have reported that HCB acts as an endocrine disruptor in the mammary gland and uterus in different animal models. Estrogens play a fundamental role in the etiology of breast cancer, whose actions are mediated by their α and β isoform receptors. However, the role of ER-β is not entirely clear, with evidence of a reduction in proliferation and angiogenesis in ERα-positive breast cancer cell lines, while in triple-negative cells (RE-/RP-/HER2-) performs the opposite function. G-protein-coupled ER (GPR30) mediates the action of estrogens in breast tumors and cancer-associated fibroblasts, leading to tumor progression. In the present study, we have examined the action of HCB (0.03, 0.3 and 3 mg/kg body weight, bw) in a LM3 syngeneic breast cancer mouse model (ER-/PR-/HER2+). Our results indicated that HCB induces an increase in tumor weight and volume at 3 mg/kg bw ($p<0.05$) and promotes a rise in the metastasis number in the lung at 0.3 mg/kg bw ($p<0.001$). In the tumor tissue, HCB enhances the protein expression of AhR and Vascular Endothelial Growth Factor (VEGF) at all doses tested (Western Blot; $p<0.05$). In addition, the pesticide exposure decreases the protein levels of ER-β while increasing the GPR30 expression (Western blot; $p<0.05$). These results indicate that HCB exposure induces a dysregulation in the expression of the different types of estrogen receptors, collaborating with the promotion and tumor growth in the HER2-positive breast cancer model.

578. (462) **BIOSYNTHESIS OF IRON NANOPARTICLES BY MICROORGANISMS: CHARACTERIZATION AND EFFECTS ON HUMAN KERATINOCYTES**

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Metallic nanoparticles (NPs) can be obtained by biosynthesis using microorganisms, as bacteria. Iron is abundant in nature, an essential metal for humans, and a suitable candidate for NPs synthesis with possible microbicidal activity. In this work we biosynthesized iron NPs (FeNPs) and studied possible toxic effects in the human keratinocyte cell line HaCaT.

FeNPs were synthesized using Escherichia coli (ATCC 25922), characterized by UV-Vis spectroscopy and by transmission electron microscopy. HaCaT cells were incubated for 4 and 24 h

at different FeNPs concentrations (535; 214, 107, 53.5 and 26.75 μg/ml). Controls: culture medium,

metal precursor salt solution FeSO4 (0.1 and 0.25 mM), and bacterial growth control of biosynthesis

(CCB, dilutions 1/2, 1/5, 1/10, 1/20, 1/40). We investigated cell viability by MTT and neutral red test (NRT), reactive oxygen species (ROS) by DCF-DA and by NBT, superoxide dismutase (SOD) activity by the riboflavin-NBT method, and glutathione (GSH) content by the Ellman method.

FeNPs had a spherical shape with an average size of ≈ 20 nm. Cell viability decreased after 4

and 24 h incubation with FeNPs 535 μg/ml, and CCB 1/2. However, no changes in NRT uptake were observed at 4 and 24 h. The ROS levels were significantly increased after 4 h and 24 h

incubation with all the treatments assayed. In addition, O2- production significantly increased at 24

h of incubation with FeNPs 535 μg/ml, and CCB 1/2. SOD activity was increased at all treatments tested. Finally, the GSH content was not modified by any treatment at 24 h.

Altogether these results suggest that the highest FeNPs concentration significantly modifies the cell viability with increase in O2-, ROS, and SOD. In contrast, other stimuli were able to modify HaCaT oxidant/antioxidant cell balance, but not cell viability. Prolonged incubation studies are needed in order to determine if cell viability is altered at lower concentrations and to unravel the mechanisms underlying these alterations.

579. (471) **EFFECTS OF INTRAUTERINE EXPOSURE TO BENZOPHENONE-3 IN LACTATING MURINE MAMMARY GLAND**

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Benzophenone-3 (BP3), an ultraviolet radiation filter commonly used in sunscreens, has been shown to alter mammary gland (MG) development. Previously, we have demonstrated that exposure to BP3 alters beta-casein (CSN2) and alpha-lactalbumin (LALBA) milk protein expression during functional differentiation of the MG *in vitro*. Here, our aim was to evaluate whether intrauterine exposure to BP3 alters the lactating mammary gland in the F1 female offspring. Pregnant F0 C57BL/6 mice were dermally exposed to vehicle (sesame oil; Control), 0.15 (0.15BP3) or 50mg BP3/kg/day (50BP3) from gestation day 8.5 to 18. At 8 weeks-old, female offspring (F1) were bred and MG samples were obtained on lactation day 10. The protein expression of the myoepithelial cell biomarker alpha-smooth muscle actin, CSN2 and LALBA was assessed by immunohistochemistry. The perimeter and area of the alveoli and the myoepithelial linear density were measured to establish either the proportion of large/small alveoli per group or the maturation and differentiation of the myoepithelial cells. CSN2, LALBA and whey acidic protein (WAP) mRNA expression was evaluated by qRT-PCR. The alveolar perimeter in 0.15BP3 animals was the lowest among groups ($p<0.05$). Conversely, 50BP3 animals showed the highest alveolar area compared to 0.15BP3 ones ($p<0.05$). In addition, BP3 treatments had opposite effects on the proportion of large/small alveoli: whereas it was similar in control animals (47.3/52.7), it was

diminished in the 0.15BP3 group (31.7/68.2) and augmented in the 50BP3 group (60.7/39.2). Also, the myoepithelial linear density was lower in 0.15BP3 than in control animals ($p<0.05$). In contrast, the protein expression of CSN2 and LALBA, and the mRNA expression of CSN2, LALBA and WAP were similar between groups ($p>0.05$). In conclusion, the intrauterine exposure to BP3 altered the lactating MG, and these effects could be related to the alveolar secretory content and/or its contractile function.

580. (515) **CHRONIC ORAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE IMPAIRS FEMALE REPRODUCTIVE OUTCOMES IN WISTAR RATS**

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Glyphosate-based herbicides (GBHs) are the most globally used herbicide increasing the environmental exposure risk. The chronic effects on reproductive outcomes associated with long-term exposure to GBHs remain unexplored. In the present work, we investigated, in Wistar rats, the effects of chronic oral administration of a safe dose of a commercial GBH on: 1) body weight and food intake; 2) reproductive performance and feto-placental parameters.
Female rats were exposed to GBH through food, in a dose of 2 mg of glyphosate/kg bw/day, from postnatal day 21 (PND21) and during 11 weeks. Control group (CON) was provided with a laboratory pellet chow-based paste. Body weight and food intake were registered along the exposure. Females at the proestrus stage were caged with males with proven fertility. We evaluated the pregnancy rate by assessing the number of pregnant females/number of females housed with a male x 100. In addition, we determined the reproductive performance by quantifying the number of corpora lutea, the implantation sites (IS) and the resorption sites on gestational day 19 (GD19). The fetuses and the placentas pairs were removed and weighted. The placental index was calculated as follows: placental weight/fetal body weight. Last, fetal length and litter size were determined.
We detected an increase in body weight of the rats exposed to GBH 8 days after the beginning of treatment (PND30). However, not differences were found on food intake between CON and GBH-treated rats. Regarding reproductive performance, we detected a lower number of IS in GBH group compared to CON group. Fetal development was impaired, we detect a decrease in weight and length of the fetuses in GBH group. Neither placental weight nor placental index, were altered by GBH treatment. In conclusion, chronic exposure to GBH impaired female fertility and fetal development. We consider the importance of the evaluation of chronic oral exposure to GBH to showing additional evidence associated to GBH effects.

581. (526) **SURVEY OF X-RAY DAMAGE IN PERSONNEL OF PUBLIC HEALTH SERVICES IN ENTRE RIOS: DATA FOR LOW DOSE RADIATION.**

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Nowadays people are more often exposed to low dose(LD) ionizing radiation (IR). Knowledge on the health risks associated with exposures to ionizing radiation above 100 mGy is quite well established, while LD risks are inferred from higher level exposure by a linear non-threshold (LNT) model, the estimation of long-term chronic stochastic radiation health effects (such as cancer) by LNT extrapolations for LD-rates (<6 mGy/h) has been questioned. We provide evidence of acute DNA damage by the alkaline comet assay (ACA) in health institution personnel. We performed ACA and life style and health survey in 35 healthy volunteers with more than a year as staff of a X-ray service or working at neighboring laboratories from 2 public hospitals and primary health care services, we included personnel with no history of cancer disease or HPV infection. The assay evidenced global DNA strand (double and single strand DNA) damage as is indicated by % of DNA tails over 200 nucleus, we considered a high range damage cut off over 15% (n=30 DNA tails, p-Value <0.05).Our results showed that 30% of personnel presented DNA tails over the 15%, a significantly higher % (p-value <0.05) over the other 70% of the individuals with an average of 5% ±3 of DNA tails (n=10 DNA tails nucleus). The staff labor distribution inside the hospitals evidenced that higher % of DNA tail coincides with personnel of the X-ray imaging services and who were at immediate neighboring areas, the lifestyle and other bias variables taken into account by interviews did not allow evidencing other source of non-heritable genetic damage. Conclusions: Even if there may be significant DNA damage associated to X ray sources we cannot associate it to increase cancer risk or deviations from LNT theory. We conclude that the risks for any type of cancer could be lower than those predicted by a linear extrapolation, but they could also be higher. Until more results concerning the effects of chronic low-dose exposure are available, we pursue the determination of biomarkers for cancer risk in the same cohort and suggest a reasonable radiation protection approach as other measure such as reactive oxygen species (ROS) scavenger administration.

582. (530) **EFFECT OF HEXACHLOROBENZENE (HCB) AND PROTEIN X OF THE HEPATITIS B VIRUS (HBV) ON LIVER CELL GROWTH DYSREGULATION**

Lucia Coli[1], Verónica Mathet[2], Ezequiel Ridruejo[1], Emiliano Gentile[2], Melisa Kurtz[3], Noelia Miret[1], José Oubiña[2] y Laura Alvarez[1]
[1]Laboratorio de Efectos Biológicos de Contaminantes Ambientales (LEBCA), Departamento de Bioquímica Humana, Facultad de Medicina, UBA, [2] Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM), UBA-CONICET, [3]Laboratorio de Bio-Toxicología Ambiental, Escuela de Ciencia y Tecnología, Universidad Nacional de San Martín.

Chronic hepatitis B and exposure to persistent organic pollutants (POPs) can lead to cellular hepatocarcinoma (HCC), the most common liver tumor. HBV DNA encodes transactivator X (HBx). HCB is a COP promoter of hepatic preneoplastic foci. We have shown that HCB deregulates cell growth in rat liver and HepG2 cells, involving TGF-β1, a reversed effect with an Rβ1 inhibitor.
Objectives: To analyze in vitro 2 models of HCC generation -associated to HCB or to the expression of HBx- and in vivo the hepatic and angiogenic promoter HCB effect in Balb/c nude mice inoculated with HepG2. M&M: the HCB effect on PCNA (Western blot)/TGF-β1 (RT-PCR) was studied in vitro in: 1.1) Huh-7: HCB at various doses (0.005, 0.05, 0.5 and 5 μM) and times (15, 30, 60, 90 and 120 min); 1.2) Huh-7 transiently transfected with HBx; 2) HepG2.2.15 (with stable expression of HBV) and 3) EA-hy926 (endothelial). In 1.2, 2 and 3 5 μM HCB, 24h was used. Mice: HCB i.p. (0.3 and 3 mg/kg), and were inoculated with HepG2. Were evaluated: a) PCNA, b) TGF-β1, c) Nº. of tumor areas, d) histology (H&E) and e) Nº. of vessels/mm^2. Results: In Huh-7, TGF-β1 increased (20%, $p<0.05$; 69% and 78%, $p<0.01$, with 0.05, 0.5 and 5 μM HCB, respectively) and PCNA (45% and 60%, $p<0.01$, with 0.5 and 5 μM HCB, respectively). In Huh-7 / HBx, PCNA and TGF-β1 increased 66% and 71% ($p<0.01$). In Huh-7 / HBx and 5 μM HCB, PCNA increased 120% ($p<0.001$). In HepG2.2.15 PCNA was overexpressed 76% ($p<0.001$). In EA-hy926, PCNA (29%, $p<0.05$) and TGF-β1 (43%, $p<0.01$) increased. In mice, PCNA (39%; $p<0.01$), TGF-β1 (48%; $p<0.01$), preneoplastic areas (320%; $p<0.001$) and vascularization (35%; $p<0.01$).
Conclusions: HCB promotes preneoplastic cell proliferation and angiogenesis in nude mice inoculated with HepG2, while HCB and HBx induce in vitro cell proliferation associated with the increase of TGF-β1 in the examined lines, a proliferative effect increased to that promoted by the HCB being able to participate in the induction of HCC.

583. (531) **KINETICS OF THE LOCAL AND SYSTEMIC DAMAGE CAUSED BY A VENOMOUS INSECT FROM THE ARGENTINEAN ATLANTIC FOREST**

Karen González[1,2], Matías Sánchez[1], Silvana Maruñak[3], María Elisa Peichoto[2,4], Pamela Teibler[1]

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Envenomation by the South American *Lonomia* saturniid caterpillars, named lonomism, constitutes an emerging and somewhat neglected public health issue in Argentina, mainly in the province of Misiones covered by the Atlantic Forest. Our aim was to assess the 24-hour kinetics of the local and systemic damage induced by the Argentinean *Lonomia* sp. venom (50 μL of 1 mg/Kg body weight, i.d., dissolved in PBS prior to injection) in male CF1 mice (n = 3 for each time point). After 2, 5, 10 and 24 h of injection, animals were anesthetized with ketamine 100 mg/Kg and xilazine 10 mg/Kg (i.p.), and tissue fragments were removed for histological analysis. By direct macroscopic examination, we evidenced an erythematous reaction that developed rapidly at the injection site. The microscopical data showed detachment of the epidermis, and disorganization of the dermal structure; the latter is apparently caused by inflammatory infiltrate and edema, both of which decreased considerably by 24 h after venom injection. Systemically, we revealed liver damage characterized by infiltration of polymorphonuclear inflammatory cells, centrilobular vascular congestion, dilation of sinusoids, and necrosis of hepatocytes; the last injury was preferentially observed in zones 2 and 3 of the hepatic acinus. In lung sections, we observed loss of the alveolar wall structure associated with detachment of type II pneumocytes, and large areas of hemorrhage and atelectasis. The systemic pathological changes were more pronounced at later time points. These findings have implications within the clinical setting for lonomism in Argentina, and support the need for looking further into an effective therapy available for this envenomation in this country.

584. (544) **SOY PROTECTS AGAINST CADMIUM-INDUCED FIBROSIS IN LUNG. ROLE OF HEAT SHOCK PROTEINS (HSP)**

Gabriel Boldrini[1,2], Verónica Pérez Chaca[1], Glenda Martin Molinero[1,2], María Evelyn Córdoba[3], Gisela Pennacchio[3], Mariel Fanelli[3], Nidia Gómez[1,2], Silvina Álvarez[1,2].
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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on lung fibrosis and HSP expression under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots received soy (So) as protein sources. Within each group: 1 lot received regular water (control-Co) and the other 15ppm of Cd in the drinking water for 60days. Lungs were hydrolyzed and hydroxyproline (Hyp) was quantified by the chloramine-T method. The color was read in a spectrophotometer at 550 nm. Lung tissues were fixed, dehydrated, cleared in xylene, and embedded in paraffin. Sections of 5–6μm thickness were stained with Masson's trichrome solution for histology assessment. Immunohistochemistry was performed by using Hsp27 and Hsp70 antibodies. Hyp showed an increase in CasCd group vs CasCo ($p<0.005$); in SoCd, Hyp levels were higher than SoCo ($p<0.01$) but lesser compared with CasCd ($p<0.05$). Masson's Trichromic exposed that CasCo and SoCo have normal alveolar septa, without deposits of connective tissue. In CasCd, advanced pulmonary fibrosis and blue collagen deposits were evident throughout the lung. In SoCd, collagen deposition was observed around the intrapulmonary tree and lung vessels. Hsp27 showed an increase of its expression in Cd intoxicated groups, being only significant in CasCd vs CasCo ($p<0.05$). Hsp70 exhibited higher levels in SoCd vs SoCo, without differences among casein-fed groups. We conclude that Cd induce lung fibrosis, and soy might have a protective effect. It has been proven that Hsp27 promotes fibrosis in animal models. The higher expression of Hsp70, a demonstrated anti-fibrotic protein, induced by soy could protect against Cd-induced fibrosis.

585. (560) **LUNG AND KERATINO CYTE CELLS DIFFERENTIAL RESPONSE TO RURAL AIR PARTICULATE MATTER IN VITRO**

Orona Nadia S.[1], Astort Francisco[1], Fenoy Ignacio[1]; Palavecino Imanol[1]; Ramirez Haberkon Nancy B.[2], Mendez Mariano[2], Randi Andrea[3], Panebianco Juan E. [2], Goldman Alejandra[1].
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Atopic disorders susceptibility depends on interactions between genetic and environmental factors. The prevalence of these disorders has increased in the last 5 decades indicating that environmental factors might be responsible for this increase. Airborne particulate matter (PM) is one of environmental health problems affecting the world's population. Although several studies related the exposure to urban PM with the onset or exacerbation of allergies such as asthma and atopic dermatitis, there are few studies that link rural PM with the development of these allergic disorders. Rural PM comes mainly from agricultural activity, where agrochemical compounds are frequently used. Although agrochemical exposure has been related to allergic diseases, an approach considering the synergistic effects of PM and agrochemicals is necessary. Therefore, the aim of this study was to evaluate the effect of soil generated-PM_{10} from an intensive cultivation area where the use of agrochemicals prevails (Rural PM) from the central Pampas region on pulmonary (A549) and skin (HaCaT) cells. A549 or HaCaT cells were exposed to Rural PM (1-100μg/ml) or to glyphosate (0.75–7500pg/ml). After 24h, cell metabolic activity, lactate dehydrogenase (LDH) and cytokine release (IL-8, TSLP, IL-1β) was assessed. We found that A549 cells exposed to PM showed a marked increase in IL-8 production ($P<0.001$), without alterations in cell metabolic activity or LDH release. Regarding HaCaT cells, we found that exposure to PM or glyphosate provoked an alteration in metabolic activity at the highest dose employed ($P<0.01$), without changes in the release of LDH. Moreover, the levels of TSLP, IL-1β and IL-8 increased significantly in PM exposed-HaCaT cells. These results indicate a differential response of the pulmonary and dermal epithelia against rural PM. Interestingly, the increased levels of TSLP observed in keratinocytes may point to the skin as the initial sensitization site for different atopic disorders.

586. (561) **LUNG AND KERATINO CYTE CELLS DIFFERENTIAL RESPONSE *TO* RURAL AIR PARTICULATE MATTER *IN VITRO***

Orona Nadia S.[1], Astort Francisco[1], Fenoy Ignacio[1]; Palavecino Imanol[1]; Ramirez Haberkon Nancy B.[2], Mendez Mariano[2], Randi Andrea[3], Panebianco Juan E. [2], Goldman Alejandra[1].
[1]Instituto de Tecnologías Emergentes y Ciencias Aplicadas (ITECA), UNSAM, CONICET, LIVA_CESyMA_ECyT, Colectora Avenida General Paz 5445 (B1650WAB) San Martín, Buenos Aires, Argentina. [2]Instituto de Ciencias de la Tierra y Ambientales de La Pampa (INCITAP) - CONICET - UNLPam. Mendoza 109 (L6302EPA) Santa Rosa, La Pampa, Argentina. [3]Universidad de Buenos Aires, Facultad de Medicina, Depto. de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina.

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587. (564) **SUBCHRONIC INTOXICATION WITH CADMIUM INDUCES MORPHOLOGICAL ALTERATIONS IN RAT CEREBELLUM: THE PROTECTIVE ROLE OF A SOYBEAN DIET**

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Cadmium (Cd) is an environmental contaminant. The aim of this study was to characterize its toxicity in cerebellum and the potential reversal by a soybean-based diet. The glial fibrillary acid protein (GFAP), indicator for neurotoxic effects in mature brain, was evaluated. Besides, we performed a morphometric and stereological analysis. Female Wistar rats (n=12) were fed with casein (Cas) and soybean diets (So) as protein source for 60 days. Simultaneously, half of the animals were administered either 15 ppm of Cd in water or regular tap water as control. The cerebellums were fixed and processed for immunofluorescence. The samples were visualized by fluorescence microscopy and the positive fluorescence area for GFAP was quantified by IMAGE J Software. Morphometric analysis included quantifying the number of granule cell neurons (CGn) and Purkinje cells neurons (Pkn) in serial 20µm-thick sections stained with cresyl violet along the different lobules. We performed a three-dimensional volumetric reconstruction of the tissue through the use of the software Stereo Investigator. The thicknesses of the molecular and granular layers of the cerebellar cortex of lobules I-X were determined on digital images. We found a decrease in the number of CGn in the CasCd group vs. CasCo group ($p<0.05$) and SoCd group ($p<0.01$). On the contrary, the number of Pkn remained unchanged. In addition, there was a significant reduction in the internal granular layer of CasCd group vs. SoCd group, while no effect was observed in the thickness of the molecular layer. The cortical thicknesses of the different regions of each folium base from cerebellar lobules I-X did not show significant differences between the groups. Overall, these results unmask an irreversible toxic effect of low dose-sub chronic Cd intoxication on cerebellum and identify a protective role of a soy-based diet with potential as a therapeutic strategy for those individuals exposed to this dangerous environmental contaminant.

588. (578) **ONTOGENIC VARIATION ON CYTOTOXIC POTENTIAL OF *Bothrops alternatus* (SERPENTES, VIPERIDAE)**

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Argentina presents a vast richness of snakes species, but only eighteen are venomous (Viperidae and Elapidae families). Snake venom plays a critical role in food acquisition, digestion, and defense. Venoms are known to change throughout the life of some snake species, but little is known about the venom composition during juvenile stage. In the northeast (NEA), venom from *B. alternatus* species, are known to display ontogenetic variation in both, biochemical and biological activities. We reported a comparative cytotoxic analysis of venoms from juvenile and adult specimens of *B. alternatus* and correlate it with the histological evaluation of crucial venom injuries from juvenile. Cytotoxic activity was assessed with undifferentiated myoblasts as cultured murine muscle cells (C2C12). After 3 h of venom incubation, cell viability was quantified by crystal violet staining. To support the *in vitro* damage, myotoxicity in gastrocnemius muscle from mice was assayed (6.9 μg/g) and submitted to routine histological processing and H&E staining. The venoms of *B. alternatus* juvenile and adult exhibited concentration-dependent cytotoxic activities toward the C2C12 cell line tested. Both venoms exhibited differential cytotoxic effects, with venom from juvenile species being significantly more potent in inhibiting the growth of the myoblast cells (~55%) with venom concentrations of 5 μg/mL. The histological effects from juvenile muscle envenomation exhibited marked and persistent hemorrhage, edema forming, polymorphonuclear infiltrations and necrosis. We demonstrated that the ontogenetic shift in diet, from ectothermic prey in early life to endothermic prey in adulthood, and in animal size are associated with changes in the venom proteome in *B. alternatus* species. Altogether, these results point out the different toxic features between juvenile and adult snakes in ontogenetic development.

TRANSDUCCIÓN DE SEÑALES

589. (010) **THIOREDOXIN-LIKE PROTEINS AS MEDIATORS OF FORMALDEHYDE TOXICITY AND THEIR ROLE IN CANCER**

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Formaldehyde (FA) is a mutagen and a carcinogen also produced inside cells as a byproduct of essential biological processes such as epigenetic demethylations and the one carbon cycle. We have previously shown that FA toxicity can not only be inflicted by damaging the DNA but also through the accumulation of reactive oxygen species.

To further identify the mechanism by which FA is triggering cell death through oxidative stress, we searched for factors that are affected upon FA treatment. We found that the protein thioredoxin like-1 (TXNL1), which has been found associated with the 19S proteasome, is downregulated upon acute exposure to FA in HCT116 colorectal cancer cells. The protein level of TXNL1 drops 31.7±7.9% upon 5-h exposure to 200 uM FA, and 48.1±14.4% upon 5-h ex-

posure to 400 uM FA as determined by western blot. On the other hand, chronic exposure to lower levels of FA does not significantly affect TXNL1 expression. In order to understand how TXNL1 is downregulated, we performed a bioinformatic analysis of the promoter region, which revealed an Antioxidant Response Element (ARE) site, suggesting the oxidative stress master regulator NRF2 might be involved in the control of TXNL1. In contrast to the canonical genes controlled by NRF2, the ARE site detected in the promoter of *TXNL1* locates -7 pair bases upstream from TSS (Eukaryotic promoter database, p-value: 0.00001), suggesting that NRF2 might be directly repressing the expression of TXNL1 in response to FA.
We explored The Cancer Genome Atlas Program (TCGA) database and found that TXNL1 is significantly more expressed in many cancer biopsies when compared to normal tissues. Moreover, we explored the GEPIA database and detected that reduced TXNL1 expression correlates with poor prognosis in colorectal cancer (p-value=0.04). This result suggests that downregulation of TXNL1 might increase aggressiveness of tumor cells.

590. (041) **RSUME IMPAIRS SECURIN DEGRADATION BY THE PROTEASOME SYSTEM**

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Introduction: Securin is a sister chromatid separation inhibitor that in vertebrates is called Pituitary tumor transforming gene (PTTG). On the onset of anaphase, PTTG is ubiquitinated by the APC/C complex and degraded rapidly allowing progression of cell division. Increased expression of PTTG was associated with enhanced tumor cell growth, aneuploidy and malignant status. We reported a stabilization of PTTG protein by RWD-containing SUMOylation enhancer (RSUME) in pituitary tumor cells. As a consequence, RSUME enhances PTTG transcription factor and securin activities.
Objective: We explore the molecular mechanism of PTTG up-regulation in tumoral cells by RSUME focusing in its action on PTTG conjugation to ubiquitin (Ub).
Methods: We investigated PTTG degradation in COS-7 cells transfected with His-Ub vector, by Niquel affinity chromatography (Ni^{2+}-NTA) assay purifying ubiquitinated proteins and posterior western blot with specific PTTG-antibody. To inhibit the proteasome system, we used 10 or 20μM MG-132 for 6h; to accelerate PTTG degradation we used 5 or 10μM Reversine for 24-48h; while an expression vector of Gam1, a viral enzyme, was used to block SUMOylation.
Results: By Ni^{2+}NTA we observed that RSUME promotes a decrease on Ub conjugation to PTTG, stepping up its protein abundance. RSUME knockdown decreases PTTG protein but had no effect on PTTG stability when the proteasome degradation is inhibited by MG-132. Adding Gam1 we restored PTTG ubiquitination levels by preventing SUMOylation, even in the presence of RSUME. Reversine accelerates PTTG degradation in a dose and time-dependent manner and in combination with RSUME, Reversine could not accelerate PTTG degradation.
Conclusion
RSUME diminishes PTTG degradation by the proteasome through a mechanism that involves the sumoylation of PTTG, contributing by this mechanism to the abundance of PTTG in tumor cells.

591. (061) **ROLE OF GPR75 RECEPTOR IN 20-HYDROXYEICOSATETRANOIC ACID (20-HETE)- INDUCED SUBCELLULAR LOCALIZATION AND TRANSCRIPTIONAL ACTIVITY OF THE ANDROGEN RECEPTOR (AR) IN HUMAN ANDROGEN- SENSITIVE PROSTATE CANCER CELLS (LNCAP)**

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We have previously shown that 20-HETE promotes AR nuclear translocation and transcriptional activity (TrAc) in LNCaP cells. The aim of this study was to assess the role of the 20-HETE receptor, GPR75, in these actions.
Expression and i.c. localization of GPR75 or AR were studied by western blot (WB) or immunofluorescence (IF), and TrAc of the AR by gene reporter assay. RNA-seq gene expression data from Genomic database TCGA-Prostate Cancer were analyzed by UCSC Xena browser. Statistics: t-student test or one-way ANOVA/Tukey's.
Incubation of LNCaP cells with the GPR75 antagonist, 19-HEDE (5uM, 36h) decreased AR expression by 20% (WB), or by 43% (IF) ($p<0.05$ and $p<0.01$ vs control). Moreover, knockdown of GPR75 (LNCaP/siGPR75) reduced AR expression by 80% ($p\leq0.01$ vs LNCaP/siControl). 20-HETE increased AR TrAc by 559% in siControl cells ($p\leq0.0001$), and this response was abrogated in siGPR75 cells ($p<0.0001$ vs siControl+20-HETE).
Besides, 20-HETE increased by 41% the nuclear localization of AR (IF, $p<0.01$ vs control), which was abolished by 19-HEDE ($p<0.0001$ vs 20-HETE). 20-HETE- induced AR nuclear redistribution was confirmed by subcellular fractionation and WB (20-HETE $p<0.0001$ vs control; 19-HEDE+20- HETE $p<0.0001$ vs 20-HETE). Also, inhibitors of oncogenic pathways known to be activated by GPR75 (PKA, H-89 50uM; PKC, RO318220, 100nM; PI3K, LY294002, 25µM) reverted 20-HETE-
induced AR nuclear translocation ($p<0.0001$ vs 20-HETE for all).
Importantly, a high positive correlation between AR and GPR75 genes was found in human prostate cancer samples from TCGA, (Pearson's r= 0.66, p=2.59e-69; Spearman's r= 0.58, p=1.75e-50). Moreover, the correlation of the expression level of eight out of eleven AR- dependent genes with the expression of GPR75 was statistically significant according to Pearson and Spearman analysis.
Our data strongly suggest a role of the 20-HETE/GPR75 axis in the availability of transcriptionally active androgen receptors in prostate cancer cells.

592. (119) **ANTIDEPRESSANTS INHIBIT FKBP51 REGULATION OF THE GLUCOCORTICOID RECEPTOR ACTIVITY**

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Glucocorticoids are the main effectors of the hypothalamic-pituitary-adrenal axis and key mediators of the stress response. They exert their function by binding to the glucocorticoid receptor (GR), which in turn translocates to the nucleus and modulates gene transcription. FK506-binding protein 51 (FKBP51) is an Hsp90 co-chaperone that tightly regulates the activity of the GR. Abnormal FKBP51 function has been associated to stress-related disorders and to antidepressant (AD) treatment response. Our group has demonstrated the key role of FKBP51 SUMO conjugation in the regulation of Hsp90-mediated inhibitory effect on GR activity. Our data shows that ADs, particularly clomipramine, inhibit FKBP51 SUMOylation by inhibiting its interaction with the SUMO E3 ligase PIAS4. The inhibition of FKBP51 SUMOylation by these drugs diminishes the formation of the inhibitory complex between FKBP51, Hsp90 and GR. To confirm the importance of PIAS4 in this context we performed co-immunoprecipitation assays between FKBP51 and Hsp90 in the presence of clomipramine and increasing amounts of PIAS4. We observed that PIAS4 over-expression dose dependently dampens the inhibitory effect of clomipramine on FKBP51 and Hsp90 interaction (53,6±16,7%; $p<0,01$). We also silenced PIAS4 expression in astrocyte cell cultures and observed an inhibition between GR and FKBP51 interaction (46,2± 10,6%; $p<0,05$). To analyze if ADs could

restore GR activity we performed luciferase reporter gene assays. Using MMTV-luc and POMC-luc reporters we observed that ADs restore GR transcriptional activity in the presence of FKBP51 (MMTV-luc: 74,1±4,3%, p<0,01; POMC-luc: 35,9±6,4%, p<0,01). Moreover we observed that when we over-expressed PIAS4, clomipramine could no longer sustain its inhibitory effect on FKBP51 activity. Similar results were obtained by analyzing AD effect on GR transcription performed by real-time PCR assays. Our findings provide a novel molecular mechanism by which ADs can restore GR activity.

593. (120) **MECHANISM OF P53 INHIBITION BY MAGEC2 TUMOR PROTEIN**

Franco Andrés Pascucci 1, Maria Fátima Ladelfa 1 2, Micaela Escalada 1, Melisa Suberborde 1, Martín Monte 1 2.
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MageC2 is a tumor-specific protein that belongs to the big family of MAGE-I proteins. Under physiological conditions, MAGE-I genes are expressed in testis and silenced in somatic cells, but become aberrantly reactivated in cancer cells playing pro-oncogenic activities. Recent studies pointed to MageC2 as a potent inhibitor of the p53 tumor suppressor depending on TRIM28/KAP1 protein. The current model proposes that MageC2-KAP1 interaction could regulate p53 transactivation function and its degradation, however, this mechanism has not been fully evaluated and compared to other potential mechanisms. In this work we have expanded the knowledge in this regard, by evaluating Mdm2 (the main p53 repressor) as well as KAP1 and HDACs (MAGE-I interacting proteins able to regulate p53), as potential factors involved in p53 regulation by MageC2. p53 transactivation activity was assessed by a p53-Luciferase gene reporter assay. To get the independence of Mdm2, we performed the assay in double KO MEFs (mdm2 -/-, p53 -/-) exogenously expressing MageC2 and p53. To evaluate the relevance of KAP1, we used the MageC2-L152,153A mutant unable to bind KAP1 in wt-p53 U2OS cells. Both experiments showed a strong p53 inhibition by MageC2 and unaltered p53 levels, concluding Mdm2 and KAP1 are no required for p53 inhibition in our conditions. Instead, inhibition of HDAC activity by Trichostatin A impaired the ability of MageC2 in inhibiting p53 function. We also observed that MageC2 interacts with HDAC1 and HDAC3 in a co-immunoprecipitation assay. Functionally, the co-expression of MageC2 and HDAC1 and HDAC3 enhanced MageC2 inhibition of p53 activity. In conclusion, we showed for the first time that MageC2 binds HDACs to impair p53 transcription factor function. So, we suggest that MageC2 could inhibit p53 by an alternative mechanism to that reported depending on KAP1. Further studies should be performed to determine the substrate of HDACs recruited by MageC2 that causes p53 inhibition.

594. (126) **MECHANISM OF P53 INHIBITION BY MAGEC2 TUMOR PROTEIN**

Franco Andrés Pascucci 1, Maria Fátima Ladelfa 1 2, Micaela Escalada 1, Melisa Suberbordes 1, Martín Monte 1 2.
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. 2 Instituto IQUIBICEN, UBA-CONICET.

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595. (191) **CFTR CORRECTION WITH LUMACAFTOR/IVACAFTOR REDUCED THE MITOCHONDRIAL FRAGMENTATION IN CYSTIC FIBROSIS CULTURED EPITHELIAL CELLS**

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The impairment of the CFTR channel, a cAMP-activated chloride (Cl^-) channel responsible for cystic fibrosis (CF), has been associated with a variety of mitochondrial alterations. Recently, we reported an increase in mitochondrial fragmentation induced by the inhibition of the CFTR activity. The cAMP treatment to stimulate the CFTR activity worsen this phenotype in CF cells. Here, we analysed the mitochondrial network in CF cells treated with the CFTR modulators Lumacaftor (VX-809)/Ivacaftor (VX-770) VX-809 act as pharmacological chaperone, increasing the amount of CFTR at the cell membrane surface; while VX-770 increase the activation of the CFTR. Single, combined and sequential treatments with VX-809 (10 µM, CFTR corrector) and VX-770 (10, 1 and 0.1 µM, CFTR enhancer) were performed in IB3-1 cells (CF cells). Also, cAMP was tested as a CFTR stimulator to compare its effect with VX-770. Mitochondria were labelled with MitoTracker Orange, imaged by confocal microscopy, and analysed with Mitochondrial Network Analysis (MiNA) plugin for Fiji. A single treatment with VX-80948 h, but not with VX-770, reduced the mitochondrial fragmentation in IB3-1 cells, while a combined treatment with VX-809/ VX-770 for 48 h had no effect. However, the preincubation with VX-809 24 h previous to VX-770 for another 24 h reduced significantly the mitochondrial fragmentation. Similar results were observed using cAMP to stimulate the CFTR activity. These results suggest that CFTR potentiators could impair the mitochondrial dynamics when the CFTR is not expressed on the membrane surface. Further research is needed to identify the mechanisms involved in this regulation to improve the therapy by using these drugs.

596. (346) **CHARACTERIZATION OF MAGEB2 AS A RIBOSOME-ASSOCIATED PROTEIN**

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In addition to ribosomal proteins (RP), non-ribosomal proteins associates with ribosomes, contributing to ribosomal heterogeneity and ribosomal specialization, that is, different ribosomes selectively translating mRNAs.
Through Immunoprecipitation (IP) and mass spectrometry assays we identified a large number of RP associated with MageB2. Likewise, co-IP assays showed that MageB2 co-immunoprecipitates with RPS6 and LP2. MageB2 presents cytoplasmic, nuclear and nucleolar localization, with the latter being where ribosomal biogenesis begins. Besides, we observed a correlation between MageB2 expression and protein synthesis. Here, we hypothesized that MageB2 could be a ribosome-associated protein. In this sense, the purification of ribosomes by ultracentrifugation in sucrose cushions showed MageB2 co-sedimentation with cytoplasmic ribosomes. Then, we studied whether MageB2 could associate with the ribosome in the

nucleolus. As a first step, we ectopically express MageB2 constructions with different degree of nucleolar localization (MageB2-GFP, MageB21-118-GFP, MageB229-319-GFP and MageB2118-319-GFP) and we observed that those with marked nucleolar localization (MageB2-GFP and MageB21-118-GFP) co- sediment better with cytoplasmic ribosomes, suggesting that the nucleolar localization of MageB2 favors its association with the ribosome. In line with this, we observed that MageB2 co-sediments with ribosomal subunits in the nuclear fraction. Previously, we reported that ribosomal stress causes MageB2 relocalization from the nucleolus to the nucleo. Here we investigate the effect of ribosomal stress on MageB2 recruitment to ribosomes. So far, we have not observed differences after treatment with BMH-21 (RNA-PolI inhibitor).
Results presented here suggest that MageB2, a tumor-specific protein, associates with ribosomes under both normal and ribosomal stress conditions. It would be very attractive to know if this association promotes selective translation in cancer cells.

597. (364) **MECHANISM OF P53 INHIBITION BY MAGEC2 TUMOR PROTEIN**

Franco Andrés Pascucci[1], Maria Fátima Ladelfa[1 2], Micaela Escalada[1], Melisa Suberbordes[1], Martín Monte[1 2].
1 Laboratorio de Oncología Molecular, Dpto. Química Biológica, FCEN-UBA. 2 Instituto IQUIBICEN, UBA-CONICET.

MageC2 is a tumor-specific protein that belongs to the big family of MAGE-I proteins. Under physiological conditions, MAGE-I genes are expressed in testis and silenced in somatic cells, but become aberrantly reactivated in cancer cells playing pro-oncogenic activities. Recent studies pointed to MageC2 as a potent inhibitor of the p53 tumor suppressor depending on TRIM28/KAP1 protein. The current model proposes that MageC2-KAP1 interaction could regulate p53 transactivation function and its degradation, however, this mechanism has not been fully evaluated and compared to other potential mechanisms. In this work we have expanded the knowledge in this regard, by evaluating Mdm2 (the main p53 repressor) as well as KAP1 and HDACs (MAGE-I interacting proteins able to regulate p53), as potential factors involved in p53 regulation by MageC2. p53 transactivation activity was assessed by a p53-Luciferase gene reporter assay. To get the independence of Mdm2, we performed the assay in double KO MEFs (mdm2 -/-, p53 -/-) exogenously expressing MageC2 and p53. To evaluate the relevance of KAP1, we used the MageC2-L152,153A mutant unable to bind KAP1 in wt-p53 U2OS cells. Both experiments showed a strong p53 inhibition by MageC2 and unaltered p53 levels, concluding Mdm2 and KAP1 are not required for p53 inhibition in our conditions. Instead, inhibition of HDAC activity by Trichostatin A impaired the ability of MageC2 in inhibiting p53 function. We also observed that MageC2 interacts with HDAC1 and HDAC3 in a co-immunoprecipitation assay. Functionally, the co-expression of MageC2 and HDAC1 and HDAC3 enhanced MageC2 inhibition of p53 activity. In conclusion, we showed for the first time that MageC2 binds HDACs to impair p53 transcription factor function. So, we suggest that MageC2 could inhibit p53 by an alternative mechanism to that reported depending on KAP1. Further studies should be performed to determine the substrate of HDACs recruited by MageC2 that causes p53 inhibition.

598. (365) **BENZNIDAZOLE PROMOTES M1-TO-M2 POLARIZATION OF MURINE MACROPHAGES IN A CLASS I PI3K DEPENDENT MANNER**

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Benznidazole (Bz), the drug of choice in many countries for the treatment of Chagas disease, leads to parasite clearance in the early stages of infection and contributes to immunomodulation. In addition to its parasiticidal effect, Bz inhibits the NF-κB pathway through IL-10/STAT3/SOCS3. In this work we further studied the mechanisms involved in immunomodulation.
To dissociate the antiparasitic effect of Bzl from its anti-inflammatory properties, we used an *in vitro* model of RAW 264.7 macrophages stimulated with LPS. Treatment with Bz not only inhibited the release of NO but also increased the expression of Arginase I ($p<0.05$). Moreover, mRNA expression of M1 and M2 macrophage markers was evaluated. Bz treatment not only reversed the increase of pro-inflammatory cytokines but also increased the expression of M2-macrophage markers like Mannose Receptor, TGF-β, and VEGF-A ($p<0.05$). Moreover, Bz increased the expression of PPAR-γ and PPAR-α, known as key regulators of macrophage polarization ($p<0.05$).
PI3K directly regulates M1-to-M2 macrophage polarization. Thus, to assess the participation of PI3K in Bz effects, experiments were performed in presence of LY294002, an inhibitor of the PI3K activity. M2 polarization was precluded by LY294002, as shown by the inhibition of Bz-mediated increase of M2-markers expression ($p<0.05$). Furthermore, in presence of LY294002, Bz could neither increase SOCS3 expression nor prevent IκBα degradation ($p<0.05$).
Since the PI3Kδ isoform is expressed in immune system cells and regulates the inflammatory response, experiments were carried out in presence of CAL-101, a specific inhibitor of this subunit. Under this condition, Bz not only failed to inhibit the expression of pro-inflammatory cytokines but also could not increase M2 markers ($p<0.05$).
Taken together, these results demonstrate, for the first time, that Bz-mediated polarization to M2 macrophages involves the action of the p110δ catalytic subunit of PI3Kδ.

599. (479) **EFFECT OF HUMAN SERUM ON THE EXPRESSION LEVELS OF THE δ-AMINOLEVULINATE SYNTHETASE 1 PROTEIN IN C3A HEPATOMA CELLS**

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Studies carried out in our laboratory, in an *in vitro* model, demonstrated that the presence of fetal bovine serum (FBS) in the culture medium caused an increase in the amount of δ-aminolevulinate synthetase (ALA-S1) protein, the first and regulatory enzyme of the heme pathway, due to an increase in protein translation. It has also been described that the addition of insulin influences markedly the induction of ALA-S1 through the PI3K / Akt pathway. Therefore, in order to continue studying *in vitro* the effects obtained with FBS and to extend the research to human serum (Sh), it was decided to evaluate the effects of serum in cultures of the established line of human hepatoma C3A.The cells were grown in low glucose DMEM medium (5 mM) and without serum for 18 h. The cells were then treated with different concentrations of Sh (1, 2.5, and 10%), with 2% Sh inactivated (Shi) by heat or stripped with activated carbon (SHe) and 2% FBS. The addition of Sh caused a significant increase in ALA-S1 protein (100%) at all concentrations. Heat inactivation or activated carbon treatment had no effect on the induction of ALAS1 by Sh. However, the expression levels of ALA-S1 mRNA were significantly decreased (30%). This result agrees with the observed increase of the phosphorylation levels of Akt (Ser473), an enzyme that inactivates the transcription factor needed for the transcription of ALA-S1 mRNA. The phosphorylation levels of 4-EBP1 were 172% increased. When 4-EBP1 is phosphorylated, the protein synthesis is favored, thus the obtained result would suggest that the increase in ALA-S1 protein is due to a greater translation. These results here presented constitute a significant contribution to the understanding of the molecular mechanisms that lead to changes in the expression of ALA-S1. Moreover, the *in vitro* model used is adequate to further research the effect of Sh on ALA-S1.

600. (590) **MODULATION OF EXTRACELLULAR PH IN CYSTIC FIBROSIS CELLS BY EGFR**

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Cystic fibrosis (CF) is an inherited disease caused by mutations in the CFTR (CF transmembrane conductance regulator) gene. It is principally characterized by chronic infections and persistent inflammation in lungs. One of the hypotheses that tried to explain the increased susceptibility to lung infections is the reduction in the airway surface liquid (ASL) pH. The changes observed in the extracellular pH (pHe) could be a consequence of a reduced bicarbonate transport through CFTR and also an increase in lactic acid secretion. Considering that the EGFR pathway is affected in the CF phenotype, the aim of the present work was to determine if the EGFR pathway is involved in the extracellular pH regulation in CF cells. Two cellular models were used: IB3-1 cells (CF cells) and C38 cells (IB3-1 "corrected" cells). Together with the extracellular pH and lactic acid (lactate) secretion measurements, LDH expression and activity were performed by RT-PCR, Western Blot, flow cytometry, confocal microscopy and spectrophotometric techniques. The results reported a decrease in pH in the extracellular medium culture ($p<0.05$) in IB3-1 cells concomitantly with an increased in lactate secretion and both LDH expression and activity ($p<0.05$) compared with C38 cells. Studying the role of EGFR in pH regulation, we observed that inhibition of EGFR restores the pH in the extracellular medium ($p<0.05$), the lactate secretion ($p<0.05$) and the LDH expression and activity ($p<0.05$) in CF cells. In conclusion, our results confirmed that a constitutive EGFR activation is partially involved in the regulation of pHe, lactic acid secretion and LDH expression and activity by the CFTR channel. The low pH microenvironment observed in CF cells could interfere with immunological functions and promote the establishment of infections in patients with CF. Acknowledgements: ANPCYT, UCA and CONICET.