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SUMMARY AND CONCLUSION

EXCELLENCE OF CANCER RESEARCH IN BUENOS AIRES

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From Wednesday 6th to Friday 8th of June, 2007, the *Academia Nacional de Medicina de Buenos Aires* welcomed about 200 researchers working in the field of experimental and clinical cancer research to the vibrant and inspiring atmosphere of the capital of Argentina, Buenos Aires. Dr. **Christiane Dosne Pasqualini**, Conference and Scientific Chairman, together with the Co-chairmen Dr. **Kurt S. Zänker** (Germany) and Dr. **Enrico Mihich** (USA) designed a scientific program at the cutting edge of the state of the art of cancer research, including a most prestigious keynote lecture: "Mechanisms of Malignant Control", delivered by Dr. **Robert Weinberg** (Cambridge, USA).

This conference discussed in six sessions basic and translational science in cancer research and effective novel strategies in clinical oncology. The six sessions included the topics: Basic Mechanisms of Cancer Control, Therapeutic Exploitations of New Targets (A and B), Microenvironment and Cancer and Hematopoietic Diseases (A and B).

Robert Weinberg (Cambridge, USA) gave a magnificent overview on mechanisms of malignant progression. He addressed four major issues in respect to metastasis formation extending the Vogelstein model of carcinogenesis. i) Is the development of metastases a selective or adaptive process? ii) What are the rate limiting determinants of metastases? iii) Do cancer cells learn to colonize while in the primary tumor (cell-of-origin) or at the sites of dissemination? iv) Does a partial or complete Epithelial-Mesenchymal-Transition (EMT) program underlie the invasive/metastatic phenotype of all high-grade human tumors? Dr. Weinberg further pointed out that 5 pathways are mutated within the process of tumorigenesis: i) loss of growth control, ii) apoptosis genomic instability, iii) nutrient metabolism, iv) telomere maintenance and v) proliferation stimuli. Dr. Weinberg also included in his key lecture the molecular features and influences of transcription factors on EMT, such as: Twist, Goosecoid, Slug and FOXC2. In summary, to extend the Vogelstein model on carcinogenesis, additional mutations have to occur in order to form a metastatic phenotype.

William Kaelin (Boston, USA) focused on the functions of the VHL tumor suppressor protein involving oxygen sensing and cancer. The von Hippel-Lindau (VHL) tumor-suppressor gene is mutated or silenced in most clear cell renal carcinomas (RCCs). pVHL loss results in the stabilization of the heterodimeric transcription factor hypoxia-inducible factor (HIF) and enhanced transactiva-tion of HIF target genes which are, among others, addressing glucose uptake (GLUT 1), anaerobic glycolysis, angiogenesis and erythropoiesis. Downregulation of HIF is both necessary and sufficient for pVHL to suppress the growth of RCCs in preclinical models. A number of drugs have been developed that target HIF-responsive gene products, e.g. endothelial growth factor, platelet-derived growth factor. Inhibitors of the vascular-endothelial growth factor (VEGF) and vascular-endothelial growth factor receptors have shown to be effective against RCCs, emphasizing the biological significance of HIF-induced upregulation of VEGF in oncogenesis and tumor development.

Stephen Baylin (Baltimore, USA) presented epigenetic modifications as a major regulator of eukaryotic gene expression, and aberrant epigenetic silencing of gene expression which contributes to tumorigenesis. Both regional DNA methylation and global chromatin packaging are interrelated partners that function in concert to control gene transcription. Histone modifications include acetylation, phosphorylation, and methylation, resulting in a combination of histone marks known collectively as the histone code. The chromatin marks at a given promoter determine, in part, whether specific promoters are in an open/active conformation or closed/repressed conformation. Dimethyl-l-lysine4 histone H3 (H3K4me2) is a transcription-activating chromatin mark at gene promoters, and demethylation of this mark by the lysine-specific demethylase (LSD1) may broadly repress gene expression. Novel biguanide and bisguanidine polyamine analogues are potent inhibitors of LSD1 and candidates for reversing aberrant repression of gene transcription. Dr. Baylin pointed out that on an average/tumor cancer specific methylation is within a number of

300 compared to cancer specific mutated genes, which is on an average/tumor of 4. Drugs which kill tumor cells cause likely re-growth of tumors, whereas drugs killing tumor stem cells will likely erradicate the tumor. For the future it is important in cancer research to distinguish between epigenetic and genetic gatekeepers.

David Goodrich (Buffalo, USA) spoke about one major problem in gene expression: How is the protein production from genes of divergent structures coordinated? Thoc1 encodes an essential component of the mammalian TREX protein complex. TREX is an evolutionary conserved complex that couples elongating RNA polymerase II with RNA processing and nuclear RNA export factors to facilitate regulated gene expression. Thoc1 deficiency leads to defects in transcriptional elongation, defective nucleotide excision repair, increased sensitivity to DNA damage and is lethal with topoisomerase mutations. Thoc1 loss causes apoptosis but not cell cycle arrest. He asked the question: Are Thoc1 levels relevant to human cancers? Indeed, clinical studies for prostate, lung and pancreatic cancer indicate a dependency of a decreased survival rate from the expression level of Thoc1. Thoc1 compromised the replication potential of stem cells/progenitor cells and might be a selective therapeutical target for cancer stem cells.

Feyruz Rassool (Baltimore, USA) spoke about chromosomal instability as a characteristic feature of myeloid malignancies and the preleukemic syndrome that predisposes to these leukemias. Genetic instability in myeloid malignancies may be driven by a combination of ongoing constitutive DNA damage coupled with increased activity of the error-prone non homologous end-joining pathway which results in improper repair of double stranded break (DSB). Very likely increased reactive oxygen species (ROS) lead to increased complex DSB and to improper repair which contributes to carcinogenesis.

Gabriel Rabinovich (Buenos Aires, Argentina) introduced galectins as a taxonomically widespread family of glycan-binding proteins, defined by at least one conserved carbohydrate-recognition domain with a canonical amino acid sequence and affinity for ß-galactosides. The mammalian galectins play an important role in cell adhesion, spreading, migration of immune competent cells and tumor cells and the crossregulation of these functions. Galectin-1 is found at peripheral lymphoid organs and inflammatory sites. Treatment with galectin-1 in vitro differentially regulates constitutive and inducible FcgammaRI expression on human monocytes and FcgammaRI-dependent phagocytosis. In addition, galectin-1 inhibits IFN-gamma, induces MHC class II expression and MHC-II dependent antigen presentation in a dose dependent manner. Galectin-1 seems to play an up to know unre-vealed role in the control of monocyte/macrophage physiology with potential implications at the crossroad of innate and adaptive immunity.

Alberto Mantovani (Milan, Italy) *was unable to attend because of a last moment indisposition but he has agreed that the manuscript he had previously sent, be included* herein. He considers that macrophages are key orchestrators of chronic inflammation responding to genetic and functional programs. M1 macrophages which are classically activated by microbial products and interferon, are potent effector cells which kill microorganisms and tumors. In contrast, M2 macrophages tune inflammation and adaptive immunity, promote cell proliferation by producing growth factors and products of the arginase pathway, express scavenger receptors, promote angiogenesis, tissue remodelling and repair. M1 and M2 cells represent simplified extremes of a continuum of functional states. Available information suggests that tumor associated macrophages (TAM) are a prototypic M2 population. M2 polarization of phagocytes would orchestrate the smouldering chronic inflammation associated to established neoplasia. Recent studies have shown that rearrangement of the RET oncogene (RET/PTC) is a frequent, causative and sufficient event in papillary carcinoma of the thyroid and activates a pro-inflammatory genetic program in primary human thyrocytes. These molecules are also expressed *in vivo* and more so in metastatic tumors. These results highlight a direct connection between an early, causative and sufficient oncogene rearrangement along with an activation of a pro-inflammatory program in a human tumor.

Raffaela Giavazzi (Bergamo, Italy) addressed the question of how the vascular endothelium can be modulated through the use of pharmacologic agents. The ability to modulate vascular endothelium for the purpose of cancer treatment and prevention is in an early stage. She presented a concept for therapeutic efficacy by using SU6669, a small molecule receptor tyrosine kinase inhibitor together with paclitaxel in an assay either with human umbilical vein endothelial cells or human microvascular endothelial cells derived from lungs, endothelial cells, aortic smooth muscle cells, and human ovarian carcinoma cells sensitive (1A9) and resistant (1A9-PTX22) to paclitaxel. She could show that the activity of angiogenesis inhibitors on vascular cells could be potentiated when administered in combination with chemotherapeutic agents.

Thomas Kipps (San Diego, USA) embarked on the zeta-associated protein 70kDa (ZAP-70) which is expressed in patients with aggressive chronic lymphocytic leukemia (CCL). ZAP-70 positive CLL cells express activated heat-shock protein 90 (Hsp90) with high binding affinity for Hsp90 inhibitors, such as 17-allyl-aminode-methoxy-geldanamicyin (17-AAG), whereas normal lymphocytes or ZAP-70 CLL cells express non activated Hsp90. Activated Hsp90 binds and stabilizes ZAP-70, which behaves like an

Hsp90 client protein only in CLL cells. Treatment with Hsp90 inhibitors induces ZAP-70 degradation and apopotosis in CLL cells but not in T cells, and also impairs B-cell receptor signaling in leukemia cells, suggesting that Hsp90 inhibitors could be valuable therapeutically in patients with aggressive CLL.

Mario Mariano (Sao Paulo, Brazil) gave further insights into B-1 cell biology. At least three B cell subsets, B-1a, B-1b and B-2 are present circulating peripherally in the mouse. B-1 cells are a minor fraction of B cells in spleen and are absent in lymph nodes although they represent a B cell population in peritoneal and pleural cavities. Currently these cells are identified by a surface phenotypic repertoire: Mac-1(high) and B220(low). B-1 cells migrate from the peritoneal cavity of mice and home to distant site of inflammation to become macrophage-like cells. B-1 cells have macrophage activities *in vitro*. From *in-vivo* experiments, it can be deduced that B-1 cells can influence the effector functions of macrophages in vitro via IL-10 secretion.

Mirta Giordano (Buenos Aires, Argentina) is interested in the association between chronic lymphocytic leukemia (CLL) and autoimmune hemolytic anemia (AHA). In AHA, autoimmune antibodies are mainly directed to the erythrocyte proteins band 3 (B3). She explored the possibility that CLL cells could initiate an autoimmune response by presenting B3 to T cells in the context of appropriate costi-mulation. Her results show that CLL cells can specifically bind and capture B3 and, after being activated, they can induce T cell proliferation. She proposes that the leukemic clone initiates the autoaggressive response to erythocytes by acting as a population of B3 antigen presenting cells.

Slobodanka Klein (Buenos Aires, Argentina) spoke on the role of inflammation in cancer, a most important issue, which was recently summarized by *T.Dittmar, K.S. Zaenker and A. Schmidt (volume editors) in: Contributions to Microbiology (Editors: S. Schmidt, H. Herwald): Infection and Inflammation: Impacts on Oncogenesis, vol. 13: 2006, Basel Karger*. Klein mentioned that murine lung adenocarcinoma LP07 tumor bearers develop lung metastases, leukocytosis and cachexia during tumor growth. As polymorphonuclear neutrophils (PMN) was the main leukocyte population in peripheral blood, their activation was examined during tumor development. Depletion of PMN by specific antibody and treatment with antiinflammatory drugs (AINES) resulted in inhibition of tumor growth and proteolytic enzymes. LP07 tumor cells produce large amounts of IL6, IL1, GMCSF and PGE2, which were also inhibited by AINE treatment The mechanism by which AINE decreases tumor growth is not only through COX2 inhibition but mostly by reducing NFkB tranfer factor activity.

Marina Simian (Buenos Aires, Argentina) described the stromal-epithelial interactions in tamoxifen resistance. She introduced crosstalk between estrogen signaling and growth factors and pointed out that the estrogen receptor can be activated in the absence of estrogens. Within this line, she presented a new estrogen receptor positive, tamoxifen sensitive mouse mammary tumor model where the response to tamoxifen can be studied in the context of stromal-epithelial interactions. She further talked about cancer associated fibroblasts, and showed interesting findings that suggest a role for microenvironmental factors, such as extracellular matrix components, in the protection of tumor cells from tamoxifen induced cell death.

Claudia Lanari (Buenos Aires, Argentina) decribed, using a murine model in which tumors express high levels of estrogen and progesterone receptors, that carcinoma associated fibroblasts from hormone independent tumors show increased FGF-2 levels as compared with those from hormone dependent tumors suggesting that stroma is involved in the acquisition of hormone independence. She also showed that FGF-2 activated progesterone receptors in the epithelial cells and induced their cell proliferation. The finding that FGF-2 or progestin treatment increased nuclear co-localization of progesterone receptors and FGFR suggests new crosstalks between both pathways.

Hugues de Thé (Paris, France) spoke on the patho-genesis of acute promyelocytic leukemia (APL). The ability of retinoic acid (RA) and arsenic trioxide to directly target the oncogenic promyelocytic leukemia retinoic receptor A (PML-RARA) fusion protein made this disease the first model for oncogene targeted therapy. Dr. de Thé has shown that the K160 sumolation site in PML moiety of PML/RARA is redirected for efficient immortalization/differentiation arrest *ex-vivo*, implying that RARA homo-dimerization is insufficient to fully immortalize primary hematopoietic progenitor cells. Similarly, PML/RARA-K160R transgenic mice develop myeloproliferative syndromes, but never APL. Elevating the levels of cyclic AMP (cAMP) confers onto retinoid X receptor (RXR)-selective agonists and the ability to induce terminal granulocyte differentiation and apoptosis of all-trans retinoic acid resistant and insensitive APL cells and blasts of patients. APL patients´ blasts responded to rexinoid-cAMP combination treatment with induction of maturation and apoptosis, independent of karyotype, immunophenotype, and French-American-British classificaton status. Clonogenic assays revealed complete inhibition of blast clonogenicity in four out of five tested samples. All in all, his findings –from the bench to the clinic– suggest that the combination of rexinoids: i) (RA), ii)arsenic trioxide and iii) cAMP-elevating drugs, such as phosphodiesterase inhibitors might lead to a novel therapeutic option for APL patients.

Raquel Bengio (Buenos Aires, Argentina) introduced the ongoing multicentre study for detection of abl kinase domain mutations in Argentina. Imatinib inhibits the oncogenic fusion protein bcr/abl, a tyrosine kinase, involved in the pathogenesis of chronic myeloid leukemia (CML). Patients with advanced stages of CML are often resistent to Imatinib and overexpression of scr is also related to resistance. In order to understand the profile of resistance it is necessary to determine the bcr/abl transcripto-mes and the mutations, because point mutations are critical of Imatinib binding. Preliminary results indicate in 18 patients a mutation in the P-loop, in 1 patient a mutation in the A-loop and in 5 patients a doubling of the Ph-chromosome. Up to now 40% of the patients enrolled, who were resistant to Imatinib, showed genetic abnormalities.

Asher Chanan-Khan (Buffalo,USA) spoke about myeloma therapies: a peak in the future. The clinical characteristic of malignant myeloma (MM) are: incurability, cancer of the plasma cell, bone marrow plasmacytosis, osteolytic bone lesions, renal dysfunction and failure, aberrant immunoglobulin production, amyloid deposits and an immune compromised state which challenges infections and often leads to death. The intramedullary MM cells are stroma cell dependent for their propagation, the extramedullary MM cells are L-6 dependent. Thalidomide and the new derivative Lenalidomide show strong activity against the disease. Future molecular and therapeutic targets might be: anti-CD56, Hsp90, MAPK inhibitors as well as Telomerase and Proteasome inhibitors.

Frans C.S. Ramaekers (Maastricht, The Netherlands) gave new insights into the molecular and cellular mechanisms by which mistletoe extracts (*Viscum album L.*) act on the cytotoxic and apoptotic machinery of cancer cells. *Viscum album L.* extracts (Iscador®) causes early cell cycle arrest followed by apoptosis in a dose dependent manner. Apoptosis is induced by the activation of the mitochondrial but not the death receptor dependent pathway; also certain preparations of Iscador®M (malus) might also activate the death receptor dependent pathway.

Srini V. Kaveri (Paris, France) has shown several lines of evidence for the mode of action of *Viscum album L.* extracts (Iscador®). As in the case of other members of the RIP II family, *Viscum album* (VA) extracts exert cytotoxicity towards cell lines from both human and rodent origins, although to a lesser extent compared to ricin. VA extracts and purified mistletoe lectins (ML I, II, III) also induce activation of transcription and secretion of pro-inflammatory cytokines in peripheral blood mononuclear cells and endothelial cells. In the C57Bl6-mouse B16-melanoma model, Iscador® inhibits *in vivo* angiogenesis, suppressing tumor growth by decreasing the number of blood vessels oriented towards the tumor mass.

Thomas Dittmar (Witten, Germany) spoke about the influence of Iscador® preparations if applied in-vitro together with growth factors. Iscador®P, which is used in B-Non-Hodgkin´s Lymphoma treatment, efficiently counteracts the IL-6 induced prolifertion of WSU-NHL and Sc-1 B-NHL cell lines. Thereby, clinical relevant doses of Iscador®P plus IL-6 induces apoptosis in WSU-NHL cells, whereas the IL-6 induced proliferation of Sc-1 B-NHL cells is likely inhibited by a Iscador®P-mediated interference with the cell cycle machinery. Microarrays revealed that Iscador®P led to a down-regulation of several G1-, S-, and M-cell cycle genes in Iscador®P plus IL-6 co-treated Sc-1 cells. In accordance with the above mentioned data, clinical relevant doses of Iscador®M, which is used in breast cancer treatment, inhibits the EGF induced proliferation of the MDA-MB-468-HER2 breast cancer cell line and the M13HS-2 breast cancer cell line. The latter cell line was derived from a spontaneous fusion between the breast stem cell line M13SV1 and the breast cancer cell line HS578T. Cell fusion increases the genetic instability of tumor cells and is generally accepted to be a crucial process for tumor tissue heterogeneity. Additionally, the fusion between (tissue) stem cells and tumor cells has been postulated as one mechanism explaining the origin of cancer stem cells. If breast stem/breast cancer cell hybrids truly carry cancer stem cell characteristics, here, it is shown for the first time that *Viscum album* might be useful not only for the treatment of parenchymal tumor cells but also for cancer stem cells.

Isabelle Wagschal (Wädenswill, Switzerland) spoke about gene expression profiles of different breast cancer cells and compared the profiles with the *in vitro* responsiveness to *Viscum album L.* extracts. Cytotoxicity assays *in vitro* exhibited that the breast cancer cell lines Kpl-1, MCF-7 and Mfm-223 responded differently to the cytotoxic activity of mistletoe extracts (Iscador® Quercus, Abies, Malus and Pinus). The gene expression profiles of these mammary cancer cell lines were determined from Iscador® treated and untreated cells. The results of the transcriptome analysis indicate that different Iscador® preparations influence genes regulating the immune competence, the stress response, the apoptosis and cell-cell adhesion. Iscador®Qu(Quercus) and Iscador®M(Apple Tree) have a greater influence on the immune defense and stress response genes whereas Iscador®A (Abies) tends to affect the cell-cell adhesion pathway and genes of the cytoskeleton. The gene profile/protein expression for relevant genes/proteins was futher substantiated by PCR-analysis and Western blotting in order to reduce the biological informations from the Human Whole Genome Microarray Chips (41000 genes) to clinical feasible/rel-

evant informations and eventually personalize application of one mistletoe subspecies preparation derived from one of the different host trees. Furthermore, genetic analytic approach allows good manufacturing and pharmaceutical practice, because each lot can be analysed for its functional activity besides its pharmacological quantification.

J. Kuehn (Arlesheim, Switzerland) introduced retrospectively analysed clinical data in Non-Hodgkin Lymphoma, obtained from patients treated with Iscador®P (Pini) alone or in combination with (prior) chemotherapy regimens. Basically, he presented retrospective data obtained between May 1999 and April 2007 from patients suffering from follicular (61 pts) and non-follicular (130 pts) B-cell lymphoma. The Lukas Clinic, Arlesheim, Switzerland, recruits also patients for oncological therapy options who are borderliners between conventional therapy regimens and complementary and alternative treatment strategies, for different reasons, e.g. non-compliant with chemo- and/or radiation therapy protocols, beliefs and worldview or who wish to combine both treatment options with the hope to increase quality of life during and after treatment regimens exhibiting high scores of side effects. The clinical results clearly indicate that *Viscum album L*. therapy can be safely administered (safety proof) and does not stimulate IL-6 production of the tumor cells, although, it is well known that Il-6 is a mandatory growth factor for B-cell lymphoma. The *Viscum album L*. treatment can lead in follicular B-cell lymphoma to partial remissions and even to complete and long lasting remissions as demonstrated in a best case series. *Viscum album L*. can be given in an out-patient modus over a long time and can be combined with conventional therapies, thereby, maintaining a reasonable quality of life status, which is most important for elderly patients suffering from comorbidity. An extended retrospective study in these tumor entities with increased number of patients is underway in order to design, on the basis of those results, a prospective randomized clinical trial with the aim of validating specific indications of the *Viscum album L.* therapy in oncology or to close the books.

Kurt S. Zänker (Witten, Germany) summarized the meeting by recalling in essence what the distinguished speakers had presented. What has been neglected, and even dismissed for decades, is the concept of study designs for elderly patients suffering not only from cancer but who are also burdened with co-morbidity. This is now an up-coming issue, because these patients need to have a more personalized treatment, not only with cytotoxic drugs but also with drugs modifying the tumor-stroma cell interactions together with a fine tuning of their innate and adaptive immune competence. For these patients the indication to use *Viscum album L.* preparations as one therapeutical option, either as monotherapy or in combination with any other conventional therapy, might lead to a renaissance; almost 40% of cancer patients in Europe (e.g. Germany, Switzerland) are already taking *Viscum album L.* preparations during the course of their disease. However, before such a concept is generally accepted by the scientific community, more fundamental proofs of clinical activity for *Viscum album L*. preparations have to be presented. Hereby, molecular results with *Viscum album L*. preparations should provide more insight - as a proof of principles - into eventually novel aspects of tumor-immune and tumor-stromal interactions which do occur in a complex interplay involving matrix proteins, cytokines, chemokines and neurokines. Dr. Gabriel Rabinovich addressed very elegantly the multi-facet protein-glycan interactions in immune regulation and tumor-immune escape, focusing on Galectin-1. *Viscum album* L. preparations, are, among other biological active compounds, a source for “biologically embedded lectins”, e.g. not separated or purified from their “natural molecular environment”. Interestingly, it has been shown that Iscador®M (special) and purified mistletoe lectin-1 do show similar cytotoxic activity in-vitro in a panel of tumor cell lines, derived from gastric, lung, mammary, prostate, renal and uterus cancer (Kelter G, Fiebig H.H. (2006), *Arzneim.-Forsch./Drug Res*. 56, 435-440,). Furthermore, it is highly likely that *Viscum album* L. derived lectins and/or carbohydrate compounds in a biochemically well defined extract could modulate the immune competence by differential activation of dendritic cells via Toll-like (TLRs) and/or C-type lectin (CLRs) receptors. *Viscum album* L. compounds (extract) might mimic conserved pathogen-associated molecule patterns, like lipopolysaccharides and peptidoglycans. Dendritic cells are equipped with a certain repertoire of TLRs and CLRs allowing for specialized responses to them. These unique responses, tailored by each dendritic cell subset, serve to enhance innate immune responses at the site of inflammation and guide adaptive immunity. Therefore, well characterized (for molecular pathogen recognition patterns) *Viscum album* extracts are candidates for bridging the innate and the adaptive immune competence by fine tuning the appropriate immune response.

Kurt S. Zänker ended his Summary by thanking the audience for attending this meeting and he expressed his thankfulness to Dres Christiane Dosne Pasqualini and Enrico Mihich for their scientific and social impact to make this meeting successful and memorable.

Conflict of interest statement: None declared