ISSN 0025-7680

International Symposium
NEW DIRECTIONS IN CANCER MANAGEMENT
Academia Nacional de Medicina
Buenos Aires, 7-8 August 2000

MEDICINA (Buenos Aires) 2000; 60 (Supl. II): 41-47

INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS A NOVEL APPROACH FOR CANCER THERAPY

JEANETTE M. WOOD

Oncology Research, Novartis Pharma AG, Basel, Switzerland

Abstract Of the numerous growth factors and cytokines that have been shown to have angiogenic effects, vascular endothelial growth factor (VEGF), also known as vascular permaebility factor (VPF), appears to be a key factor in pathological situations which involve neovascularization as well as enhanced vascular permeability. Our aim was to design a low molecular weight synthetic molecule that potently and selectively blocks the VEGF/VEGF receptor system after oral administration, suitable for the chronic therapy of VEGF-dependent pathological neovascularization. PTK787 / ZK 222584 is a potent inhibitor of VEGF receptor tyrosine kinases, active in the submicromolar range. It also inhibits other class III kinases, like the PDGFR-ß tyrosine kinase, c-Kit and c-Fms, but at higher concentrations. It is not active against kinases from other receptor families such as EGFR, FGFR-1, c-Met and Tie-2 or intracellular kinases like c-Src, c-Abl, PKC-alpha. PTK787 / ZK 222584 inhibits VEGF-induced autophosphorylation of KDR, and endothelial cell proliferation, migration and survival in the nanomolar range in cell based assays. In concentrations up to 1 µM, PTK787 / ZK 222584 does not have any cytotoxic or anti-proliferative effect on cells that do not express VEGF receptors. After oral dosing (50 mg/kg) to mice, plasma concentrations of PTK787 / ZK 222584 remain above 1 µM for more than 8 h. PTK787 / ZK 222584 induces dose-dependent inhibition of VEGF- and PDGF-induced angiogenesis in a growth factor implant model, as well as a tumor cell-driven angiogenesis model after once daily oral dosing (25-100 mg/kg). In the same dose range, it also inhibits the growth of several human carcinomas, grown subcutaneously in nude mice, as well as a murine renal carcinoma and its metastases in syngeneic, orthotopic models. Histological examination of tumors reveals inhibition of microvessel formation in the interior of the tumor. PTK787 / ZK 222584 also significantly inhibits ascites formation induced by a human ovarian carcinoma grown in the peritoneum of nude mice as well as pleural effusion induced by a human lung adenocarcinoma in nude mice. PTK787 / ZK 222584 is very well tolerated and does not impair wound healing. It also does not have any significant effects on circulating blood cells or bone marrow leukocytes as a single agent, or impair hematopoetic recovery following concomitant cytotoxic anti-cancer agent challenge. These studies indicate that compounds that inhibit the effects of VEGF, such as PTK787 / ZK 222584, have the potential to provide a novel, effective and well-tolerated therapy for the treatment of solid tumors. These agents may also provide a new therapeutic approach for the treatment of other diseases where angiogenesis plays an important role.

Key words: cancer therapy, VEGF, VPF

Resumen ***Inhibición del factor de crecimiento endotelial vascular (VEGH) como nueva propuesta terapéutica en cáncer.*** De los numerosos factores de crecimiento y citoquinas que han demostrado tener efecto angiogénico, el factor de crecimiento del endotelio vascular (VEGF) también conocido como factor de permeabilidad vascular (VPF) surge como un punto clave en situaciones patológicas que implican neovascularización así como aumento de la permeabilidad vascular. Nuestro propósito fue diseñar una molécula sintética de bajo peso molecular que bloquee en forma potente y selectiva el sistema VEGF/VEGF luego de administración oral apropiada para la terapia crónica de la neovascularización patológica VEGF dependiente. PTK787/ZK222584 es un potente inhibidor del receptor de tirosin quinasa del VEGF activo en índice submicromolar. Inhibe además quinasas de clase III como el PDGFR-b tirosín quinasa, c-kit y cFms pero a más altas concentraciones. No es activo contra quinasas de otras familias de receptores como el EGFR, FGFR-1 c-MET y Tie-2 o quinasas intracelulares como c-Src, c-Abl, Pkz-alfa. El PTK787/ZK222584 inhibe la autofosforilación del KDR inducida por el VEGF y la proliferación endotelial, migración y sobrevida en índice nanomolar en ensayos celulares. En concentraciones de hasta 1mM, el PTK787/ZK222584 no tiene ningún efecto citotóxico ni antiproliferativo sobre células que no expresan receptores VEGF. Luego de administración oral (50mg/kgr) en ratones las concentraciones plasmáticas de PTK787/ZK222584 permanecen por encima de1mM por más de 8hs. El PTK787/ZK222584 inhibe en forma dosis dependiente la angiogénesis inducida por el VEGF y el PDGF en modelos experimentales así como en modelos de angiogénesis dirigidos por células tumorales después de la dosificación oral diaria (25-100mg/kgr). En el mismo

Postal address: Dr. Jeanette M. Wood, Oncology Research, Novartis Pharma AG, Basel, CH 4002, Switzerland
Fax: 0041 61 696 62 42 e-mail: jeanette.wood@pharma.novartis.com

espectro de dosis también inhibe el crecimiento de numerosos carcinomas humanos implantados en forma subcutánea en ratones *nude* así como carcinoma renal murino y sus metástasis en modelos singeneicos ortotópicos. El exámen histológico de los tumores revela inhibición de la formación microvascular en el interior del tumor. El PTK787/ZK222584 también inhibe significativamente la formación de ascitis inducida por el crecimiento del carcinoma ovárico humano en el peritoneo del ratón *nude* así como la efusión pleural inducida por un adenocarcinoma humano de pulmón en el ratón *nude.* PTK7877ZK222584 es bien tolerado y no empeora la curación de la lesión. Inclusive no tiene efecto significativo sobre células sanguíneas circulantes o leucocitos de médula ósea como agente único ni perjudica el rescate o recuperación hemopoyética concomitante al uso de agentes citotóxicos antitumorales. Estos estudios indican que los compuestos que inhiben los efectos del VEGF como el PTK787/ZK222584 tienen el potencial de proveer una nueva, efectiva y bien tolerada terapia para el tratamiento de los tumores sólidos. Estos agentes también proveen una nueva propuesta terapéutica para el tratamiento de otras enfermedades dónde la angiogénesis juega un rol importante.

Angiogenesis, the formation of new vessels from an existing vascular network, is an essential event in a variety of physiological and pathological processes. Under physiological conditions, angiogenesis is restricted to processes such as embryogenesis, ovulation and wound healing. Angiogenesis also occurs in pathological processes such as inflammation[1-4], rheumatoid arthritis[5-8], ocular neovascularization[9-13], psoriasis[14-16] and tumor growth and the formation of metastases[17-18].

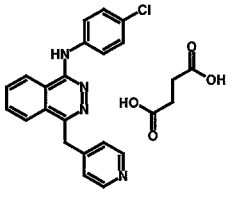
Of the numerous growth factors and cytokines that have been shown to have angiogenic effects, vascular endothelial growth factor (VEGF), also known as vascular permaebility factor (VPF), appears to be a key factor in pathological situations which involve neovascularization as well as enhanced vascular permeability[19, 20]. The VEGF receptors, Flt-1 (Fms-like tyrosine kinase, VEGF-R1)[21] and KDR (kinase insert domain-containing receptor, VEGF-R2)[22], are almost exclusively located on endothelial cells[23]. Expression of these receptors is low in normal tissues and only upregulated during the development of these pathological states when neovascularization occurs[24, 25]. Both receptors have seven immunoglobulin-like domains in their extracellular region, a single transmembrane-spanning domain, and an intracellular split tyrosine kinase domain and belong to the same family of receptors as PDGFR, c-Kit, c-Fms, Flt-3 and Flt-4. Flt-1 binds VEGF-A and -B[26, 27] and the related placenta growth factor[28, 29], whereas KDR binds VEGF-C and -D (30,31) in addition to VEGF-A, VEGF-C and -D are both ligands and activators of Flt-4 (VEGF-R3) which is expressed on endothelial cells of lymphatic vessels[32-34]. Although activation of Flt-1 was shown to mediate biological responses, such as endothelial and monocyte cell migration and tissue factor induction[27, 28, 35-38], in cells expressing only Flt-1 or in Flt-1 deficient cells transfected with Flt-1 cDNA, stimulation with VEGF induces only weak receptor phosphorylation and no significant mitogenic response[37, 38]. In contrast to Flt-1, KDR is strongly autophosphorylated upon VEGF-stimulation and mediates a mitogenic response[39, 40].

Gene knock-out experiments for VEGF[41, 42] as well as its receptors[43-46] have highlighted the pivotal role of VEGF/VEGF-receptor system in the development of the embryonic vascular system. Various different approaches have been used to interfere with the VEGF/VEGF-receptor system in adult animals and thereby determine the role of VEGF receptors in the various pathological states. These approaches include VEGF-neutralizing antibodies[47-49], antibodies against the VEGF receptors[50, 51] recombinant soluble VEGF receptor proteins[52, 53], a tetracycline-regulated VEGF expression system[54, 55], dominant negative mutants of the VEGF receptors[56] and VEGF receptor tyrosine kinase inhibitors[57-59]. Results from these approaches suggest that the VEGF/VEGF-receptor system is a novel and attractive therapeutic target for suppression of pathological neovascularization and, in particular, for inhibiting tumor growth (Figure 1).

Our aim was to design low molecular weight synthetic molecules that potently and selectively block the VEGF/VEGF receptor system after oral administration, suitable for the chronic therapy of VEGF-dependent pathological neovascularization. A series of 1-anilino-(4-pyridylmethyl)-phthalazines have been synthesized that are inhibitors of the VEGF-receptor tyrosine kinase and have favourable pharmacokinetic properties following oral administration to animals[60]. One of these compounds, PTK787 / ZK 222584 (1-[4-Chloroanilino]-4-[4-pyridylmethyl] phthalazine succinate) (Figure 2)[60] has been selected for further characterization[61] and is currently in phase I clinical trials in patients with advanced cancer[62]. PTK787 / ZK 222584 was discovered and profiled as part of a collaboration between the Department of Oncology Research, Novartis Pharmaceuticals, Basle, Switzerland, the Oncology Research Laboratories of Schering AG, D-13342 Berlin, Germany and the Institute of Molecular Medicine, Tumor Biology Center, D-79106 Freiburg, Germany.

Using enzymatic assays with recombinant GST-fused kinase domains and synthetic substrates, PTK787 / ZK 222584 has been shown to be a potent inhibitor of VEGF receptor tyrosine kinases, active in the submicromolar range (Figure 2)[60, 61]. PTK787 / ZK 222584 also inhibits other class III kinases, like the PDGFR-ß tyrosine kinase, c-Kit and c-Fms, but at higher concentrations[61]. However, it is not active against kinases from other receptor families such as EGFR, FGFR-1, c-Met and Tie-2 or intracellular kinases like c-Src, c-Abl, PKC-alpha. Since a kinase inhibitor must enter cells in order to inhibit the ki-

Fig. 1.- Concept of the role of vascular endothelial growth factor (VEGF) in tumor vascularization, tumor growth and the formation of metastases. As clusters of transformed cells proliferate, the environment of the cells becomes hypoxic and this is a potent stimulus for the formation and secretion of angiogenic growth factors, such as VEGF. VEGF induces existing blood vessels near the transformed cells to form capillary sprouts that grow towards the cells and then form vessels that will supply the cells with oxygen and nutrients, thereby allowing a tumor to form and begin to grow exponentially. An established vascular network also allows tumor cells to travel away from the primary site and establish metastases at other sites in the body. Inhibition of the actions of VEGF, by inhibition of the tyrosine kinase on the intracellular domain of the VEGF receptor (which is located selectively on activated endothelial cells), will prevent VEGF-induced receptor phosphorylation and therefore block the signalling pathway. This should prevent VEGF-mediated effects on endothelial cells and prevent tumor vascularization.



PTK787 / ZK222584

	Kinase Inhibition IC50 [nM]
KDR-R	37 ±2
Flt-1	77 ±12
Flk	270 ±40
PDGF-Rβ	580 ±80
c-Kit	730 ±50
Tek	$14 \cdot 10^3$
FGF-R1	$>10 \cdot 10^3$
c-Met	$>8 \cdot 10^3$
EGF-R	$>10 \cdot 10^3$
v-abl	$>10 \cdot 10^3$
c-src	$>10 \cdot 10^3$
PKC-c	$>10 \cdot 10^3$

Fig. 2.- Structure and kinase inhibitory profile of PTK787 / ZK222584 (1-[4-Chloroanilino]-4-[4-pyridylmethyl] phthalazine succinate), a potent and selective inhibitor of VEGF receptor tyrosine kinases. The kinase assays were performed, using the recombinant GST-fused kinase domains of the receptor. ^{33}P-ATP was used as the phosphate donor and poly-(Glu:Tyr 4:1) peptide was used as the acceptor. Values are mean ± SEM.

nase domain of the receptor, the effects of PTK787 / ZK 222584 were tested in cell-based receptor autophosphorylation assays using human umbilical vein endothelial cells (HUVEC) that naturally express KDR, or KDR-transfected CHO cells. PTK787 / ZK 222584 inhibits VEGF-induced autophosphorylation of KDR in both cell systems. Potential anti-proliferative effects of PTK787 / ZK 222584 unrelated to VEGF inhibition, were tested using cells that do not express the VEGF receptors. In concentrations up to 1 µM, PTK787 / ZK 222584 does not have any cytotoxic or anti-proliferative effect on these cells. In contrast, PTK787 / ZK 222584 selectively inhibits VEGF-mediated functions of endothelial cells, which express the KDR receptor. Inhibition of VEGF-mediated proliferation, migration and survival is observed in the nanomolar range. PTK787 / ZK 222584 inhibits angiogenesis in an *in vitro* assay; the formation of capillary sprouts from pieces of blood vessel cultured in a fibrin gel (Figure 3).

Since our aim was to develop a compound that would inhibit VEGF-induced angiogenesis after oral administration, we tested whether PTK787 / ZK 222584 is absorbed after oral administration in mice. After oral dosing (50 mg/kg) to mice, plasma concentrations of PTK787 / ZK 222584 remain above 1 µM for more than 8 h[61]. The compound also has excellent oral bioavailability in rats, dogs and humans. To determine whether PTK787 / ZK 222584

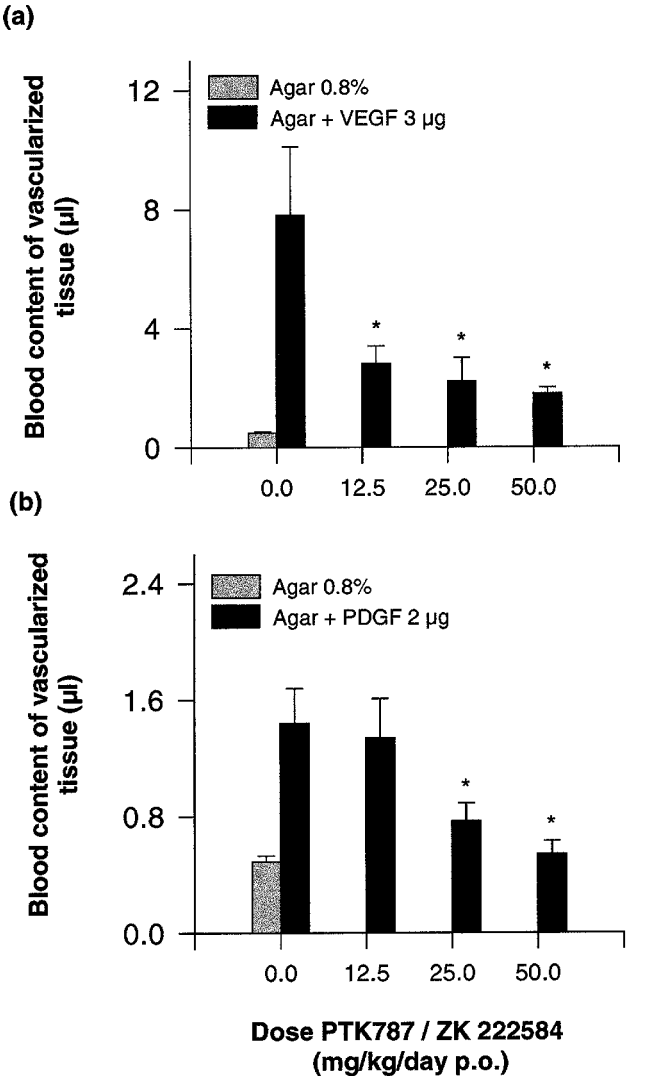


Fig. 4.- Effects of PTK787 / ZK 222584 on angiogenesis induced in a growth factor implant model. Mice were treated with PTK787/ZK 222584 (dihydrochloride) from one day before implantation of chambers containing (a) VEGF (3 µg/ml) or (b) PDGF (2 µg/ml) and 5 days thereafter. The angiogenic response was quantified by measurement of the weight and blood content of the vascularized tissue around the implant. Values are mean ± SEM.* = $p < 0.05$, statistical significance of inhibition, Dunnett's test. Figure from Reference 61 reproduced with permission from *Cancer Research*.

Fig. 3.- Effects of PTK787 /ZK 222584 on the formation of capillary-like sprouts from pieces (1 mm^2) of rat aorta in a fibrin gel cultured in MCDB 131 medium containing 10% FCS and 300 µg/ml aminocaproic acid at 37^0 C and 5% CO2 for 6 days. The images viewed under an inverse microscope show sprout like formations in (a) the absence or (b) presence of PTK787 / ZK 222584 (1 µM). Figure from Reference 61 reproduced with permission from *Cancer Research*.

inhibits VEGF mediated angiogenesis *in vivo*, we tested the effects of PTK787 / ZK 222584 on the angiogenic response induced by VEGF in a growth factor implant model in mice. To test the specificity of the response, the effects on PDGF-induced angiogenesis were also tested. Consistent with its *in vitro* inhibitory profile, PTK787 / ZK 222584 induces dose-dependent inhibition of VEGF- and PDGF-induced angiogenesis in this growth factor implant model, with greater potency against VEGF (Figure 4). To determine whether PTK787 / ZK 222584 inhibits an angiogenic response mediated by tumor cell *in vivo*, we tested the effects of PTK787 / ZK 222584 on the angiogenic response induced by epithelial carcinoma A431 encapsulated in alginate beads and implanted subcutaneously on the dorsal flank of nude mice. This study revealed that PTK787 / ZK 222584 also inhibits tumor cell-driven angiogenesis. In the same dose range (25-100 mg/kg), it also inhibits the growth of several human carcinomas, grown subcutaneously in nude mice, as well as a murine renal carcinoma and its metastases in syngeneic, orthotopic models (Figure 5). Histological examination of tumors reveals inhibition of microvessel formation in the interior of the tumor. Consistent with its potential to block the effects of VEGF on vascular permeability, PTK787 / ZK 222584 also significantly inhibits ascites

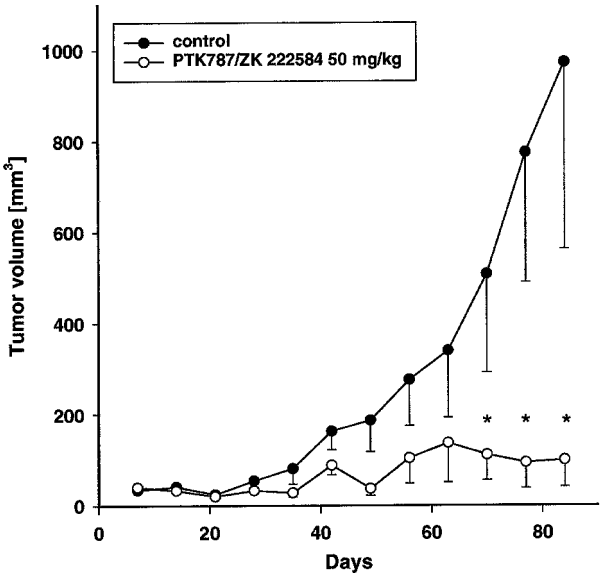


Fig. 5.- Effect of PTK787 / ZK 222584 on the volume of primary human CWR-22 prostate carcinoma xenografts. Primary tumor growth was induced by subcutaneous implantation of tumor fragments into male nude mice. All mice were substituted with testosterone by subcutaneously implanted pellets containing testosterone two days prior tumor implantation. Mice were treated with vehicle or PTK787 / ZK 222584 (dihydrochloride 50 mg/kg, p.o.) once daily from day 7 after tumor implantation until day 84. Values are mean ± SEM, n=10. * Statistically significant difference compared to the control, Dunnett's test. Figure from Reference 61 reproduced with permission from *Cancer Research*.

formation induced by a human ovarian carcinoma grown in the peritoneum of nude mice[63] as well as pleural effusion induced by a human lung adenocarcinoma in nude mice[64].

PTK787 / ZK 222584 was very well tolerated in all the animal models in which it was tested. Surprisingly, although it inhibits the effects of 2 angiogenic growth factors (VEGF and PDGF) it did not impair wound healing in any of the animal models involving surgery or in a dermal incisional wound in the rat[61]. Despite its inhibition of c-Kit, the receptor for stem cell factor, PTK787 / ZK 222584 does not have any significant effects on circulating blood cells or bone marrow leukocytes as a single agent, or impair hematopoetic recovery following concomitant cytotoxic anti-cancer agent challenge[61].

These studies indicate that compounds that inhibit the effects of VEGF, such as PTK787 / ZK 222584, have the potential to provide a novel, effective and well-tolerated therapy for the treatment of solid tumors. These agents may also provide a new therapeutic approach for the treatment of other diseases where angiogenesis plays an important role, such as diabetic retinopathy, macular degeneration and rheumatoid arthritis. Phase I trials with PTK787 / ZK 222584 in patients with advanced cancers have confirmed its excellent tolerability and kinetic properties after oral administration[62].

References

1. Taichman NS, Young S, Cruchley AT, Taylor P, Paleolog E. Human neutrophils secrete vascular endothelial growth factor. *Leukoc Biol J* 1997; 62: 397-400.
2. Horiuchi T, Weller PF. Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte macrophage colony-stimulating factor and interleukin-5. *Am J Respir Cell Mol Biol* 1997; 17: 70-7.
3. Amano S, Rohan R., Kuroki M, Tolentino M, Adamis AP. Requirement for vascular endothelial growth factor in wound- and inflammation-related corneal neovascularization. *Invest Ophthalmol Vis Sci* 1998; 39: 18-22.
4. Griga T, Tromm A, Spranger J, May B. Increased serum levels of vascular endothelial growth factor in patients with inflammatory bowel disease. *Scand J Gastroenterol* 1998; 33: 504-8.
5. Fava RA, Olsen NJ, Spencer GG, et al. Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. *J Exp Med* 1994; 180: 341-6.
6. Nagashima M, Yoshino S, Ishiwata T, Asano G. Role of vascular endothelial growth factor in angiogenesis of rheumatoid arthritis. *J Rheumatol* 1995; 22: 1624-630.
7. Paleolog E. Target effector role of vascular endothelium in the inflammatory response: insights from the clinical trial of anti-TNF alpha antibody in rheumatoid arthritis. *Mol Pathol* 1997; 50: 225-33.
8. Koch AE. Review: angiogenesis: implications for rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 951-962.
9. Alon T, Hemo I, Itin APe, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995; 1: 1024-8.
10. Aiello LP, Pierce EA., Foley ED, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc Natl Acad Sci USA* 1995; 92: 10457-61.
11. Robinson GS, Pierce EA, Rook SL, Foley E, Webb R, Smith LE. Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy. *Proc Natl Acad Sci USA* 1996; 93: 4851-6.
12. Vinores SA, Youssri AI, Luna JD, et al. Upregulation of vascular endothelial growth factor in ischemic and non-ischemic human and experimental retinal disease. *Histol Histopathol* 1997; 12: 99-109.
13. Armstrong D, Augustin AJ, Spengler RAl JA, Nickola T, Grus F, Koch F. Detection of vascular endothelial growth factor and tumor necrosis factor alpha in epiretinal membranes of proliferative diabetic retinopathy, proliferative vitreoretinopathy and macular pucker. *Ophthalmologica* 1998; 212: 410-4.
14. Detmar M, Brown LF, Claffey KP, et al. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med* 1994; 180: 1141-6.
15. Weninger W, Rendl M, Mildner M, Tschachler E. Retinoids downregulate vascular endothelial growth factor/vascular permeability factor production by normal human keratinocytes. *J Invest Dermatol* 1998; 111: 907-11.
16. Detmar M, Brown LF, Schon MP, et al. Increased microvascular density and enhanced leukocyte rolling and

adhesion in the skin of VEGF transgenic mice. *J Invest Dermatol* 1998; 111: 1-6.
17. Plate KH, Breier G, Millauer B, Ullrich A, Risau W. Up-regulation of vascular endothelial growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis. *Cancer Res* 1993; 53: 5822-7.
18. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; 1: 27-31.
19. Brown LF, Detmar M, Claffey K, et al. Vascular permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. EXS 1997; *79*: 233-69.
20. Esser S, Wolburg K, Wolburg H, Breier G, Kurzchalia T, Risau W. Vascular endothelial growth factor induces endothelial fenestrations in vitro. J Cell Biol 1998; 140: 947-59.
21. Shibuya M, Yamaguchi S, Yamane A, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. *Oncogene* 1990; 5: 519-24.
22. Terman BI, Dougher VM, Carrion ME, et al. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem Biophys Res Commun* 1992; 187: 1579-86.
23. Jakeman LB, Winer J, Bennett GL, Altar CA, Ferrara N. Binding sites for vascular endothelial growth factor are localized on endothelial cells in adult rat tissues. *J Clin Invest* 1992; 89: 244-53.
24. Brown LF, Berse B, Jackman RW, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res* 1993; 53: 4727-35.
25. Brown LF, Berse B, Jackman RW, et al. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. *Am J Pathol* 1993; 143: 1255-62.
26. Olofsson B, Pajusola K, Kaipainen A, et al. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc Natl Acad Sci USA* 1996; 93: 2576-81.
27. Olofsson B, Korpelainen E, Pepper MS, et al. Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. *Proc Natl Acad Sci USA* 1998; 95: 11709-14.
28. Clauss M, Weich H, Breier G, et al. The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. *J Biol Chem* 1996; 271: 17629-34.
29. Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem* 1994; 269: 25646-54.
30. Joukov V, Kaipainen A, Jeltsch M et al. Vascular endothelial growth factors VEGF-B and VEGF-C. *J.Cell Physiol* 1997; 173: 211-215.
31. YamadaY, Nezu J, Shimane M, Hirata Y. Molecular cloning of a novel vascular endothelial growth factor, VEGF-D. *Genomics* 1997; 42: 483-8.
32. Achen MG, Jeltsch M, Kukk E, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci USA* 1998; 95: 548-53.
33. Joukov V, Pajusola K, Kaipainen A et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases [published erratum appears in EMBO J 1996 Apr 1;15(7): 1751]. *EMBO J* 1996; 15: 290-8.
34. Kukk E, Lymboussaki A, Taira S, et al. C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* 1996; 122: 3829-37.
35. Maru Y, Yamaguchi S, Shibuya M. Flt-1, a receptor for vascular endothelial growth factor, has transforming and morphogenic potentials. *Oncogene* 1998; 16: 2585-95.
36. Keyt BA, Nguyen HV, Berleau LT, et al. Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective VEGF variants by site-directed mutagenesis. *J Biol Chem* 1996; 271: 5638-46.
37. Waltenberger J, Claesson WL, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem* 1994; 269: 26988-95.
38. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood* 1996; 87: 3336-43.
39. Takahashi T, Shibuya M. The 230 kDa mature form of KDR/Flk-1 (VEGF receptor-2) activates the PLC-gamma pathway and partially induces mitotic signals in NIH3T3 fibroblasts. *Oncogene* 1997; 14: 2079-89.
40. Kroll J, Waltenberger J. The vascular endothelial growth factor receptor KDR activates multiple signal transduction pathways in porcine aortic endothelial cells. *J Biol Chem* 1997; 272: 32521-7.
41. Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996;*380*: 435-9.
42. Ferrara N, Carver MK, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996; 380: 439-42.
43. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995; 376: 66-70.
44. Fong GH, Klingensmith J, Wood CR, Rossant J, Breitman ML. Regulation of flt-1 expression during mouse embryogenesis suggests a role in the establishment of vascular endothelium. *Dev Dyn* 1996; 207: 1-10.
45. Shalaby F, Rossant J, Yamaguchi TP, et al. Role of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995; 376: 62-6.
46. Shalaby F, Ho J, Stanford WL, et al. A requirement for Flk1 in primitive and definitive hematopoiesis and vasculogenesis. *Cell* 1997; 89: 981-90.
47. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* 1993; 362: 841-4.
48. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res* 1997; 57: 4593-9.
49. Borgstrom P, Hillan KJ, Sriramarao P, Ferrara N. Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. *Cancer Res* 1996; 56: 4032-9.
50. Witte L, Hicklin DJ, Zhu Z, Pytowski B, Kotanides H et al. Monoclonal antibodies targeting the VEGF receptor-2 (Flk1/KDR) as an anti-angiogenic therapeutic strategy. *Cancer Metastasis Rev* 1998; 17: 155-61.
51. Skobe M, Rockwell P, Goldstein N, Vosseler S, Fusenig NE. Halting angiogenesis suppresses carcinoma cell

invasion. *Nat Med* 1997; 3: 1222-7.
52. Lin P, Sankar S, Shan S, et al. Inhibition of tumor growth by targeting tumor endothelium using a soluble vascular endothelial growth factor receptor. *Cell Growth Differ* 1998; 9: 49-58.
53. Goldman CK, Kendall RL, Cabrera G, et al. Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis, and mortality rate. *Proc Natl Acad Sci USA* 1998; 95: 8795-800.
54. Yoshiji H, Harris SR Thorgeirsson UP. Vascular endothelial growth factor is essential for initial but not continued in vivo growth of human breast carcinoma cells. *Cancer Res* 1997; 57: 3924-8.
55. Benjamin LE, Keshet E. Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. *Proc Natl Acad Sci USA* 1997; 94: 8761-6.
56. Millauer B, Longhi MP, Plate KH, et al. Dominant-negative inhibition of Flk-1 suppresses the growth of many tumor types in vivo. *Cancer Res* 1996; 56: 1615-20.
57. Strawn LM, McMahon G, App H et, al. Flk-1 as a target for tumor growth inhibition. *Cancer Res* 1996; 56: 3540-5.
58. Fong TAT, Shawver LK, Tang C, et al. SU5416 is apotent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res* 1999; 59*:* 99-106.
59. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995; 55: 3964-8.
60. Bold G, Altmann KH, Brüggen J, et al. New anilino-phthalazines as potent and orally well absorbed inhibitors of the VEGF receptor tyrosine kinases useful as antagonists of tumor driven angiogenesis. *J Med Chem* 2000; 43: 2310-23.
61. Wood J, Bold G, Buchdunger E, et al. PTK787/ZK 222584, a novel and potent inhibitor of VEGF receptor tyrosine kinases, impairs VEGF-induced responses and tumor growth after oral administration. *Cancer Res* 2000; 60: 2178-89.
62. Drevs J, Mross P, Reusch P, Peng B, Ball H, Henry A, Laurent D, Dugan M, Marme D, Unger C. Phase I dose escalation, pharmacokinetic (pk) study of a novel vascular endothelial growth factor (VEGF) receptor inhibitor, PTK787 / ZK 222584(PTK/ZK). Proceedings ECCO 10. Vienna, Austria, 1999.
63. Xu L, Yoneda J, Herrera C, Wood J, Killion J, Fidler I. Inhibition of malignant ascites and growth of human ovarian carcinoma by oral administration of a potent inhibitor of VEGF receptor tyrosine kinases. *Int J Onc* 1999; 16: 445-54.
64. Yano S, Herbst R, Shinoharam H, et al. Novel therapeutic strategy of malignant pleural effusion of human lung adenocarcinoma by oral treatment with an inhibitor of vascular endothelial growth factor receptor tyrosine kinase phosphorylation. *Clin Cancer Res* 2000; 6: 957-65.

- - - -

The painful reality is that there is no holy grail, no magic cure-all bullet, and no quick fix (for cancer). And the paradox is that all the complexity, now that the fog is lifting, has a coherent pattern and makes a great deal of sense. And, as often when seen in retrospect, it is difficult to imagine how it could have been otherwise. Demystifying the disease is to travel over a new and more realistic landscape. It's not the easiest of journeys but it's the only ticket worth having.

La dolorosa realidad es que no hay ningún santo grial, ninguna bala mágica, y ningún arreglo (para el cáncer). Y lo paradójico es que toda esta complejidad, ahora que se levantó la bruma, tiene un patrón coherente y tiene mucho sentido. Y, como se ve tan a menudo retrospectivamente, es difícil imaginar como podría haber sido de otra manera. Desmistificar la enfermedad es emprender un camino nuevo y más realista. No es el más fácil de los viajes pero es el único boleto que vale la pena tener.

Mel Greaves

Cancer. The evolutionary legacy. Oxford: University Press, 2000, p 5