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DEVELOPMENT OF ANGIOGENESIS INHIBITORS TO VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2. CURRENT STATUS AND FUTURE PERSPECTIVE

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1. ABSTRACT

Angiogenesis, the recruitment of new blood vessels is a crucial mechanism required for both tumor growth and metastasis. Advances in the understanding of the molecular mechanisms underlying the angiogenesis process have led to the discovery of a variety of pharmaceutical agents with antiangiogenic activity. The potential application of these angiogenesis inhibitors is currently under intense clinical investigation. Decades of investigation suggest that vascular endothelial growth factor (VEGF) and its receptors, particularly VEGF receptor 2 (VEGFR2, or kinase insert domain-containing receptor, KDR), play a critical role in tumor-associated angiogenesis. KDR, therefore, represents a good target for therapeutic intervention. A number of agents designed selectively for targeting KDR are being evaluated in various phases of clinical trials in cancer patients. This manuscript reviews briefly the biology of VEGF family of ligands and receptors and of KDR in particular. The attempts to develop effective KDR antagonists, including small molecules, antibodies and others, for therapeutic purposes are discussed comprehensively with special emphasis on tumor angiogenesis.

2. INTRODUCTION

Angiogenesis, the recruitment of new blood vessels is a crucial mechanism required for a number of physiological and pathological conditions. It is a tightly regulated, multiple step process, that results in the formation of blood vessels from pre-existing vasculature (1). Under normal conditions, angiogenesis occurs during embryonic development, wound heeling and the female menstruation cycle (2-5). Uncontrolled angiogenesis is

observed in various pathological states, such as psoriasis, diabetic retinopathy, rheumatoid arthritis, chronic inflammatory and cancer (6-13). Tumor cells begin to promote angiogenesis early in tumorigenesis to allow proper nourishment and removal of metabolic wastes from tumor site. In contrast to normal cells, which form a single layer around capillary blood vessels, multiple layers of tumor cells surround the microvasculature, effectively creating a capillary "cuff" (14-16). Although these in situ tumors may replicate rapidly, their uncontrolled growth and metastatic properties are severely restricted by the absence of adequate blood supply. Tumor cells, therefore, are going through a switch from a quiescent to an invasive phenotype. This 'switch' is invariably accompanied by the acquisition of angiogenic properties and is considered the hallmark of the malignant process, whereby pro-angiogenic mechanisms overwhelm or circumvent negative regulators of angiogenesis (17). Indeed, increased tumor vascularization and expression of pro-angiogenic factors has been associated with advanced tumor stage and poor prognosis in a variety of human cancers (15, 18, 19). Decades of investigating the molecular basis of angiogenesis has identified a number of growth factor/receptor signaling pathways that contribute to promote tumor angiogenesis. Ones, and probably the major pathways involved in this process are the vascular endothelial growth factor (VEGF) / VEGF receptors-mediated signaling cascades (20-23). This manuscript reviews briefly the biology of VEGF family of ligands and receptors and of VEGF receptor 2 (VEGFR2, or kinase insert domain-containing receptor, KDR), in particular. The attempts to develop effective KDR antagonists for therapeutic purposes are discussed comprehensively with special emphasis on tumor angiogenesis.

3. VASCULAR ENDOTHELIAL GROWTH FACTOR

VEGF is the prototype of the enlarging family of angiogenic and lymphangiogenic growth factors. The family comprised of six structurally homologous, secreted glycoproteins. These proteins share a great similarity in their primary sequence (24-26). VEGF-A (also known as VEGF) was first identified in the 1980s as a vascular permeability factor (VPF) secreted by tumor cells (27-31). Its gene undergoes alternative splicing to yield at least 6 different mature isoforms of 121, 145, 165, 183, 189 and 206 amino acids (30, 32, 33). These isoforms vary in their bioavailability, level of expression, affinity to heparin and heparan sulfate, mitogenic strength and tissue specificity. $VEGF_{121}$ and $VEGF_{165}$ are the most abundant forms (32, 34-36). Placenta growth factor (PlGF) shares 46% amino acid identity with VEGF and is predominantly expressed in the placenta (37). VEGF-B is 43% identical to VEGF and is highly expressed in skeletal and cardiac tissues (38). VEGF-C exhibits approximately 30% identity to VEGF and is a fairly selective growth factor for lymphatic vessels (22, 39). VEGF-D is most closely related to VEGF-C (40, 41) with 31% identity to VEGF (42-45). Both VEGF-C and VEGF-D have been shown to be endothelial cells mitogens (40, 41, 46). Two additional VEGF-related polypeptides were identified in the genome of the *Orf* virus (47). These polypeptides, NZ-7 VEGF (designated VEGF-E) and ORFV2-VEGF share 25% and 43% amino acid identity with VEGF, respectively (48-51). The active forms of VEGF family of ligands appear either as homodimers (40-45kDa) or as heterodimers with other VEGF family members (26, 52).

4. VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR)

VEGF ligands initiate their biological function upon binding to structurally related cell surface receptors (26, 52, 53). Two receptors were originally identified on endothelial cells, the 180 kDa fms-like tyrosine kinase (Flt1 or VEGFR1) (51, 54, 55) and the 200 kDa KDR (or VEGFR2), or its murine homolog, fetal liver kinase (Flk1) (56-62). The overall amino acid sequence identity between Flt1 and KDR is 44%. KDR binds VEGF, VEGF-C, VEGF-D, VEGF-E and ORFV2-VEGF, whereas Flt1 binds VEGF, VEGF-B and PlGF. Both Flt1 and KDR are expressed primarily on vascular cells of endothelial lineage (51, 55-57, 63-65). A third structurally related tyrosine kinase receptor is the 180 kDa Flt4 (or VEGFR3) (66-71). Flt4 binds VEGF-C and VEGF-D (42, 72). Similar to KDR, Flt4 is widely expressed on endothelial cells during early embryonic development. However, Flt4 becomes largely confined to lymphatic endothelial cells in the adult tissues (66, 68-71, 73, 74).

Two additional receptors were recently identified, a 130-140 kDa isoforms Neuropilin-1 (NRP-1) and NRP-2 (75-77). NRP-1 binds $VEGF_{165}$, PlGF-2 and ORFV2-VEGF (75, 78, 79). NRP-2 binds $VEGF_{165}$, $VEGF_{145}$, PlGF and VEGF-C (80, 81). NRP-1 and -2 differ greatly from other VEGF receptor family members. Their intracellular domain is short, and does not suffice for independent transduction of biological signals (82-86). Their activity is likely mediated as a co-receptor for VEGFR-1 and -2 by enhancing the binding affinity of ligands to the receptors (82, 86-91).

5. VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (KDR)

5.1. Structure and function of KDR

KDR is expressed in all adult vascular endothelial cells with perhaps the exception of vascular endothelial cells in the brain (56). In addition, KDR is detected on circulating endothelial progenitor cells (CEPs) (92-94), pancreatic duct cells (95), retinal progenitor cells (96), and megakaryocytes (97). Significantly increased levels of KDR are also presented on tumors derived from kidney, bladder, ovaries and brain (98-100). KDR-deficient mice have impaired blood island formation and lack mature endothelial cells (101-103). Similar to Flt1, KDR possesses a characteristic structure consisting of seven extracellular immunoglobulin-like domains, a single transmembrane domain, and a tyrosine kinase domain interrupted by an insert (51, 55-57, 104).

Recent studies have provided direct evidence that two molecules of either KDR or Flt1 bind a single VEGF homodimer (35, 105-110). Deletion mutant analysis

demonstrates that KDR extracellular immunoglobulin-like domains 2 and 3 are sufficient for high affinity binding of VEGF (106, 107). Detailed analysis of the interaction between VEGF and various KDR immunoglobulin-like domain deletion mutants suggests that the domains 2-4 might be important for VEGF association, and domains 5 and 6 are important for ligand dissociation (109). The presence of a split kinase domain places both KDR and Flt1 into the same subfamily of class III receptor tyrosine kinases (RTKs), which also includes several 5-immunoglobulin-like domain type receptors such as c-Fms, c-Kit, and the alpha and beta chains of the PDGF receptor. VEGF binding induces conformational changes within KDR, followed by receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain (111-113). The use of recombinant KDR cytosolic domain enabled the identification of four tyrosine residues, Tyr-951, Tyr-996, Tyr-1054 and Tyr-1059, as the autophosphorylation sites (114). Tyrosine phosphorylation forms high-affinity binding sites for a variety of SH2 and PTB domain-containing proteins, including PLCγ, VEGFR-associated protein (VRAP), Ras-GAP, FAK, Sck, Src family of tyrosine kinases, Grb2, PI3-kinase, Akt, PKC, Raf-1, MEK, ERK, p38MAPK, Nck, Crk, Shc, STAT3 and others (105, 111, 114-133). These proteins either possess an intrinsic enzymatic activity, or serve as docking proteins to position other signaling molecules in close proximity with the receptor, to further propagate the VEGF signal (115-118).

The role of KDR in endothelial cells has been extensively studied (103). It is suggested that interaction with KDR is a critical requirement to induce VEGF biological responses, which include cell proliferation, migration, differentiation, tube formation, increase of vascular permeability, and maintenance of vascular integrity (111-113, 134-136). However, the key molecules involved in VEGF/KDR signaling pathway remain to be completely elucidated. The identification of downstream signaling molecules may provide clues to the biochemical mechanisms used to transmit VEGF activity during angiogenesis and, therefore, guide the rational design of potent anti-angiogenesis inhibitors (136).

5.2. KDR as a target for antiangiogenesis therapy

Various angiogenesis inhibitors have been developed to target vascular endothelial cells and block tumor angiogenesis. Compelling evidence suggests that VEGF and its receptors, Flt1 and KDR, provide excellent targets for anti-angiogenesis intervention. Although there are many molecules that have been proven to be endothelial growth factors, VEGF is the one most consistently found in a wide variety of conditions associated with angiogenesis. VEGF and its receptors are over-expressed in the great majority of clinically important human cancers. These include carcinomas of gastrointestinal track, pancreas, breast, bladder, kidney, endometrium, and Kaposi's sarcoma (98, 137-147). In addition, over expression of the VEGF receptors was demonstrated among several intracranial tumors including glioblastoma multiforme (147), as well as in both sporadic and Hippel-Lindau syndrome-associated capillary hemangioblastoma (148). The mRNA for both KDR and Flt1 is greatly up-regulated in tumor-associated endothelial cells, but not in the vasculature surrounding normal tissues (139, 141, 147). Furthermore, a significant correlation between KDR expression and microvessel density has been observed in several tumors. This increased microvessel density appears to be associated with poor prognosis in patients with a wide spectrum of cancers, including carcinomas of breast, bladder, prostate, ovarian, colorectal, stomach, head and neck, non-small cell lung, and uterine cervix, as well as melanomas, testicular germ cell and pediatric brain tumors (149-162).

A retrovirus-mediated expression of a dominant negative Flk1 (mouse KDR analog) mutant inhibited the growth of eight of nine tumor cell lines tested in nude mice, along with significant reduction of vessel density in the tumors (163). Furthermore, inhibition of endothelial cell mitogenesis in vitro and tumor growth in vivo have also been achieved by using anti-KDR/Flk1 antibodies (164-169) and small molecule KDR/Flk1 kinase inhibitors (170-177). Additionally, accumulating evidences suggest the existence of a VEGF/KDR autocrine loop in mediating growth and metastasis of several types of tumors (178-181). Treatment with a neutralizing anti-KDR antibody effectively inhibited VEGF activities both in vitro and in vivo (169, 182-184). KDR inhibitors also have greater accessibility to their targets since tumor vessel endothelium is in direct contact with the blood. In contrast to conventional therapies that require targeting individual tumor cells, local interruption of tumor vasculature by targeting KDR expressed on endothelial cells may detrimentally affect all tumor cells that are dependent upon the targeted vasculature for nutriment. Taken together, it is not surprising that KDR has became one of the most sought-after antiangiogenesis targets being pursued by various pharmaceutical and biotech companies in the recent years (Table 1).

5.3. Neutralizing Antibodies directed against KDR

5.3.1. DC101

A rat anti-mouse VEGFR2, Flk1, monoclonal antibody (DC101) was developed by ImClone Systems (New York, NY) using conventional hybridoma technique (185, 186) to conduct proof-of-concept studies both in vitro and in animal models. In vitro studies demonstrated that DC101 binds with high affinity and specificity to KDR/Flk1, functions as a potent antagonist to VEGF binding, blocks Flk1 signaling, and blocks VEGF-induced endothelial cell proliferation (187). DC101 has been studied extensively in mouse models of angiogenesis, mouse tumors and human tumor xenografts, demonstrating potent anti-angiogenic and anti-tumor activity in these models (187-199). In addition, DC101 treatment inhibits the dissemination and growth of metastases in mouse and human tumor metastasis models (187, 188, 191, 197). Histological examination of DC101-treated tumors showed evidence of decreased microvessel density, increased tumor cell apoptosis, decreased tumor cell proliferation and extensive tumor necrosis (187, 191-194, 198).

Table 1 Summary of anti-KDR antibodies and KDR-selective tyrosine kinase inhibitors (TKI) currently in clinical development

Drug	Category	Company	Phase
ZD4190	TKI	AstraZeneca plc	Discontinued
Semaxanib (SU5416)	TKI	SUGEN Inc.	Discontinued
IMC-1C11	Chimeric Antibody	ImClone Systems	Phase 1
IMC-1121B	Human Antibody	ImClone Systems	Phase 1
CDP-791	Antibody Fragment	CellTech Group plc	Phase 1
AZD2171	TKI	AstraZeneca plc	Phase 1
Bay57-9352	TKI	Bayer Yakuhin Ltd	Phase 1
XL647 (EXEL647)	TKI	Exelixis Inc.	Phase 1
XL999 (EXEL999)	TKI	Exelixis Inc.	Phase 1
CHIR258 (GFLKI258)	TKI	Chiron Corp.	Phase 1
CEP7055	TKI	Cephalon Inc.	Phase 1
AEE788	TKI	Novartis AG	Phase 1
ZK304709 (ZK-CDK)	TKI	Schering AG	Phase 1
Merck Inhibitor	TKI	Merck & Co	Phase 1
ZD6474	TKI	AstraZeneca plc	Phase 2
SU6668	TKI	SUGEN Inc.	Phase 2
GW786034	TKI	GlaxoSmithKline	Phase 2
AG13736	TKI	Pfizer	Phase 2
Vatalanib (PTK787)	TKI	Novartis AG	Phase 3
SU11248	TKI	SUGEN Inc.	Phase 3
Neovastat (Ae941)	Natural Inhibitor	AEterna Zentaris Inc.	Phase 3

This table is complied from information obtained via a variety of sources including research articles, reviews, meeting reports, conference proceedings and abstracts, company websites and press releases.

Combination therapy with anti-Flk1 antibody and chemotherapeutic drugs or radiation may be a useful strategy, since the use of these therapies alone is not able to completely eradicate tumors. In this regard, DC101 treatment has been shown to enhance the anti-tumor activity of chemotherapeutic agents such as Paclitaxel, Cyclophosphamide and Gemcitabine (188, 197). In these studies, combination therapy with DC101 and chemotherapy resulted in significant regression of implanted tumors when compared to either therapy alone. DC101 treatment has also been combined with radiotherapy showing an enhanced anti-tumor response. In one study, DC101 significantly decreased the dose of radiation (TCD_{50}) required to treat nude mice implanted with either human small cell lung carcinoma 54A or glioblastoma multiforme (GBM) U87 cells (200). Combination therapy with DC101 and chemotherapy has also been studied in the context of low-dose or "metronomic" dosing of cytotoxic agents (201). Metronomic therapy refers to frequent, low-dose administration of cytotoxic drugs with the aim of affecting new blood vessel formation. Since VEGF has been shown to act as a survival factor for endothelial cells in response to chemotherapy or radiation (191), the addition of anti-Flk1 antibody to metronomic therapy may enhance the anti-angiogenic effect of metronomic therapy on proliferating tumor vasculature. This hypothesis was tested in experiments where DC101 was combined with chronic, low-dose vinblastine treatment of human neuroblastoma xenografts in athymic mice. Remarkably, this treatment regimen resulted in complete tumor regression of large, established tumors that was sustained for greater than 6 months (202). These results have been confirmed in other tumor models with other cytotoxic agents (203-205). These data support the notion that anti-KDR/Flk1 treatment potentiates the anti-vascular effects of low-dose chemotherapy on proliferating tumor endothelium. No overt toxicity has been observed in long-term DC101 treatment experiments of tumor bearing or non-tumor bearing mice. Autopsy of DC101-treated mice revealed no abnormalities in the organs of these mice including the heart, intestine, kidney, liver, lung and spleen. These findings are important, since low levels of KDR/Flk1 expression are present on the endothelium of some normal tissues and required for normal angiogenic processes (206). Indeed, DC101 treatment does have an impact on normal angiogenesis associated with reproduction (207) and bone formation (UPD). The lack of toxicity observed during DC101 therapy may be due to the limited dependence of resting endothelium for Flk1 stimulation. In contrast, tumor angiogenesis is expected to be more dependent on upregulation and function of Flk1 on tumor vasculature and thus more susceptible to anti- Flk1blockade. The apparent lack of toxicity associated with anti-Flk1 antibody treatment can also be attributed to the high specificity of an antibody antagonist.

5.3.2. IMC-11C1

As DC101 does not cross-react with human VEGFR2, KDR, a panel of new antibodies directed against KDR was generated, using both the traditional hybridoma method and the antibody phage display technique (187, 208-210). This effort gave rise to a lead candidate, IMC-IC11 (211), a mouse/human chimeric IgG1 derived from a single chain Fv isolated from a phage display library (208-210). The antibody binds both soluble and cell-surface

expressed KDR with high affinity (Kd, ~ 300pM), and competes efficiently with radiolabeled VEGF for binding to KDR-expressing human endothelial cells. Furthermore, it strongly blocks VEGF-induced phosphorylation of both KDR and ERK, and inhibits VEGF-stimulated mitogenesis of human endothelial cells (208-210). The binding epitope(s) for IMC-1C11 are located within the first three N-terminal extracellular Immunoglobulin-like domains of the receptor, the same domains that encompass the binding site for VEGF (107). Cross-species examination revealed that IMC-1C11 cross-reacts with VEGFR2 expressed on endothelial cells of monkeys and dogs, but not with those on rat and mouse. In a canine retinopathy model, IMC-1C11 significantly inhibited retinal neovascularization in newborn dogs induced by high concentration of oxygen (212). Furthermore, administration of IMC-1C11 to primate rhesus monkey demonstrated a significant impact on the menstrual cycles, an angiogenesis-related event. (213). Finally, IMC-1C11 strongly inhibits proliferation and migration of KDR-expressing both primary and cultured human leukemic cell lines, and significantly prolonged the survival of NOD-SCID mice inoculated with these cells (169, 178, 182).

Toxicological studies in cynomolgus monkeys demonstrated that twice a week intravenous bolus injection of IMC-1C11 at dose levels of 1.0, 3.0, or 10.0mg/kg, over 4 weeks for a total of 8 doses, was well tolerated. There were no treatment-related clinical adverse signs, changes in body weight, or ocular, hematological and clinical biochemical abnormalities. Furthermore, no macroscopic or microscopic changes were observed in any of 44 organs or tissues examined from each animal (UPD). Taken together, these results suggest that IMC-1C11 is a safe compound and may have potential clinical applications in the treatment of cancer and other diseases in which pathological angiogenesis is involved.

In May 2000 ImClone Systems initiated phase I clinical trial in patients with liver metastatic colorectal cancer. The dose-finding study enrolled 14 patients. When IMC-1C11 was infused at 0.2, 0.6, 2.0 or 4.0mg/kg weekly for 4 weeks, no serious toxicities were observed. Five patients had stable disease by week 4 and continued on therapy, with one patient maintaining stable disease for 6 months (167).

5.3.3. IMC-1121B

ImClone Systems is currently developing fully human anti-KDR antibodies for the potential treatment of solid tumors and certain leukemias (165, 166, 169). These fully human anti-KDR IgG1 antibodies, IMC-2C6 and IMC-1121 were generated from Fab fragments originally isolated from a large antibody phage display library. Further effort was carried out to improve affinity by increasing the stringency of the selection conditions towards KDR (166). These fully human IgG1 antibodies bind specifically to KDR with high affinities of 50-200pM and block VEGF/KDR interaction with an IC50 value of approximately 1nM (166, 169). They strongly inhibited VEGF-stimulated receptor phosphorylation and downstream signal transduction, as well as migration and mitogenesis of human endothelial cells. Further, these antibodies efficiently inhibited proliferation and migration of KDR+ human leukemia cells in vitro, and when administered in vivo, significantly prolonged survival of NOD-SCID mice inoculated with KDR+ human leukemia cells (166, 169). It is noteworthy that the mice treated with antibody of the highest affinity, IMC-1121, survived the longest period of time (166, 169). Since these anti-KDR antibodies do not cross react with mouse Flk1, the in vivo anti-leukemia effect of these antibodies is likely to be due to a direct inhibition of cell growth via blockade of the VEGF/KDR autocrine growth loop in human leukemia cells. When the VEGF-producing leukemia cells were co-cultured in vitro with human endothelial cells, addition of IMC-2C6 blocked the leukemia (VEGF)-stimulated production of growth factors (eg., IL-6 and GM-CSF) by the endothelial cells, thus blocking the paracrine growth loop between the leukemia and the endothelial cells (183). A chronic toxicological study in cynomolgus monkeys demonstrated that once-a-week intravenous bolus injection of IMC-1121 at dose levels of 5.0, 16.0, or 50.0mg/kg, over 39 weeks (9 months) was well tolerated without apparent treatment-related clinical adverse events (UPD). Taken together, these observations suggest a great clinical potential for these antibodies in anti-angiogenesis therapy. Phase I clinical trials of IMC-1121B (IMC-1121 produced by a stable NS0 cell line) were initiated in January 2005.

5.3.4. CDP-791

CDP-791 is a pegylated antibody fragment directed against KDR under development by CellTech Group plc (Slough Berkshire, UK), as a potential treatment for cancer. CDP791 comprises a humanized anti-KDR F(ab')2 fragment conjugated to a polyethylene glycol molecule using a pegylation technology developed by Inhale (now Nektar Therapeutics, San Carlos, CA). In August 2003, Celltech Group plc initiated phase I clinical trials of CDP791 in patients with a variety of advanced solid tumors to assess the safety of ascending doses of the antibody and its pharmacological activity.

5.4. Small molecular weight tyrosine kinase inhibitors to KDR

An increasing number of small molecule tyrosine kinase inhibitors (TKI) to signal transduction pathways of KDR RTK are under various stages of development at several pharmaceutical companies (173, 175, 176, 214, 215, 216). This review summarizes the most advanced compounds in the field which are currently in different stages of clinical trails (Table 1). Noticeable, vast majority of the information on these drugs is not yet available in published manuscripts. Nevertheless, due to the significance of such data for the purpose of further investigation and therapy consideration, information gathered from individual company's web sites and press releases, proceeding of scientific and clinical meetings, for example the American Society of Clinical Oncology (ASCO), and other sources is presented here and referred collectively as unpublished data (UPD).

5.4.1. Vatalanib (PTK787)

Vatalanib is a very potent small molecule inhibitor under development by Novartis (Basel,

Switzerland) and Schering (Berlin, Germany) (172, 217-221). Vatalanib inhibits both KDR and Flt1 with IC50 values of 37 and 77nM respectively (222, 223). It inhibits other class III RTK, such as PDGFR, Flt4, c-Kit, c-fms with 10-fold higher IC50, but is not active against kinases from other receptor family. Vatalanib blocks VEGF-induced KDR phosphorylation, endothelial cell migration and proliferation at nanomolar concentration, but dose not have any cytotoxic and anti-proliferative effects on cells that do not express VEGF receptors (222). Orally, once daily administration of 25 to 100mg/kg inhibited the growth of several human xenograft tumors, as well as an orthotopic murine syngeneic renal carcinoma in mouse models, along with reduction in microvessel formation in tumors (172, 218, 222). The compound was rapidly absorbed with exposure time of 1.6h and average terminal half-life of 5.9h. Phase I dose-escalating and pharmacokinetic (PK) studies of Vatalanib were performed on a wide spectrum of tumors including colorectal, renal cell carcinoma, non-small cell lung carcinoma (NSCLC), acute myeloid leukemia (AML), glioblastoma and prostate cancer. Dose ranged up to 2000mg once-daily or 1000mg twice daily were administered orally. In most studies, results in patients with advanced solid tumors indicated that treatment was well tolerated with no drug-related serious adverse events. Tumor volume reduction was observed in some patients. The maximal tolerated dose (MTD) was not reached with doses up to 1500mg/day. The optimal dose was determined as 1250mg/day. Measurable responses of tumor volume reduction were observed in 19% and 4% of the patients with renal cell carcinoma and glioblastoma, respectively, while over 50% of patients achieved stable disease (UPD).

Phase II trials of Vatalanib in combination with 5-FU/Leucovorin/Irinotecan (IFL) in patients with treatment-naive metastatic colorectal cancer were presented at the 39th ASCO meeting, June 2003. A decrease in the extent of Irinotecan bioavailability and its metabolite (SN-38) was detected following co-administration with Vatalanib. The compound exposure decreased by approximately 40% in four of five patients at 1000mg/day. Of 11 patients evaluable for tumor response, 4 had partial response and 4 achieved stable disease. Common adverse events included nausea, fatigue, vomiting, epistaxis, diarrhea and dizziness. At 500mg/day there was one case of dose-limiting toxicity (DLT) of grade III fatigue and at 1000mg/day there was one case of DLT of grade III hypertension. No other drug-related toxicities were observed. Median time to progression for 11 evaluable patients was 6.7 months. At the median follow-up of 9 months all 16 patients were alive. An additional study on Vatalanib in combination with Oxaliplatin/5-FU/Leucovorin (FOLFOX4) was carried out in patients with metastatic colorectal cancer. Oral administrated Vatalanib was well tolerated and no PK interaction between Vatalanib and Oxaliplatin was detected. From 21 patients evaluable for tumor response, 9 had partial response. The median time to progression was 11 months. Adverse events included grade III ataxia, grade IV neutropenia, grade III thrombocytopenia and dizziness. Neurological DLTs were noted in 2 patients at the 2000mg/day and grade III expressive dysphasia and intermittent dizziness were DLT at 1500 mg/day (UPD).

In phase II study of vatalanib in patients with myelofibrosis with myeloid metaplasia the first two patients treated with 750mg/day experienced DLT of grade III dyspepsia and grade II proteinuria. Therefore, the subsequent 6 patients were treated at 500mg/day. This dose was well tolerated. Only one of six patients had a grade III dose-limiting thrombocytopenia. Other grade III/IV toxicities at this dose level included elevated liver enzymes and neutropenia, all of which occurred beyond day 28 of therapy and were reversible after drug interruption. Gastrointestinal or central nerve system toxicities were minimal or absent. In another study, oral, once-daily administration of Vatalanib was tested in 55 patients with recurrent GBM and the MTD was 1500mg/day. Median progression-free survival was 11 weeks when the dose of Vatalanib was greater than 1200mg/day, but only 8.4 weeks with a dose of smaller than 1000mg/day. DLTs included liver enzyme elevation, deep vein thrombosis, insomnia, cerebral edema, fatigue, and nausea and vomiting. Of the 47 evaluable patients, 2 had partial responses, 31 had stable disease and 14 showed disease progression. The median duration of stable disease was 12.1 weeks. Vatalanib in combination with Temozolomide demonstrated the greatest antitumor activity, with a median progression free survival of 16.1 weeks, compared to 12.1 weeks when in combination with Lomustine. Of the 51 patients who were evaluable for response, 4 had a partial response and 17 had stable disease. The median time to progression was 15.7 and 10.4 weeks for the Temozolomide- and Lomustine-treated groups, respectively. There was one grade III dizziness DLT in the 1500mg/day group treated with Temozolomide. The MTD was not reached (UPD). Phase III colorectal cancer trials were initiated in January 2003. Vatalanib was orally administered at a dose of 1250mg/day in two different studies of 1090 and 830 patients with metastatic colorectal cancer.

5.4.2. ZD4190, ZD6474 and AZD2171

ZD4190, a small TKI under investigation by AstraZeneca (Cheshire, UK), inhibits kinase activity of both KDR and Flt1. ZD4190 blocks VEGF-induced human umbilical vein endothelial cells (HUVEC) proliferation with an IC50 value of 60μM (224, 225). Chronic treatment with ZD4190 inhibited the growth of a variety of human tumor xenografts in animal models, including colon, lung, breast, prostate, and ovarian origin (225-227). However, despite its promising potential, clinical development of ZD4190 was discontinued at 2000 due to intrinsic physiochemical and pharmacokinetic properties of the compound, which were responsible for its moderate and variable bioavailability in higher animal species and patients. Structural modification of ZD4190, as well as new generation of compounds, aiming at improving its physiochemical properties led to the discovery of two new compound, ZD6474 and ZD2171 (228, 229).

ZD6474 is a structural modification of ZD4190 that possesses potent inhibitory characteristics on KDR TK

activity (224). The compound shows selectivity for KDR (IC50, 40nM) versus other RTK, such as EGFR (IC50, 500nM), PDGFR (IC50, 1.1 μM), Flt1 (IC50, 1.6 μM), Tie2 (IC50, 2.5 μM), FGFR (IC50, 3.6 μM), IGF-1R and erbB2 (IC50 > 20 μM), and serine/threonine kinases, such as CDK2, Akt and PDK (IC50, > 20 mM) (229-231). ZD-6474 showed a broad spectrum of dose-dependent antitumor activity against lung, prostate, colon, breast, ovarian and vulval cell lines in vitro (171, 232-234). ZD6474 is approximately 500-fold more soluble than ZD4190 in phosphate buffer at pH 7.4, which led to a significant improvement in oral bioavailability as shown in dogs (224). ZD6474 has a half-life of 15 h and 8 h in rat and dog, respectively, following iv injection (228). When orally given once-daily using several different dosing regimens, ranging from 25 to 100 mg/kg/day, ZD6474 has demonstrated an antitumor activity in a variety of human xenograft models including central nerve system tumors and intestinal adenomas (230, 232). Dynamic contrast-enhanced MRI assessment indicated that acute ZD6474 treatment significantly reduced vascular permeability in tumor tissue (226, 233). Chronic administration of ZD6474 was generally well tolerated. However, similar to ZD4190 and other anti-VEGF agents, ZD6474 induced a dose-dependent increase in the femoral epiphyseal growth plate area in young rat (225). In phase I trials, a total of 49 patients with malignant solid tumors were treated with a single oral dose of ZD6474 (50 to 600mg/kg/day) followed by a 7 day washout period and continuation of daily oral dosing, until disease progression or dose-limiting toxicity. Drug-related toxicity has been minimal. The MTD was reached at 600mg in one patient who developed grade III thrombocytopenia. Adverse events were otherwise limited to mild diarrhea and rash increasing with dose. The estimated half-life of the orally administered ZD6474 was 130 h (ranging from 82 to 206 h). Under these conditions, stable disease was observed in two patients. In a follow up study, a total of 18 patients with malignant solid tumors received single oral doses (100 to 400mg/kg/day) followed by 7 days of rest and then daily dosing at the same dose for a total of 28 days. Partial responses were observed in four of nine patients with NSCLC. MTD was 400mg with 100 to 300mg doses recommended for phase II studies.

ZD6474 is currently in phase II clinical trail in patients with malignant solid tumors. Phase II trials in small cell lung cancer (SCLC), NSCLC and myeloma patients were initiated in November 2002. Data were presented at the 40th ASCO meeting, May 2004. In a two-part, open label, randomized, phase II study, 15 patients with NSCLC were administered once daily with 100 and 300mg/kg of ZD6474 in combination with Docetaxel. Serious adverse events were recorded in eight patients among which toxic-induced encephalopathy, nail infection, non-Q wave myocardial infraction and bacterimia were considered to be therapy related. Nine patients had dose reductions or interruptions in treatment mainly due to QTc prolongation or grade III rash. Among the four patients that received 100mg ZD6474, there were two cases of stable disease for duration greater or equal to 12 weeks and two cases of disease progression. From the 11 patients who received 300mg of ZD6474, 10 were valuable for response. Among these, 2 achieved a partial response, 2 had stable disease for duration of 6 to 12 weeks and 2 had stable disease for at least 12 weeks, while 3 patients experienced disease progression. The median time to progression was 15.1 and 18.6 weeks, respectively, for the 100 and 300mg groups. AstraZeneca has announced that they planned to file ZD6474 for marketing authorization application (MAA) in Europe and new drug application (NDA) in the US in 2007.

As of today, AZD2171 has the potential to be a 'best in class' angiogenesis therapy. It is one of a new generation series of orally available, highly potent inhibitors of KDR TK. The compound inhibited VEGF-stimulated HUVEC cells proliferation with an IC50 value of 0.4nM and was specific for this type of proliferation. AZD2171 demonstrated selectivity greater than 2000 fold for the inhibition of KDR versus EGFR phosphorylation in cells (UPD). AZD2171 exhibited pharmacokinetic properties in animals compatible with once-daily oral dosing. Preclinical studies demonstrated a broad spectrum of antitumor activity that extended to a range of histologically distinct xenografts, including lung, colon, breast, prostate and ovarian. In an orthotopic murine renal cell carcinoma model, treatment with 6.3mg/kg/day of AZD2171 resulted in a significant inhibition of primary tumor growth and microvessel density, with a notable decrease in lung metastases. Similar treatment prevented growth plate ossification in the long bones of growing rats and inhibited luteal development in the ovary, a physiological processes that are highly dependent on neovascularization. Administration of 3mg/kg/day AZD2171 inhibited tumor xenograft growth by 69% to 100% in a variety of animal models. When administered concomitantly with other drugs (Gefitinib , ZD6126, or Irinotecan), AZD2171 resulted in a greater tumor growth inhibition, with tumor regression induced in all cases and a 41% lower mean tumor volume when compared to pretreatment volume at 18 days of dosing. The drug was well tolerated in all studies (UPD). AZD2171 is currently in phase I clinical trail in patients with advanced cancer and liver metastases. Clinical data were presented at the 40th ASCO meeting, June 2004. Cohorts of 3 to 4 patients received a single oral dose of the compound (0.5-20 mg/kg) followed by a 7-day washout period. An equal daily dosing, was then administered for a total of 28 days. PK data of 16 patients indicated that AZD2171 is rapidly absorbed with a median time to maximal plasma concentration of 3h and a half-life of 20h. The PK profile appeared to be linear following single and multiple doses. Treatment was well tolerated at the dose levels and the MTD has not been established at this time. Grade II dizziness was the only toxicity noted, in one patient from the 10mg cohort.

5.4.3. SU5416, SU6668 and SU11248

SU5416 (Semaxanib) was one of the most advanced agents in clinical development as an anti-angiogenic agent developed by SUGEN (Ownership of SUGEN passed to Pfizer Inc. as part of its acquisition of Pharmacia in April 2003.) (18, 235). SU5416 is a KDR TK antagonist that exhibits inhibitory activities against

PDGFR, Flt1 and Flt4 as well. Biochemical studies indicated that SU5416 possesses ATP mimetic properties, and exerts its inhibitory effects on the signaling pathway of KDR/Flk1 in an ATP-competitive manner by localizing in the ATP binding pocket of the RTK (18, 236). SU5416 blocks VEGF-stimulated mitogenesis and migration of human endothelial cells, and induces apoptosis of endothelial cells. It inhibited the growth of a variety of xenograft tumors in mice, along with reducing tumor vascular density (18, 237-242). Phase I studies of SU5416 were carried out in 69 cancer patients with advanced diseases. The drug was given intravenous twice weekly at dose level of 4.4 to 190mg/kg/day. Objective responses were observed in three patients, among which 7 remained on study for more than 6 months and 2 for over 18 months (242-245).

Phase II and III trials were carried out alone or in combinations with standard chemotherapy regimens, in patients with cancers of colorectal, breast and lung, malignant mesothelioma, melanoma, AML and Kaposi's sarcoma. Major toxicities associated with SU5416 have been projectile vomiting (dose-limiting toxicity), grade III diarrhea, nausea, fatigue, headache, and pulmonary emboli (246-252). In February 2002, SUGEN (then owned by Pharmacia) made the decision to discontinue the drug based on interim results from phase III trials involving colorectal cancer patients. Analysis of the data showed that the study would not achieve the defined trial endpoints due to lack of clinical benefit. The company closed its phase III trials and discontinued development for all indications of SU5416 (253).

SU6668 is a broader RTK inhibitor that targets KDR, PDGFR, and FGFR (235, 254-257). SU6668 is structurally similar to SU5416 with better toxicity profiles and oral availability. It offers two different mechanisms of action of both anti-angiogenic and anti-tumor effects, by affecting several targets simultaneously. SU6668 blocks recombinant KDR and FGFR kinase activity with IC50 values of 2.4 and 3nM, respectively. SU6668 induced tumor inhibition or regression following oral administration to mice bearing a variety of tumor xenografts (254, 255). In a metastatic colorectal cancer model, SU6668 increased median survival of tumor-bearing mice by 58% and led to a time-dependent endothelial cell apoptosis and decrease in tumor volume (258). In addition, pericyte vessel coverage and tumor vascularity were significantly decreased in SU6668-treated mice. Combination of SU6668 with Paclitaxel affects ascites formation and tumor spread in ovarian carcinoma xenografts growing orthotopically (259). Furthermore, it was suggested that SU6668 sensitizes radiation via targeting survival pathways of vascular endothelium in Lewis lung carcinoma and GL261 xenografts, possibly through reducing the survival of tumor endothelium (260, 261).

In phase I studies, SU6668 was administrated orally once daily to 68 patients with advanced malignancies at dose levels between 100 to 2400mg/kg/day (262). No serious drug-related toxicities have been observed. Mild-moderate side effects included nausea, diarrhea and fatigue. Median time on study was 13 weeks (range 2 to 86 weeks), and no MTDs were reached. In a dose escalation pharmacological study, SU6668 was administered at 100 or 200mg/kg to 16 patients with advanced solid tumors. No significant toxicities were observed. SU6668 was extensively bound to plasma proteins with a half-life of 3.6h. A 3 times-a-day dose regime suggested an MTD of 100 mg/kg when administered with food. In another study, a dose of 300mg/kg administered daily with food was well tolerated among 35 patients, with adverse effects including fatigue and joint pains. Dose limiting toxicity was 400 and 800mg/kg with grade III thrombocytopenia. Four patients had stable disease for more than 6 months. Phase I data were presented at the 39th ASCO meeting, June 2003. A group of 24 patients with advanced solid tumors were given 200-500mg/kg/day of SU6668 for 28 days. Grade I and II toxicities were edema, nausea, vomiting, fatigue, anorexia and abdominal pain. One patient had grade IV pericardial effusion at the 400mg dose. Plasma concentration was lower on day 28 than day 1. Among this group, 10 patients achieved stable disease, but no objective responses were observed (UPD). SUGEN (Pharmacia) initiated a US phase II trial and a collaborative (Taiho) Japan phase II trial for SU6668 on February 2003 and June 2004 respectively, with a recommended dose of 300mg/kg twice daily with food.

SU11248 (263) displays selectivity for members of the split kinase domain subgroup, KDR, PDGFR-alpha, PDGFR-beta, c-Kit and Flt3, with in vitro IC50 values in the nanomolar range (4-14nM) (175, 264-267). In biological and cellular assays, SU11248 competitively inhibited ligand-dependent KDR and PDGFR-beta autophosphorylation with IC50 values of 10nM (267-270). In mouse xenograft models, SU11248 inhibited the phosphorylation of PDGFR-beta, KDR and c-Kit time- and dose-dependently as well. SU11248 demonstrated broad and potent antitumor activity, including regression in murine models of human epidermal (A431), colon (Colo205 and HT-29), lung (NCI-H226 and H460), breast (MDA-MB-435), prostate (PC3-3M-luc) and renal (786-O) cancers, and suppressing or delaying the growth of many others, including the C6 rat and SF763T human glioma xenografts and B16 melanoma lung cancer. Tumor inhibition ranged from 11 to 93%, and was found to be dose-dependent between oral doses of 20 and 40mg/kg/day. In mice bearing established A431 tumors, administration of 80mg/kg/day of SU11248 for 21 days resulted in a complete tumor regression in 6 of 8 mice during the first round of treatment, and in the remaining 2 mice upon retreatment. The tumors did not regrow for duration of 110 days. In Colo205 tumors, SU11248 treatment induced a dose- and time-dependent, rapid decrease in tumor microvessel density and tumor-cell proliferation, and an associated increase in tumor-cell apoptosis, culminating in tumor regression. In SF763T tumor models, on the other hand, SU11248 decreased tumor vascularization and proliferation, with no overt tumor tissue destruction, resulting in tumor growth delay (267). SU11248 has also demonstrated synergy with both radiation therapy and chemotherapeutic drugs such as Docetaxel, Cisplatin, 5-FU or Doxorubicin in a number of in vitro and in vivo studies (271).

Phase I and II clinical data confirmed that orally administered SU11248 is well absorbed and has a half-life of 40h. The compound was well tolerated in clinical studies and little toxicity was reported (271). SU11248 was shown to be effective as second-line therapy for patients with metastatic renal cell carcinoma whose disease had progressed despite standard therapy. All patients were given repeated cycles of SU11248 at 50mg/day for 4 weeks followed by a 2-week rest period. Partial responses were observed in 33% patients while 37% had stable disease for over 3 months. At 6 months, 14 out of 63 of the patients were still under treatment with an ongoing partial response. In another study, SU11248 was administered at 25-75mg/day for a duration of 2 weeks and followed by 2 weeks rest to 18 patients with Imatinib (STI571 or Gleevec)-resistant gastrointestinal stromal tumors (GIST). This regimen resulted in 2 partial tumor responses and 10 stable diseases, the longest being 6 months. Biopsy and imaging studies showed that 10 out of 17 valuable patients had a reduction in metabolic activity in their tumors. The MTD was determined to be 50mg, as 2 patients treated with 75mg experienced transient DLT including fatigue, nausea, and vomiting during the first cycle. In a similar study, administration of 50mg/day of SU11248 for 4 weeks was followed by 2 weeks of rest. Analysis had been conducted in 48 progressing patients, 26 of whom showed a clinical benefit. Administration of 50mg/day of SU11248 for duration of 4 weeks in patients with refractory, metastatic RCC induced mostly grade I or II toxicities, including fatigue, asthenia, nausea and diarrhea. Grade III and IV toxicities included lymphopenia and elevated lipase and amylase, but no clinical signs of pancreatitis. Two patients were taken off the study for decreases in left ventricular ejection fraction greater than 20% without clinical symptoms. In a leukemia trial, AML patients were treated with repeated doses of 25-100mg/day of SU11248 for 2 weeks. Grade III fatigue was the DLT in 2 of 22 patients. Other drug-related adverse events included nausea and vomiting, diarrhea, headache, altered blood counts and lipase elevations; most were grade I and II and were considered manageable. SU11248 is presently in phase III trials. A randomized, double-blind, placebo-controlled study for the treatment of Imatinib-resistant GIST and RCC was initiated in July 2004, with an expected enrollment of 690 patients.

5.4.4. Neovastat (Ae941)

Neovastat is a naturally occurring orally bioavailable antiangiogenic compound, extracted from shark cartilage, under investigation by AEterna Zentaris Inc. (Quebec, Canada). Neovastat possesses multiple antiangiogenic mechanisms of action that provide broad therapeutic potential for a number of diseases (273). The development of Neovastat first began due to the mistaken belief that sharks, whose skeletons consist mostly of cartilage, are not affected by cancer. Despite the fact that this assumption is not correct, several substances isolated from shark cartilage have been found to possess antitumor activity. Fractionation of liquid shark cartilage led to the characterization of some active components that have been tested for direct antitumor activity in vitro. As yet, however, no reports have identified the active components in Neovastat. Neovastat blocks two main mechanisms of angiogenesis activation, VEGF and matrix metalloproteinase (MMP)-2 and MMP-9. At the molecular level, Neovastat was shown to compete against the binding of VEGF to its receptor in endothelial cells and significantly inhibited the VEGF-dependent tyrosine phosphorylation of KDR, whereas it had no significant effect on Flt1 activity (274-278). Moreover, the inhibition of receptor phosphorylation was correlated with a marked decrease in the ability of VEGF to induce pERK activation (274). Neovastat in a concentration of up to 0.2mg/ml inhibited VEGF-induced endothelial cell sprouting in a dose-dependent fashion. It also inhibited endothelial cell migration and vessel formation (274). Neovastat (85µg/ml) induced 50 and 100% cell death following 24 and 48h treatment, respectively, in bovine aortic endothelial cells (BAECs) (111, 279). Sub-chronic toxicity studies in animals did not indicate any significant toxicity associated with the administration of Neovastat. Toxicology studies in rats and monkeys demonstrated no DLT or target organ damage after 1 year of chronic exposure.

A US open-label, multicenter phase I/II study suggested that Neovastat was efficacious in the treatment of refractory metastatic lung cancer (235, 280, 281). The study did not demonstrate any serious adverse events. Analysis of data from a group of 48 patients with unresectable late-stage NSCLC from phase I/II dose-tolerance trial showed that those receiving more than 2.6 mg/kg/day Neovastat were 50% less likely to die than those who received less than 2.6 mg/kg/day(280, 282). Neovastat has now been monitored in over 800 patients, some of whom have taken the drug for over four years. Overall, Neovastat has an excellent safety profile with few side effects. Although one serious adverse event (hypoglycemia) was noted in a type 2 diabetic patient, other grade III to IV toxicities have not been observed. Phase I/II trial of Neovastat (30 to 240 ml/day) conducted in 331 solid-tumor patients demonstrated the most frequent adverse events of nausea (7%), vomiting (3%), dyspepsia (2%) and anorexia (2%) (283, 284). Phase I/II trial on patients with renal cell carcinoma, multiple myeloma and prostate cancer performed in Canada and the US, showed no DLT, good patient compliance, and improved conditions or disease stabilization were noted in some of the patients. Nevertheless, development of Neovastat for indications other than lung cancers were suspended or discontinued as a result of budget issues. A recent phase III study found that 200 patients with NSCLC given Neovastat in combination with induction chemotherapy and concomitant chemo-radiotherapy noted granulocytopenia as a common toxicity in the induction phase, and one patient suffered a myocardial infarction in the chemo-radiotherapy phase. It appears that, therefore, Neovastat is suitable for long-term use either alone or in combination with other anticancer agents (235, 280-282, 285).

Identification of the active component of Neovastat may elucidate its specific mode of action and potentially limit the side effects identified at the present time. This is particularly pertinent if, as expected, life-long administration is required, because the effects of chronic

exposure and interactions between Neovastat and other therapies are not yet known. The positive safety profile and the oral administration route of Neovastat, however, are advantages in comparison with current therapies and some angiogenesis inhibitors.

5.4.5. GW786034

GW786034 is a KDR TK inhibitor under development by GlaxoSmithKline (Brentford, UK) for the potential treatment of solid tumors (286). Clinical data on GW786034 were presented at the 40th ASCO meeting, June 2004. In a phase I, open label, non-randomized, dose-escalating trial, 37 patients with various solid tumors were orally administered GW786034 as part of a three-times a week schedule (50 or 100mg each dose) or a daily administration schedule (50 to 2000mg each dose). Four cases of stable disease were noted for patients in the trial for more than 27 weeks. Partial response was observed in a patient who received treatment for 46 weeks. Minimal responses of 15- 18% tumor shrinkage were noted in two patients on the study for at least 12 weeks. An unconfirmed partial response was observed in a patient on the study for over 14 weeks. Apparent correlation between increase in blood pressure and GW786034 dose was observed. Dose-limiting toxicity of grade III fatigue was obtained in the 200mg dose. For the once daily administration schedule the half-life was approximately 35 h. Phase II clinical trials of GW786034 were initiated in November 2004.

5.4.6. AG13736

Pfizer (New York, NY), in collaboration with its wholly-owned subsidiary Agouron Pharmaceuticals, is developing AG13736, a potent inhibitor of the VEGF/PDGF receptor TK, as an anti-angiogenic agent for the potential treatment of cancer. AG13736 is active against Flt1, KDR, Flt4, PDGFR-beta and c-Kit with IC50 values of 1.2nM, 0.25nM, 0.25nM, 2.5nM, and 2.0nM, respectively (UPD). The compound showed potent activity and specificity for the recombinant KDR kinase at sub-nanomolar concentrations. It was shown to inhibit VEGF-stimulated HUVEC cells proliferation and survival. In a human colon carcinoma mouse model, oral administration of AG13736 twice daily inhibited tumor growth associated with a significant decrease in microvessel density and increased necrosis. AG13736 significantly inhibited metastasis to the lung and lymph nodes in an orthotopically implanted human melanoma tumor in SCID mice with half-life of 2h. Co-administration of AG13736 with Docetaxel resulted in a higher antitumor efficacy compared to that achieved by either agent alone. Quantitative MRI analysis revealed that AG13736 treatment produced changes in vascular permeability and antiangiogenic effects (UPD).

Phase I trails were initiated in April 2002 and data were presented at the 40th ASCO meeting, June 2004. AG13736 was orally administered at escalating doses to patients with various solid tumors including breast, thyroid, renal cell, lung and other for cycles of 28 days. The MTD was found to be 5mg/kg among fasted patients. DLTs at doses higher than the MTD were hypertension, seizures, elevated liver functions, mesenteric vain thrombosis and pancreatitis and stomatitis. One patient with a cavitating lung lesion died from hemoptysis while on the treatment. At doses less than or equal to the MTD, the only DLTs observed were one case of stomatitis and 6 cases of dose-limiting hypertension. Durable responses were achieved with 2 patients and 7 patients had stable disease for more than 4 months. PK studies showed that peak plasma concentrations occurred between 2 and 4h and the terminal half-life was between 3 and 5h. The dose of 5mg/kg to fasted patients was recommended for phase II trials (UPD). AG13736 entered phase II studies in breast cancer and RCC in November 2004.

5.4.7. BAY57-9352

BAY57-9352 is a KDR inhibitor under development by Bayer Yakuhin Ltd (Leverkusen, Germany) for the potential treatment of cancer (170). It potently and selectively inhibited KDR, Flt4, c-Kit and mouse PDGFR TK in vitro with IC50 values of 6, 4, 1 and 15nM, respectively. BAY57-9352 blocked VEGF-dependent receptor autophosphorylation in mouse fibroblasts expressing human KDR, with an IC50 value of 19nM. Its affect on KDR phosphorylation was detected in endothelial and smooth muscle cells as well. BAY57-9352 inhibited the proliferation of HUVEC cells and human aortic smooth muscle cells with an IC50 value of 26nM and 249nM respectively, with no effect on proliferation of MDA-MB-231 breast carcinoma, LS17T colorectal carcinoma, HCT-116 colorectal carcinoma or PC-3 prostate carcinoma cells. Nevertheless, administration of 20mg/kg BAY57-9352 reduced MDA-MB-231, Colo-205, DU-145 and H460 xenografts tumor growth in NCr nu/nu mice by 91, 79, 61 and 78%, respectively. Moreover, microvascular density and endothelial cell content around the MDA-MB-231 and Colo-205 tumor xenografts were significantly reduced within 24h of the first administration (UPD). BAY57-9352 is currently in phase I clinical trail in patients with malignant solid tumors.

5.4.8. XL647 and XL999

XL647 (EXEL-647) and XL999 are two potent 'Spectrum Selective' inhibitors under development by Exelixis Inc. (South San Francisco, CA). These compounds are aiming at targeting both the tumor and its vasculature by inhibiting different RTKs implicated in driving tumor proliferation and vascularization. XL647 simultaneously inhibits the EGFR, HER2, KDR and EphB4 TK with high potency and demonstrates excellent activity in target-specific cellular functional assays. Administration of XL647 resulted in a dose-dependent and sustained inhibition of KDR, EGFR and ErbB2 phosphorylation. XL647 has good oral bioavailability and showed potent anticancer activity and sustained inhibition of target RTKs in vivo, following a single oral dose. XL647 induced tumor regression in established MDA-MB-231 and PC3 xenografts models. A single oral dose treatment in MDA-MB-231 xenograft model resulted in a complete and rapid loss of microvessels in the tumor, a decrease in cell proliferation and an increase in necrosis and hypoxia over time. In athymic, xenograft-bearing mice, treatment with 100mg/kg of XL647 produced over 85% suppression of tumor growth. The compound has moderate clearance and

a half-life of more than 8 h (UPD). Exelixis initiated a phase I trial for XL647 in June 2004.

XL999 simultaneously inhibits the FGFR, KDR, PDGFR and Flt3 TK with high potency and demonstrates excellent activity in target-specific cellular functional assays. In preclinical models of major tumor types, including human breast, lung, colon and prostate cancer, XL999 demonstrated potent inhibition of tumor growth and has been shown to cause tumor regression. XL999 is suitable for both oral and intravenous dosing and shows sustained inhibition of target RTKs in vivo following a single oral dose. An in vitro functional angiogenesis assays demonstrated XL999-induced inhibition of tubule formation and migration on endothelial cells in culture in response to VEGF or bFGF. In nude mice, a single oral dose resulted in potent inhibition of KDR, PDGFR-beta, FGFR1, Flt3 and c-Kit. Daily administration of XL999 to nude mice bearing MDA-MB-231 xenograft resulted in a rapid destruction of the tumor vasculature with tumor and endothelial cell death evident 2 to 4h post administration of the first dose. Longer exposure caused large decreases in vessel density and proliferating cells and large increases in tumor necrosis. Endothelial cells in the tumor vasculature were selectively targeted as endothelial cells elsewhere were not affected (UPD). Exelixis initiated a phase I trial for XL999 in September 2004.

5.4.9. CHIR258

CHIR258 is a potent VEGF, FGF and PDGF receptor kinase inhibitor, for the potential treatment of cancer, under development by Chiron Corp. (Emeryville, CA). CHIR258 has shown potent activity against several growth factor-related kinases, with IC50 values of 27, 2, 0.1, 10 and 8nM against PDGFR-beta, c-Kit, Flt3, VEGFR1/2/3 and FGFR1/3, respectively. It showed minimal activity against 25 other kinases. The compound had a significant antitumor activity in more than 10 models, including the KM12L4A human colon cancer. It was also showed to induce regression in large tumors, and had potent anti-angiogenic activity in vitro and in vivo. The oral bioavailability of CHIR258 was greater than 70% in mice, rats and monkeys; and 34% in dogs. Maximum plasma and tissue concentrations occurred approximately 4h following an oral dose in mice and rats. The elimination half-life ranged from 2.7 to 3.6h in plasma following an intravenous administration. CHIR258 inhibited the proliferation of a subset of cancer cell lines, with IC50 values of less than 25nM. In in vivo studies, human colon tumor (KM12L4a) xenografts treated with CHIR258, demonstrated significant tumor regression and inhibition. Tumor regression and/or disease stabilization was observed in 90 to 100% of animals. In a mouse model of murine breast cancer (4T1), CHIR258 inhibited primary tumor growth in a dose-dependent manner (20 to 82%) and liver metastases were inhibited by more than 75% at all doses greater than 10mg/kg/day. In further studies, CHIR258 was shown to potentiate the antitumor activity of the standard cytotoxic therapeutics Irinotecan, Trastuzumab and Gefitinib. Analysis of KM12L4a tumors after CHIR258 treatment indicated that phosphorylation of Flt1, KDR, PDGFR-beta and FGFR were inhibited in a time-and-dose-dependent manner (UPD).

Phase I studies in solid tumor and AML patients were initiated in UK in January and October 2004, respectively. A reduction in phosphorylated ERK was observed in patient peripheral blood lymphocytes 4 to 24 h following the first dose. Pharmacodynamic studies showed dose-dependent plasma concentration and supported once-daily dosing. In September 2004, the third cohort had completed treatment with 75mg/day of CHIR-258 and a 100mg/day dose level had been initiated. No clinically significant toxicities had been detected at this point.

5.4.10. CEP7055

CEP7055 is the lead compound in a series of KDR TKI for the potential treatment of prostate and pancreatic cancers, under development by Cephalon Inc. (West Chester, PA) and Sanofi-Aventis (Paris, France) (174, 216, 287). It is a fully synthetic orally active ester of CEP5214, a very potent KDR inhibitor with a poor water solubility (174, 216, 287). CEP7055 demonstrated antitumor efficacy, as well as antiangiogenic and antimetastatic activity in animal models. It is 20% orally bioavailable in rats, and has a half-life of 4 to 5h in monkeys. Chronic oral administration of CEP7055 at doses of 10 to 20mg/kg/day resulted in significant inhibition of a variety of established murine and human subcutaneous tumor xenografts in nude mice. No DLT was noted following 10 or 28 days administration in monkeys. No adverse neurological, cardiac or respiratory effects were observed. Treatment of human pancreatic ductal carcinoma-bearing mice with CEP7055 was well tolerated, and resulted in a significant reduction in primary pancreatic tumor mass, incidence of ascites and the magnitude and extent of hepatic and peritoneal lymph node metastases. Oral administration of CEP7055 at 3 and 20mg/kg/ to BALB/C mice inoculated with renal cancer cells (RENCA) tumors was well tolerated as well and resulted in a decrease in metastatic score. Administration of CEP7055 in combination with Temozolomide led to an improvement in median survival of human GBM-bearing mice versus mice receiving Temozolomide monotherapy. In a dose-response study in the same model, chronic oral administration of CEP7055 alone at 24 to 95mg/kg/day demonstrated a dose-related reduction in brain edema and hemorrhagic lesions. Significant reductions in neurological dysfunction were observed in GBM-bearing mice receiving CEP7055 alone and to a greater extent, in combination with Temozolomide. Phase I data were presented at the 39th ASCO meeting, June 2003. A group of 19 patients with various solid tumors were given 10-120mg/kg/day CEP7055 continuously for a duration of 28 days followed by a 14-day washout period. Adverse events were generally mild, including hypertension occurred in one patient on the 120mg dose towards the end of the washout period.

5.4.11. AEE788

AEE788 is a potent multi-target inhibitor of both EGF and VEGF RTK family members under development by Novartis AG (Basel, Switzerland) (177, 288). At the enzymatic level, AEE788 inhibited EGFR, ErbB2, KDR and Flt1 TK activity with IC50 values of 2nM, 6nM, 77nM and 59nM respectively (177). AEE788 demonstrated an anti-proliferative activity against a range of EGFR and

ErbB2-overexpressing cell lines and inhibited the proliferation of EGF- and VEGF-stimulated HUVEC cells (177). Oral administration of AEE788 to tumor-bearing mice resulted in high and persistent compound levels within the tumor tissues. AEE788 also inhibited VEGF-induced angiogenesis in a murine implant model. Antiangiogenic activity was also apparent by measurement of tumor vascular permeability and interstitial leakage space using dynamic contrast enhanced magnetic resonance imaging methodology (177, 288). In an in vitro study using the cell line JMAR SCCHN, AEE788 inhibited cell growth with an IC50 value of 7μM at 72h and induced 50% cell death after treatment with 14μM at 48h. Treatment of KAT-4 anaplastic thyroid cancer cells with AEE788 for duration of 1h inhibited autophosphorylation of EGFR and KDR, phosphorylation of ERK and AKT and cell proliferation in a dose-dependent manner with an IC50 value of 7 μM (288). Administration of AEE788 to nude mice implanted with JMAR tumors resulted in significant reduction in tumor growth (288). A combinatorial treatment of AEE788 and Everolimus increased the antiproliferative effects of the drugs in comparison to that of single agents alone. Cell death, confirmed to occur via apoptosis, was dramatic at optimal concentrations of the combination.

Phase I clinical trials were initiated in April 2003 in patients who have not previously received treatment directed against EGFR, ErbB2 and VEGFR. Results were presented at the 16th EORTC-NCI-AACR meeting, September 2004. A group of 50 adult patients with advanced solid tumors received continuous, oral, daily administration of AEE788 at doses of 25-550mg/day. A total of 41 patients were assessed. The mean exposure increased with dose duration, as did the exposure of the metabolite of AEE788, AQM-674. Exposure of the parent compound and active metabolite increased with dose until day 15 when steady state was achieved. The metabolite, AQM-674, was rapidly formed and eliminated in comparison to AEE788. At a dose of 300 to 400mg, a predicted 80% inhibition of KDR phosphorylation occurred. The drug was widely distributed within the tissues and extensively metabolized. The once-daily regimen of up to 400mg/day was found to be safe and well tolerated, achieving a therapeutic exposure profile. The half-life of the compound was noted to be above 24h. DLTs were observed at the 550mg dose. The most frequent advert effects were diarrhea, fatigue anemia and nausea, which were experienced by 66, 50 and 42% of patients, respectively. A total of 14 out of 41 (34%) patients achieved stable disease and remained on the study for more than two cycles (UPD).

5.4.12. ZK304709 (ZK-CDK)

ZK304709 is an orally available dual specific CDK and VEGFR kinase inhibitor, under development by Schering AG (Berlin, Germany) for the potential treatment of cancer. ZK304709 inhibited CDK2 and KDR kinase activity with IC50 values of 4 and 30nM, respectively. In xenograft mouse models ZK304709 reduced tumor blood supply and strongly induced apoptosis. Its dual kinase activity enables blocking of cell cycle followed by preferential tumor cell apoptosis through CDK1 and 2 and blocking neoangiogenesis through VEGFR1/2/3 and PDGFR-beta. IC50 values for CDK2, CDK1, VEGFR1/2/3 and PDGFR-beta were 5nM, 60nM, 20nM and 55nM, respectively. Phase I trials were initiated in the first half of 2004.

5.4.13. Merck & Co compound

Merck & Co (West Point, PA) is developing small molecular weight KDR and KDR/Flt3 kinase inhibitors for the potential treatment of cancer and other angiogenic disorders. Preclinical data on two classes of KDR TK inhibitors showing that the lead compound demonstrated good inhibition of VEGF stimulated HUVEC cells mitogenesis with an IC50 value of 18nM and in vivo inhibition of KDR with an IC50 value of 130nM (289, 290). The compound had an IC50 value of 3nM in an in vitro KDR kinase assay and a half-life of 5.1h. Treatment of human HT1080 fibrosarcoma nude mouse xenograft model with the most potent compound (IC50 values of 19.5nM in vitro, and 21nM in vivo) was associated with partial or nearly complete inhibition of KDR phosphorylation and inhibition of tumor growth (289, 290). Histology characterization revealed that treatment led to inhibition of tumor angiogenesis and cell proliferation, with an enhancement in tumor cell death (289, 290). The inhibitors significantly blocked unstaged and staged growth of mammary and glioma tumor growth in vivo. Antitumor efficacy was associated with decreased levels of phosphorylated KDR in lungs and tumors, reduced microvessel density and vessel maturity and decreased endothelial cell proliferation. Treatments were tolerated with no significant changes in body weight. Phase I studies were initiated by July 2004.

5.5. Additional approaches

An escalating number of novel therapeutic compounds is still under discovery or preclinical studies, including RWJ-417975, a KDR TKI of Celltech Group Inc. (Slough Berks, UK) and Johnson & Johnson, IDDBCP151962, a dual KDR/FGFR TKI of Hoffman La-Roche and OSI-930, a dual c-Kit/KDR inhibitor of OSI Pharmaceuticals Inc. (Melville, NY), DX-1235, a peptide inhibitor of KDR, by Dyax Corp (Cambridge, MA) and others. In addition, a wide spectrum of compounds and antibodies targeting VEGF ligands exist under different developmental and clinical stages. Avastin® (Bevacizumab), an anti-VEGF monoclonal antibody was developed and launched by Genentech (South San Francisco, CA) in February 2004, as an anti-angiogenesis therapy for the treatment of colorectal cancer (291-294). VEGF trap (AVE-0005), a soluble decoy receptor comprising portions of VEGFR-1 and 2, by Regeneron Pharmaceuticals Inc. (Tarrytown, NY) together with Sanofi-Aventis, is currently in phase I trail in patients with solid tumors and non-Hodgkin's lymphoma (295-297). Veglin, an antisense oligonucleotide that inhibits VEGF signaling, by VasGene Therapeutics Inc. (Los Angeles, CA) is currently in phase I trial in patients with relapsed or refractory malignancies (298-300).

6. FUTURE PERSPECTIVE

Targeting cells that support tumor growth, for example the neovasculature of tumors, rather than cancer cells themselves, is a relatively new approach to cancer therapy. Virtually, the control of angiogenesis in general, and targeting KDR in particular offers hope in the treatment of many disorders and may have wide spectrum applicability. Our knowledge of tumor angiogenesis and its impact on conventional cancer therapies has improved tremendously during the course of the last few years (301, 302). The elucidation of structural abnormalities associated with tumor neovasculature, and of the underlying molecular mechanisms, has led to the identification of potential targets for therapeutic intervention by anti-angiogenesis approaches. Antiangiogenesis therapies may offer a number of theoretic advantages over the conventional cytotoxic regimens. In principal, conventional therapy is hindered by the development of drug-resistant cancer cells. Due to the fact that endothelial cells possess a normal complement of chromosomes and are genetically stable, and therefore less likely to accumulate mutations that allow them to develop drug resistance in a rapid manner (303-306). In addition, endothelial cells are more sensitive than tumor cells to most cytotoxic agents. Thus, a low-dose chronic chemotherapy (or the so-called "metronomic approach"), designed for preferential anti-angiogenic activity rather than tumoricidal activity, could be more efficacious and less toxic than conventional high-dose therapy (202, 203). It should be taken under consideration, however, that tumor cells might reduce their sensitivity or become 'resistant' to individual antiangiogenic therapy, by increasing production of, or switching to other angiogenic factors (307). Combinational use of multiple anti-angiogenic agents should prove to be more effective in this scenario (308). Finally, complete eradication of cancer cells is often unfeasible, partially due to an aberrant tumor vasculature that prevents uniform delivery of therapeutic doses of anticancer agents to the tumor tissues. By disrupting local blood supply, the antiangiogenic agents might detrimentally affect all tumor cells that are dependent upon the vessels, thus minimizing the chance of residual tumor cells escaping (309).

Antiangiogenic agents may be predominantly effective in the context of combination therapy as they may enhance the delivery and therapeutic efficacy of other treatment modalities that directly target cancer cells. Despite the concern that a reduction of tumor blood supply would interfere with the delivery of chemotherapeutic agents and oxygen to the tumor tissues, it was suggested that that antiangiogenic therapies, when used properly, could 'normalize' the tumor vasculature, thereby improving the efficiency of delivery of concurrently administered cytotoxic agents (310-312). To this end, antiangiogenic therapies have been shown to potentate the antitumor effects of several conventional cytotoxic therapies (including both chemotherapies and radiation) in various animal models (188, 200, 313, 314).

The majority of drugs directed against KDR currently in clinical investigation are small molecular weight TKI. These molecules are oral available and therefore maybe more convenient for patients to handle. However, in contrast to antibodies, which are characterized by their high specificity to their targets and their minimal systemic toxicities to patients, small TKI molecules are less specific and often affect more than one kinase simultaneously, thus often leading to increased toxicity and lack of tolerance in clinical settings. Beneficially, antibodies and small TKI molecules are not mutual exclusive. For example, combinations of growth factor receptor-specific antibody with receptor kinase-selective small molecule inhibitor have recently been shown to be more efficacious than each individual agent (315, 316).

Antiangiogenesis therapy is clearly an exciting area of research with potential for improving the care of patients with numerous cancers. The variety of antiangiogenic compounds ranks predominantly amongst novel and promising strategies to fight cancer as well as other pathologies. With the recent approval of the anti-VEGF antibody, Avastin®, the clinical antiangiogenesis approaches now look an increasingly realistic prospect. In particular, a number of carefully designed clinical trials are underway and it is hoped that answers to some of the open questions raised in this review will soon be forthcoming. Interestingly, there are currently more anti-cancer drugs in clinical trials that are anti-angiogenesis than those that fit into any other mechanistic category. The next few years are clearly going to be an exciting period for further testing and validation of the concept.

7. REFERENCES

1. Smith, S. K.: Angiogenesis. *Semin Reprod Endocrinol*, 15, 221-7(1997)
2. Breier, G.: Angiogenesis in embryonic development--a review. *Placenta*, 21 Suppl A, S11-5(2000)
3. Tonnesen, M. G., X. Feng & R. A. Clark: Angiogenesis in wound healing. *J Investig Dermatol Symp Proc*, 5, 40-6(2000)
4. Gargett, C. E. & P. A. Rogers: Human endometrial angiogenesis. *Reproduction*, 121, 181-6(2001)
5. Smith, S. K.: Regulation of angiogenesis in the endometrium. *Trends Endocrinol Metab*, 12, 147-51(2001)
6. Creamer, D., D. Sullivan, R. Bicknell & J. Barker: Angiogenesis in psoriasis. *Angiogenesis*, 5, 231-6(2002)
7. Funatsu, H., H. Yamashita, H. Noma, E. Shimizu, T. Yamashita & S. Hori: Stimulation and inhibition of angiogenesis in diabetic retinopathy. *Jpn J Ophthalmol*, 45, 577-84(2001)
8. Koch, A. E.: The role of angiogenesis in rheumatoid arthritis: recent developments. *Ann Rheum Dis*, 59 Suppl 1, i65-71(2000)
9. Tonini, T., F. Rossi & P. P. Claudio: Molecular basis of angiogenesis and cancer. *Oncogene*, 22, 6549-56(2003)
10. Arbiser, J. L.: Molecular regulation of angiogenesis and tumorigenesis by signal transduction pathways: evidence of predictable and reproducible patterns of synergy in diverse neoplasms. *Semin Cancer Biol*, 14, 81-91(2004)
11. Folkman, J.: Tumor angiogenesis: therapeutic implications. *N Engl J Med*, 285, 1182-6(1971)
12. Folkman, J.: Role of angiogenesis in tumor growth and metastasis. *Semin Oncol*, 29, 15-8(2002)

13. Folkman, J.: Angiogenesis-dependent diseases. *Semin Oncol*, 28, 536-42(2001)
14. Folkman, J. & D. Hanahan: Switch to the angiogenic phenotype during tumorigenesis. *Princess Takamatsu Symp*, 22, 339-47(1991)
15. Giordano, F. J. & R. S. Johnson: Angiogenesis: the role of the microenvironment in flipping the switch. *Curr Opin Genet Dev*, 11, 35-40(2001)
16. Huss, W. J., C. F. Hanrahan, R. J. Barrios, J. W. Simons & N. M. Greenberg: Angiogenesis and prostate cancer: identification of a molecular progression switch. *Cancer Res*, 61, 2736-43(2001)
17. Bergers, G. & L. E. Benjamin: Tumorigenesis and the angiogenic switch. *Nat Rev Cancer*, 3, 401-10(2003)
18. Fong, T. A., L. K. Shawver, L. Sun, C. Tang, H. App, T. J. Powell, Y. H. Kim, R. Schreck, X. Wang, W. Risau, A. Ullrich, K. P. Hirth & G. McMahon: SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res*, 59, 99-106(1999)
19. Blagosklonny, M. V.: Antiangiogenic therapy and tumor progression. *Cancer Cell*, 5, 13-7(2004)
20. Ferrara, N.: Vascular endothelial growth factor and the regulation of angiogenesis. *Recent Prog Horm Res*, 55, 15-35; discussion 35-6(2000)
21. Karkkainen, M. J. & T. V. Petrova: Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. *Oncogene*, 19, 5598-605(2000)
22. Tille, J. C., X. Wang, K. E. Lipson, G. McMahon, N. Ferrara, Z. Zhu, D. J. Hicklin, J. P. Sleeman, U. Eriksson, K. Alitalo & M. S. Pepper: Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates VEGF-C(deltaNdeltaC)- and VEGF-A-induced angiogenesis in vitro. *Exp Cell Res*, 285, 286-98(2003)
23. Veikkola, T., M. Karkkainen, L. Claesson-Welsh & K. Alitalo: Regulation of angiogenesis via vascular endothelial growth factor receptors. *Cancer Res*, 60, 203-12(2000)
24. Hanrahan, V., M. J. Currie, S. P. Gunningham, H. R. Morrin, P. A. Scott, B. A. Robinson & S. B. Fox: The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. *J Pathol*, 200, 183-94(2003)
25. Klagsbrun, M. & P. A. D'Amore: Vascular endothelial growth factor and its receptors. *Cytokine Growth Factor Rev*, 7, 259-70(1996)
26. Neufeld, G., T. Cohen, S. Gengrinovitch & Z. Poltorak: Vascular endothelial growth factor (VEGF) and its receptors. *Faseb J*, 13, 9-22(1999)
27. Connolly, D. T., J. V. Olander, D. Heuvelman, R. Nelson, R. Monsell, N. Siegel, B. L. Haymore, R. Leimgruber & J. Feder: Human vascular permeability factor. Isolation from U937 cells. *J Biol Chem*, 264, 20017-24(1989)
28. Ferrara, N. & W. J. Henzel: Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun*, 161, 851-8(1989)
29. Keck, P. J., S. D. Hauser, G. Krivi, K. Sanzo, T. Warren, J. Feder & D. T. Connolly: Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*, 246, 1309-12(1989)
30. Leung, D. W., G. Cachianes, W. J. Kuang, D. V. Goeddel & N. Ferrara: Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*, 246, 1306-9(1989)
31. Senger, D. R., B. B. Asch, B. D. Smith, C. A. Perruzzi & H. F. Dvorak: A secreted phosphoprotein marker for neoplastic transformation of both epithelial and fibroblastic cells. *Nature*, 302, 714-5(1983)
32. Houck, K. A., N. Ferrara, J. Winer, G. Cachianes, B. Li & D. W. Leung: The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol*, 5, 1806-14(1991)
33. Tischer, E., R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J. C. Fiddes & J. A. Abraham: The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem*, 266, 11947-54(1991)
34. Keyt, B. A., L. T. Berleau, H. V. Nguyen, H. Chen, H. Heinsohn, R. Vandlen & N. Ferrara: The carboxyl-terminal domain (111-165) of vascular endothelial growth factor is critical for its mitogenic potency. *J Biol Chem*, 271, 7788-95(1996)
35. Keyt, B. A., H. V. Nguyen, L. T. Berleau, C. M. Duarte, J. Park, H. Chen & N. Ferrara: 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*, 271, 5638-46(1996)
36. Soker, S., S. Gollamudi-Payne, H. Fidder, H. Charmahelli & M. Klagsbrun: Inhibition of vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation by a peptide corresponding to the exon 7-encoded domain of VEGF165. *J Biol Chem*, 272, 31582-8(1997)
37. Maglione, D., V. Guerriero, G. Viglietto, P. Delli-Bovi & M. G. Persico: Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci U S A*, 88, 9267-71(1991)
38. Olofsson, B., K. Pajusola, A. Kaipainen, G. von Euler, V. Joukov, O. Saksela, A. Orpana, R. F. Pettersson, K. Alitalo & U. Eriksson: Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc Natl Acad Sci U S A*, 93, 2576-81(1996)
39. Kukk, E., A. Lymboussaki, S. Taira, A. Kaipainen, M. Jeltsch, V. Joukov & K. Alitalo: VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development*, 122, 3829-37(1996)
40. Onogawa, S., Y. Kitadai, S. Tanaka, T. Kuwai, S. Kimura & K. Chayama: Expression of VEGF-C and VEGF-D at the invasive edge correlates with lymph node metastasis and prognosis of patients with colorectal carcinoma. *Cancer Sci*, 95, 32-9(2004)
41. Li, X. & U. Eriksson: Novel VEGF family members: VEGF-B, VEGF-C and VEGF-D. *Int J Biochem Cell Biol*, 33, 421-6(2001)
42. Achen, M. G., M. Jeltsch, E. Kukk, T. Makinen, A. Vitali, A. F. Wilks, K. Alitalo & S. A. Stacker: 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 U S A*, 95, 548-53(1998)
43. Krishnan, J., V. Kirkin, A. Steffen, M. Hegen, D. Weih, S. Tomarev, J. Wilting & J. P. Sleeman: Differential in vivo and in vitro expression of vascular endothelial growth factor (VEGF)-C and VEGF-D in tumors and its relationship to lymphatic metastasis in immunocompetent rats. *Cancer Res*, 63, 713-22(2003)
44. Wong, D., J. Luckhurst, H. Toma, N. Kuipers, S. Loo, A. Suarez, J. E. Wilson & B. M. McManus: Vascular endothelial growth factor-D (VEGF-D) is an endothelial hyperpermeability inducing growth factor differentially expressed in human cardiac allografts. *J Heart Lung Transplant*, 20, 156(2001)
45. Yamada, Y., J. Nezu, M. Shimane & Y. Hirata: Molecular cloning of a novel vascular endothelial growth factor, VEGF-D. *Genomics*, 42, 483-8(1997)
46. Van Trappen, P. O., D. Steele, D. G. Lowe, S. Baithun, N. Beasley, W. Thiele, H. Weich, J. Krishnan, J. H. Shepherd, M. S. Pepper, D. G. Jackson, J. P. Sleeman & I. J. Jacobs: Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis. *J Pathol*, 201, 544-54(2003)
47. Lyttle, D. J., K. M. Fraser, S. B. Fleming, A. A. Mercer & A. J. Robinson: Homologs of vascular endothelial growth factor are encoded by the poxvirus orf virus. *J Virol*, 68, 84-92(1994)
48. Kiba, A., H. Sagara, T. Hara & M. Shibuya: VEGFR-2-specific ligand VEGF-E induces non-edematous hyper-vascularization in mice. *Biochem Biophys Res Commun*, 301, 371-7(2003)
49. Meyer, M., M. Clauss, A. Lepple-Wienhues, J. Waltenberger, H. G. Augustin, M. Ziche, C. Lanz, M. Buttner, H. J. Rziha & C. Dehio: A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. *Embo J*, 18, 363-74(1999)
50. Ogawa, S., A. Oku, A. Sawano, S. Yamaguchi, Y. Yazaki & M. Shibuya: A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain. *J Biol Chem*, 273, 31273-82(1998)
51. Shibuya, M.: Vascular endothelial growth factor receptor-2: its unique signaling and specific ligand, VEGF-E. *Cancer Sci*, 94, 751-6(2003)
52. Ferrara, N., H. P. Gerber & J. LeCouter: The biology of VEGF and its receptors. *Nat Med*, 9, 669-76(2003)
53. Robinson, C. J. & S. E. Stringer: The splice variants of vascular endothelial growth factor (VEGF) and their receptors. *J Cell Sci*, 114, 853-65(2001)
54. Barleon, B., G. Siemeister, G. Martiny-Baron, K. Weindel, C. Herzog & D. Marme: Vascular endothelial growth factor up-regulates its receptor fms-like tyrosine kinase 1 (FLT-1) and a soluble variant of FLT-1 in human vascular endothelial cells. *Cancer Res*, 57, 5421-5(1997)
55. de Vries, C., J. A. Escobedo, H. Ueno, K. Houck, N. Ferrara & L. T. Williams: The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science*, 255, 989-91(1992)
56. Millauer, B., S. Wizigmann-Voos, H. Schnurch, R. Martinez, N. P. Moller, W. Risau & A. Ullrich: High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell*, 72, 835-46(1993)
57. Terman, B. I., M. Dougher-Vermazen, M. E. Carrion, D. Dimitrov, D. C. Armellino, D. Gospodarowicz & P. Bohlen: Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem Biophys Res Commun*, 187, 1579-86(1992)
58. Lenton, K.: VEGFR-2 (KDR/Flk-1). *J Biol Regul Homeost Agents*, 16, 227-32(2002)
59. Meyer, R. D. & N. Rahimi: Comparative structure-function analysis of VEGFR-1 and VEGFR-2: What have we learned from chimeric systems? *Ann N Y Acad Sci*, 995, 200-7(2003)
60. Birnbaum, D.: VEGF-FLT1 receptor system: a new ligand-receptor system involved in normal and tumor angiogenesis. *Jpn J Cancer Res*, 86, inside cover(1995)
61. Terman, B., L. Khandke, M. Dougher-Vermazan, D. Maglione, N. J. Lassam, D. Gospodarowicz, M. G. Persico, P. Bohlen & M. Eisinger: VEGF receptor subtypes KDR and FLT1 show different sensitivities to heparin and placenta growth factor. *Growth Factors*, 11, 187-95(1994)
62. Wang, D., D. B. Donner & R. S. Warren: Homeostatic modulation of cell surface KDR and Flt1 expression and expression of the vascular endothelial cell growth factor (VEGF) receptor mRNAs by VEGF. *J Biol Chem*, 275, 15905-11(2000)
63. Peters, K. G., C. De Vries & L. T. Williams: Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. *Proc Natl Acad Sci U S A*, 90, 8915-9(1993)
64. Quinn, T. P., K. G. Peters, C. De Vries, N. Ferrara & L. T. Williams: Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. *Proc Natl Acad Sci U S A*, 90, 7533-7(1993)
65. Sato, Y., S. Kanno, N. Oda, M. Abe, M. Ito, K. Shitara & M. Shibuya: Properties of two VEGF receptors, Flt-1 and KDR, in signal transduction. *Ann N Y Acad Sci*, 902, 201-5; discussion 205-7(2000)
66. Galland, F., A. Karamysheva, M. J. Pebusque, J. P. Borg, R. Rottapel, P. Dubreuil, O. Rosnet & D. Birnbaum: The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. *Oncogene*, 8, 1233-40(1993)
67. Kaipainen, A., J. Korhonen, K. Pajusola, O. Aprelikova, M. G. Persico, B. I. Terman & K. Alitalo: The related FLT4, FLT1, and KDR receptor tyrosine kinases show distinct expression patterns in human fetal endothelial cells. *J Exp Med*, 178, 2077-88(1993)
68. Pajusola, K., O. Aprelikova, J. Korhonen, A. Kaipainen, L. Pertovaara, R. Alitalo & K. Alitalo: FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. *Cancer Res*, 52, 5738-43(1992)
69. Andre, T., L. Kotelevets, J. C. Vaillant, A. M. Coudray, L. Weber, S. Prevot, R. Parc, C. Gespach & E. Chastre: Vegf, Vegf-B, Vegf-C and their receptors KDR, FLT-1 and FLT-4 during the neoplastic progression of human colonic mucosa. *Int J Cancer*, 86, 174-81(2000)

70. Hewett, P. W. & J. C. Murray: Coexpression of flt-1, flt-4 and KDR in freshly isolated and cultured human endothelial cells. *Biochem Biophys Res Commun*, 221, 697-702(1996)
71. Smith, G., D. McLeod, D. Foreman & M. Boulton: Immunolocalisation of the VEGF receptors FLT-1, KDR, and FLT-4 in diabetic retinopathy. *Br J Ophthalmol*, 83, 486-94(1999)
72. Hughes, D. C.: Alternative splicing of the human VEGFGR-3/FLT4 gene as a consequence of an integrated human endogenous retrovirus. *J Mol Evol*, 53, 77-9(2001)
73. Joukov, V., K. Pajusola, A. Kaipainen, D. Chilov, I. Lahtinen, E. Kukk, O. Saksela, N. Kalkkinen & K. Alitalo: A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *Embo J*, 15, 290-98(1996)
74. Lee, J., A. Gray, J. Yuan, S. M. Luoh, H. Avraham & W. I. Wood: Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. *Proc Natl Acad Sci U S A*, 93, 1988-92(1996)
75. Wise, L. M., T. Veikkola, A. A. Mercer, L. J. Savory, S. B. Fleming, C. Caesar, A. Vitali, T. Makinen, K. Alitalo & S. A. Stacker: Vascular endothelial growth factor (VEGF)-like protein from orf virus NZ2 binds to VEGFR2 and neuropilin-1. *Proc Natl Acad Sci U S A*, 96, 3071-6(1999)
76. Romeo, P. H., V. Lemarchandel & R. Tordjman: Neuropilin-1 in the immune system. *Adv Exp Med Biol*, 515, 49-54(2002)
77. Tordjman, R., Y. Lepelletier, V. Lemarchandel, M. Cambot, P. Gaulard, O. Hermine & P. H. Romeo: A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. *Nat Immunol*, 3, 477-82(2002)
78. Mamluk, R., Z. Gechtman, M. E. Kutcher, N. Gasiunas, J. Gallagher & M. Klagsbrun: Neuropilin-1 binds vascular endothelial growth factor 165, placenta growth factor-2, and heparin via its b1b2 domain. *J Biol Chem*, 277, 24818-25(2002)
79. Migdal, M., B. Huppertz, S. Tessler, A. Comforti, M. Shibuya, R. Reich, H. Baumann & G. Neufeld: Neuropilin-1 is a placenta growth factor-2 receptor. *J Biol Chem*, 273, 22272-8(1998)
80. Gluzman-Poltorak, Z., T. Cohen, Y. Herzog & G. Neufeld: Neuropilin-2 is a receptor for the vascular endothelial growth factor (VEGF) forms VEGF-145 and VEGF-165 [corrected]. *J Biol Chem*, 275, 18040-5(2000)
81. Gluzman-Poltorak, Z., T. Cohen, Y. Herzog & G. Neufeld: Neuropilin-2 is a receptor for the vascular endothelial growth factor (VEGF) forms VEGF-145 and VEGF-165. *J Biol Chem*, 275, 29922(2000)
82. Soker, S., S. Takashima, H. Q. Miao, G. Neufeld & M. Klagsbrun: Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell*, 92, 735-45(1998)
83. Neufeld, G., O. Kessler & Y. Herzog: The interaction of Neuropilin-1 and Neuropilin-2 with tyrosine-kinase receptors for VEGF. *Adv Exp Med Biol*, 515, 81-90(2002)
84. Kreuter, M., D. Bielenberg, Y. Hida, K. Hida & M. Klagsbrun: Role of neuropilins and semaphorins in angiogenesis and cancer. *Ann Hematol*, 81 Suppl 2, S74(2002)
85. Nakamura, F. & Y. Goshima: Structural and functional relation of neuropilins. *Adv Exp Med Biol*, 515, 55-69(2002)
86. Neufeld, G., T. Cohen, N. Shraga, T. Lange, O. Kessler & Y. Herzog: The neuropilins: multifunctional semaphorin and VEGF receptors that modulate axon guidance and angiogenesis. *Trends Cardiovasc Med*, 12, 13-9(2002)
87. Ikeda, M., Y. Hosoda, S. Hirose, Y. Okada & E. Ikeda: Expression of vascular endothelial growth factor isoforms and their receptors Flt-1, KDR, and neuropilin-1 in synovial tissues of rheumatoid arthritis. *J Pathol*, 191, 426-33(2000)
88. Gagnon, M. L., D. R. Bielenberg, Z. Gechtman, H. Q. Miao, S. Takashima, S. Soker & M. Klagsbrun: Identification of a natural soluble neuropilin-1 that binds vascular endothelial growth factor: In vivo expression and antitumor activity. *Proc Natl Acad Sci U S A*, 97, 2573-8(2000)
89. Lee, P., K. Goishi, A. J. Davidson, R. Mannix, L. Zon & M. Klagsbrun: Neuropilin-1 is required for vascular development and is a mediator of VEGF-dependent angiogenesis in zebrafish. *Proc Natl Acad Sci U S A*, 99, 10470-5(2002)
90. Oh, H., H. Takagi, A. Otani, S. Koyama, S. Kemmochi, A. Uemura & Y. Honda: Selective induction of neuropilin-1 by vascular endothelial growth factor (VEGF): a mechanism contributing to VEGF-induced angiogenesis. *Proc Natl Acad Sci U S A*, 99, 383-8(2002)
91. Whitaker, G. B., B. J. Limberg & J. S. Rosenbaum: Vascular endothelial growth factor receptor-2 and neuropilin-1 form a receptor complex that is responsible for the differential signaling potency of VEGF(165) and VEGF(121). *J Biol Chem*, 276, 25520-31(2001)
92. Gill, M., S. Dias, K. Hattori, M. L. Rivera, D. Hicklin, L. Witte, L. Girardi, R. Yurt, H. Himel & S. Rafii: Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. *Circ Res*, 88, 167-74(2001)
93. Peichev, M., A. J. Naiyer, D. Pereira, Z. Zhu, W. J. Lane, M. Williams, M. C. Oz, D. J. Hicklin, L. Witte, M. A. Moore & S. Rafii: Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood*, 95, 952-8(2000)
94. Solovey, A., Y. Lin, P. Browne, S. Choong, E. Wayner & R. P. Hebbel: Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med*, 337, 1584-90(1997)
95. Oberg, C., J. Waltenberger, L. Claesson-Welsh & M. Welsh: Expression of protein tyrosine kinases in islet cells: possible role of the Flk-1 receptor for beta-cell maturation from duct cells. *Growth Factors*, 10, 115-26(1994)
96. Yang, K. & C. L. Cepko: Flk-1, a receptor for vascular endothelial growth factor (VEGF), is expressed by retinal progenitor cells. *J Neurosci*, 16, 6089-99(1996)
97. Katoh, O., H. Tauchi, K. Kawaishi, A. Kimura & Y. Satow: Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. *Cancer Res*, 55, 5687-92(1995)
98. Brown, L. F., M. Detmar, K. Tognazzi, G. Abu-Jawdeh & M. L. Iruela-Arispe: Uterine smooth muscle cells express functional receptors (flt-1 and KDR) for vascular permeability factor/vascular endothelial growth factor. *Lab Invest*, 76, 245-55(1997)
99. Hatva, E., A. Kaipainen, P. Mentula, J. Jaaskelainen, A. Paetau, M. Haltia & K. Alitalo: Expression of endothelial cell-

specific receptor tyrosine kinases and growth factors in human brain tumors. *Am J Pathol*, 146, 368-78(1995)
100. Boocock, C. A., D. S. Charnock-Jones, A. M. Sharkey, J. McLaren, P. J. Barker, K. A. Wright, P. R. Twentyman & S. K. Smith: Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. *J Natl Cancer Inst*, 87, 506-16(1995)
101. Carmeliet, P., V. Ferreira, G. Breier, S. Pollefeyt, L. Kieckens, M. Gertsenstein, M. Fahrig, A. Vandenhoeck, K. Harpal, C. Eberhardt, C. Declercq, J. Pawling, L. Moons, D. Collen, W. Risau & A. Nagy: Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature*, 380, 435-9(1996)
102. Shalaby, F., J. Ho, W. L. Stanford, K. D. Fischer, A. C. Schuh, L. Schwartz, A. Bernstein & J. Rossant: A requirement for Flk1 in primitive and definitive hematopoiesis and vasculogenesis. *Cell*, 89, 981-90(1997)
103. Shalaby, F., J. Rossant, T. P. Yamaguchi, M. Gertsenstein, X. F. Wu, M. L. Breitman & A. C. Schuh: Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature*, 376, 62-6(1995)
104. Matthews, W., C. T. Jordan, M. Gavin, N. A. Jenkins, N. G. Copeland & I. R. Lemischka: A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. *Proc Natl Acad Sci U S A*, 88, 9026-30(1991)
105. Cunningham, S. A., C. C. Stephan, M. P. Arrate, K. G. Ayer & T. A. Brock: Identification of the extracellular domains of Flt-1 that mediate ligand interactions. *Biochem Biophys Res Commun*, 231, 596-9(1997)
106. Fuh, G., B. Li, C. Crowley, B. Cunningham & J. A. Wells: Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. *J Biol Chem*, 273, 11197-204(1998)
107. Lu, D., P. Kussie, B. Pytowski, K. Persaud, P. Bohlen, L. Witte & Z. Zhu: Identification of the residues in the extracellular region of KDR important for interaction with vascular endothelial growth factor and neutralizing anti-KDR antibodies. *J Biol Chem*, 275, 14321-30(2000)
108. Muller, Y. A., B. Li, H. W. Christinger, J. A. Wells, B. C. Cunningham & A. M. de Vos: Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. *Proc Natl Acad Sci U S A*, 94, 7192-7(1997)
109. Shinkai, A., M. Ito, H. Anazawa, S. Yamaguchi, K. Shitara & M. Shibuya: Mapping of the sites involved in ligand association and dissociation at the extracellular domain of the kinase insert domain-containing receptor for vascular endothelial growth factor. *J Biol Chem*, 273, 31283-8(1998)
110. Wiesmann, C., G. Fuh, H. W. Christinger, C. Eigenbrot, J. A. Wells & A. M. de Vos: Crystal structure at 1.7 A resolution of VEGF in complex with domain 2 of the Flt-1 receptor. *Cell*, 91, 695-704(1997)
111. Kroll, J. & J. Waltenberger: The vascular endothelial growth factor receptor KDR activates multiple signal transduction pathways in porcine aortic endothelial cells. *J Biol Chem*, 272, 32521-7(1997)
112. Seetharam, L., N. Gotoh, Y. Maru, G. Neufeld, S. Yamaguchi & M. Shibuya: A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. *Oncogene*, 10, 135-47(1995)
113. Waltenberger, J., L. Claesson-Welsh, A. Siegbahn, M. Shibuya & C. H. Heldin: Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem*, 269, 26988-95(1994)
114. Ito, N., C. Wernstedt, U. Engstrom & L. Claesson-Welsh: Identification of vascular endothelial growth factor receptor-1 tyrosine phosphorylation sites and binding of SH2 domain-containing molecules. *J Biol Chem*, 273, 23410-8(1998)
115. Abedi, H. & I. Zachary: Vascular endothelial growth factor stimulates tyrosine phosphorylation and recruitment to new focal adhesions of focal adhesion kinase and paxillin in endothelial cells. *J Biol Chem*, 272, 15442-51(1997)
116. Gerber, H. P., A. McMurtrey, J. Kowalski, M. Yan, B. A. Keyt, V. Dixit & N. Ferrara: Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem*, 273, 30336-43(1998)
117. Guo, D., Q. Jia, H. Y. Song, R. S. Warren & D. B. Donner: Vascular endothelial cell growth factor promotes tyrosine phosphorylation of mediators of signal transduction that contain SH2 domains. Association with endothelial cell proliferation. *J Biol Chem*, 270, 6729-33(1995)
118. Takahashi, T. & M. Shibuya: 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*, 14, 2079-89(1997)
119. Rousseau, S., F. Houle, H. Kotanides, L. Witte, J. Waltenberger, J. Landry & J. Huot: Vascular endothelial growth factor (VEGF)-driven actin-based motility is mediated by VEGFR2 and requires concerted activation of stress-activated protein kinase 2 (SAPK2/p38) and geldanamycin-sensitive phosphorylation of focal adhesion kinase. *J Biol Chem*, 275, 10661-72(2000)
120. Warner, A. J., J. Lopez-Dee, E. L. Knight, J. R. Feramisco & S. A. Prigent: The Shc-related adaptor protein, Sck, forms a complex with the vascular-endothelial-growth-factor receptor KDR in transfected cells. *Biochem J*, 347, 501-9(2000)
121. Wu, L. W., L. D. Mayo, J. D. Dunbar, K. M. Kessler, O. N. Ozes, R. S. Warren & D. B. Donner: VRAP is an adaptor protein that binds KDR, a receptor for vascular endothelial cell growth factor. *J Biol Chem*, 275, 6059-62(2000)
122. Takahashi, N., Y. Seko, E. Noiri, K. Tobe, T. Kadowaki, H. Sabe & Y. Yazaki: Vascular endothelial growth factor induces activation and subcellular translocation of focal adhesion kinase (p125FAK) in cultured rat cardiac myocytes. *Circ Res*, 84, 1194-202(1999)
123. Takahashi, T. & M. Shibuya: The overexpression of PKCdelta is involved in vascular endothelial growth factor-resistant apoptosis in cultured primary sinusoidal endothelial cells. *Biochem Biophys Res Commun*, 280, 415-20(2001)
124. Takahashi, T., H. Ueno & M. Shibuya: VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. *Oncogene*, 18, 2221-30(1999)

125. Takahashi, T., S. Yamaguchi, K. Chida & M. Shibuya: A single autophosphorylation site on KDR/Flk-1 is essential for VEGF-A-dependent activation of PLC-gamma and DNA synthesis in vascular endothelial cells. *Embo J*, 20, 2768-78(2001)
126. Eliceiri, B. P., R. Paul, P. L. Schwartzberg, J. D. Hood, J. Leng & D. A. Cheresh: Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol Cell*, 4, 915-24(1999)
127. Eliceiri, B. P., X. S. Puente, J. D. Hood, D. G. Stupack, D. D. Schlaepfer, X. Z. Huang, D. Sheppard & D. A. Cheresh: Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. *J Cell Biol*, 157, 149-60(2002)
128. Igarashi, K., T. Isohara, T. Kato, K. Takigawa, K. Shigeta, T. Yamano & I. I. Uno: 8-(3-oxo-4,5,6-trihydroxy-3h-xanthen-9-yl)-1-naphthoic acid inhibits MAPK phosphorylation in endothelial cells induced by VEGF and bFGF. *Int J Mol Med*, 2, 211-215(1998)
129. Igarashi, K., K. Shigeta, T. Isohara, T. Yamano & I. Uno: Sck interacts with KDR and Flt-1 via its SH2 domain. *Biochem Biophys Res Commun*, 251, 77-82(1998)
130. Dimmeler, S., E. Dernbach & A. M. Zeiher: Phosphorylation of the endothelial nitric oxide synthase at ser-1177 is required for VEGF-induced endothelial cell migration. *FEBS Lett*, 477, 258-62(2000)
131. Gliki, G., R. Abu-Ghazaleh, S. Jezequel, C. Wheeler-Jones & I. Zachary: Vascular endothelial growth factor-induced prostacyclin production is mediated by a protein kinase C (PKC)-dependent activation of extracellular signal-regulated protein kinases 1 and 2 involving PKC-delta and by mobilization of intracellular Ca2+. *Biochem J*, 353, 503-12(2001)
132. Hood, J. & H. J. Granger: Protein kinase G mediates vascular endothelial growth factor-induced Raf-1 activation and proliferation in human endothelial cells. *J Biol Chem*, 273, 23504-8(1998)
133. Zachary, I. & G. Gliki: Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. *Cardiovasc Res*, 49, 568-81(2001)
134. Gille, H., J. Kowalski, B. Li, J. LeCouter, B. Moffat, T. F. Zioncheck, N. Pelletier & N. Ferrara: Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2). A reassessment using novel receptor-specific vascular endothelial growth factor mutants. *J Biol Chem*, 276, 3222-30(2001)
135. Li, B., G. Fuh, G. Meng, X. Xin, M. E. Gerritsen, B. Cunningham & A. M. de Vos: Receptor-selective variants of human vascular endothelial growth factor. Generation and characterization. *J Biol Chem*, 275, 29823-8(2000)
136. Larrivee, B. & A. Karsan: Signaling pathways induced by vascular endothelial growth factor (review). *Int J Mol Med*, 5, 447-56(2000)
137. Brown, L. F., B. Berse, R. W. Jackman, K. Tognazzi, A. J. Guidi, H. F. Dvorak, D. R. Senger, J. L. Connolly & S. J. Schnitt: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. *Hum Pathol*, 26, 86-91(1995)
138. Brown, L. F., B. Berse, R. W. Jackman, K. Tognazzi, E. J. Manseau, H. F. Dvorak & D. R. Senger: Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. *Am J Pathol*, 143, 1255-62(1993)
139. Brown, L. F., B. Berse, R. W. Jackman, K. Tognazzi, E. J. Manseau, D. R. Senger & H. F. Dvorak: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res*, 53, 4727-35(1993)
140. Brown, L. F., B. Berse, K. Tognazzi, E. J. Manseau, L. Van de Water, D. R. Senger, H. F. Dvorak & S. Rosen: Vascular permeability factor mRNA and protein expression in human kidney. *Kidney Int*, 42, 1457-61(1992)
141. Brown, L. F., T. J. Harrist, K. T. Yeo, M. Stahle-Backdahl, R. W. Jackman, B. Berse, K. Tognazzi, H. F. Dvorak & M. Detmar: Increased expression of vascular permeability factor (vascular endothelial growth factor) in bullous pemphigoid, dermatitis herpetiformis, and erythema multiforme. *J Invest Dermatol*, 104, 744-9(1995)
142. Brown, L. F., K. Tognazzi, H. F. Dvorak & T. J. Harrist: Strong expression of kinase insert domain-containing receptor, a vascular permeability factor/vascular endothelial growth factor receptor in AIDS-associated Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol*, 148, 1065-74(1996)
143. Guidi, A. J., G. Abu-Jawdeh, B. Berse, R. W. Jackman, K. Tognazzi, H. F. Dvorak & L. F. Brown: Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in cervical neoplasia. *J Natl Cancer Inst*, 87, 1237-45(1995)
144. Guidi, A. J., G. Abu-Jawdeh, K. Tognazzi, H. F. Dvorak & L. F. Brown: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in endometrial carcinoma. *Cancer*, 78, 454-60(1996)
145. Guidi, A. J., S. J. Schnitt, L. Fischer, K. Tognazzi, J. R. Harris, H. F. Dvorak & L. F. Brown: Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. *Cancer*, 80, 1945-53(1997)
146. Olson, T. A., D. Mohanraj, L. F. Carson & S. Ramakrishnan: Vascular permeability factor gene expression in normal and neoplastic human ovaries. *Cancer Res*, 54, 276-80(1994)
147. Plate, K. H., G. Breier, B. Millauer, A. Ullrich & W. Risau: Up-regulation of vascular endothelial growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis. *Cancer Res*, 53, 5822-7(1993)
148. Wizigmann-Voos, S., G. Breier, W. Risau & K. H. Plate: Up-regulation of vascular endothelial growth factor and its receptors in von Hippel-Lindau disease-associated and sporadic hemangioblastomas. *Cancer Res*, 55, 1358-64(1995)
149. Barnhill, R. L., K. Fandrey, M. A. Levy, M. C. Mihm, Jr. & B. Hyman: Angiogenesis and tumor progression of melanoma. Quantification of vascularity in melanocytic nevi and cutaneous malignant melanoma. *Lab Invest*, 67, 331-7(1992)
150. Barnhill, R. L., M. W. Piepkorn, A. J. Cochran, E. Flynn, T. Karaoli & J. Folkman: Tumor vascularity, proliferation, and apoptosis in human melanoma micrometastases and macrometastases. *Arch Dermatol*, 134, 991-4(1998)

151. Gasparini, G., M. Barbareschi, P. Boracchi, P. Verderio, O. Caffo, S. Meli, P. D. Palma, E. Marubini & P. Bevilacqua: Tumor Angiogenesis Predicts Clinical Outcome of Node-Positive Breast Cancer Patients Treated With Adjuvant Hormone Therapy or Chemotherapy. *Cancer J Sci Am*, 1, 131(1995)
152. Gasparini, G. & A. L. Harris: Clinical importance of the determination of tumor angiogenesis in breast carcinoma: much more than a new prognostic tool. *J Clin Oncol*, 13, 765-82(1995)
153. Gasparini, G., M. Toi, P. Verderio, G. Ranieri, S. Dante, E. Bonoldi, P. Boracchi, M. Fanelli & T. Tominaga: Prognostic significance of p53, angiogenesis, and other conventional features in operable breast cancer: subanalysis in node-positive and node-negative patients. *Int J Oncol*, 12, 1117-25(1998)
154. Gasparini, G., N. Weidner, S. Maluta, F. Pozza, P. Boracchi, M. Mezzetti, A. Testolin & P. Bevilacqua: Intratumoral microvessel density and p53 protein: correlation with metastasis in head-and-neck squamous-cell carcinoma. *Int J Cancer*, 55, 739-44(1993)
155. Lindmark, G., B. Gerdin, L. Pahlman, R. Bergstrom & B. Glimelius: Prognostic predictors in colorectal cancer. *Dis Colon Rectum*, 37, 1219-27(1994)
156. Lindmark, G., B. Gerdin, C. Sundberg, L. Pahlman, R. Bergstrom & B. Glimelius: Prognostic significance of the microvascular count in colorectal cancer. *J Clin Oncol*, 14, 461-6(1996)
157. Macchiarini, P., G. Fontanini, M. J. Hardin, F. Squartini & C. A. Angeletti: Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet*, 340, 145-6(1992)
158. Olivarez, D., T. Ulbright, W. DeRiese, R. Foster, T. Reister, L. Einhorn & G. Sledge: Neovascularization in clinical stage A testicular germ cell tumor: prediction of metastatic disease. *Cancer Res*, 54, 2800-2(1994)
159. Tanigawa, N., H. Amaya, M. Matsumura, T. Shimomatsuya, T. Horiuchi, R. Muraoka & M. Iki: Extent of tumor vascularization correlates with prognosis and hematogenous metastasis in gastric carcinomas. *Cancer Res*, 56, 2671-6(1996)
160. Tanigawa, N., M. Matsumura, H. Amaya, A. Kitaoka, T. Shimomatsuya, C. Lu, R. Muraoka & T. Tanaka: Tumor vascularity correlates with the prognosis of patients with esophageal squamous cell carcinoma. *Cancer*, 79, 220-5(1997)
161. Weidner, N., P. R. Carroll, J. Flax, W. Blumenfeld & J. Folkman: Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol*, 143, 401-9(1993)
162. Weidner, N., J. Folkman, F. Pozza, P. Bevilacqua, E. N. Allred, D. H. Moore, S. Meli & G. Gasparini: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. *J Natl Cancer Inst*, 84, 1875-87(1992)
163. Fong, G. H., J. Rossant, M. Gertsenstein & M. L. Breitman: Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature*, 376, 66-70(1995)
164. Hicklin, D. J., L. Witte, Z. Zhu, F. Liao, Y. Wu, Y. Li & P. Bohlen: Monoclonal antibody strategies to block angiogenesis. *Drug Discov Today*, 6, 517-528(2001)
165. Lu, D., X. Jimenez, H. Zhang, P. Bohlen, L. Witte & Z. Zhu: Selection of high affinity human neutralizing antibodies to VEGFR2 from a large antibody phage display library for antiangiogenesis therapy. *Int J Cancer*, 97, 393-9(2002)
166. Lu, D., J. Shen, M. D. Vil, H. Zhang, X. Jimenez, P. Bohlen, L. Witte & Z. Zhu: Tailoring in vitro selection for a picomolar affinity human antibody directed against vascular endothelial growth factor receptor 2 for enhanced neutralizing activity. *J Biol Chem*, 278, 43496-507(2003)
167. Posey, J. A., T. C. Ng, B. Yang, M. B. Khazaeli, M. D. Carpenter, F. Fox, M. Needle, H. Waksal & A. F. LoBuglio: A phase I study of anti-kinase insert domain-containing receptor antibody, IMC-1C11, in patients with liver metastases from colorectal carcinoma. *Clin Cancer Res*, 9, 1323-32(2003)
168. Watanabe, H., A. J. Mamelak, B. Wang, B. G. Howell, I. Freed, C. Esche, M. Nakayama, G. Nagasaki, D. J. Hicklin, R. S. Kerbel & D. N. Sauder: Anti-vascular endothelial growth factor receptor-2 (Flk-1/KDR) antibody suppresses contact hypersensitivity. *Exp Dermatol*, 13, 671-81(2004)
169. Zhu, Z., K. Hattori, H. Zhang, X. Jimenez, D. L. Ludwig, S. Dias, P. Kussie, H. Koo, H. J. Kim, D. Lu, M. Liu, R. Tejada, M. Friedrich, P. Bohlen, L. Witte & S. Rafii: Inhibition of human leukemia in an animal model with human antibodies directed against vascular endothelial growth factor receptor 2. Correlation between antibody affinity and biological activity. *Leukemia*, 17, 604-11(2003)
170. Boyer, S. J.: Small molecule inhibitors of KDR (VEGFR-2) kinase: an overview of structure activity relationships. *Curr Top Med Chem*, 2, 973-1000(2002)
171. Ciardiello, F., R. Bianco, R. Caputo, R. Caputo, V. Damiano, T. Troiani, D. Melisi, F. De Vita, S. De Placido, A. R. Bianco & G. Tortora: Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to antiepidermal growth factor receptor therapy. *Clin Cancer Res*, 10, 784-93(2004)
172. Drevs, J., R. Muller-Driver, C. Wittig, S. Fuxius, N. Esser, H. Hugenschmidt, M. A. Konerding, P. R. Allegrini, J. Wood, J. Hennig, C. Unger & D. Marme: PTK787/ZK 222584, a specific vascular endothelial growth factor-receptor tyrosine kinase inhibitor, affects the anatomy of the tumor vascular bed and the functional vascular properties as detected by dynamic enhanced magnetic resonance imaging. *Cancer Res*, 62, 4015-22(2002)
173. Fraley, M. E., W. F. Hoffman, K. L. Arrington, R. W. Hungate, G. D. Hartman, R. C. McFall, K. E. Coll, K. Rickert, K. A. Thomas & G. B. McGaughey: Property-based design of KDR kinase inhibitors. *Curr Med Chem*, 11, 709-19(2004)
174. Gingrich, D. E., D. R. Reddy, M. A. Iqbal, J. Singh, L. D. Aimone, T. S. Angeles, M. Albom, S. Yang, M. A. Ator, S. L. Meyer, C. Robinson, B. A. Ruggeri, C. A. Dionne, J. L. Vaught, J. P. Mallamo & R. L. Hudkins: A new class of potent vascular endothelial growth factor receptor tyrosine kinase inhibitors: structure-activity relationships for a series of 9-alkoxymethyl-12-(3-hydroxypropyl)indeno[2,1-a]pyrrolo[3,4-c]carbazole-5- ones and the identification of CEP-5214 and its dimethylglycine ester prodrug clinical candidate CEP-7055. *J Med Chem*, 46, 5375-88(2003)
175. Laird, A. D. & J. M. Cherrington: Small molecule tyrosine kinase inhibitors: clinical development of

anticancer agents. *Expert Opin Investig Drugs*, 12, 51-64(2003)
176. Shawver, L. K., D. Slamon & A. Ullrich: Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell*, 1, 117-23(2002)
177. Traxler, P., P. R. Allegrini, R. Brandt, J. Brueggen, R. Cozens, D. Fabbro, K. Grosios, H. A. Lane, P. McSheehy, J. Mestan, T. Meyer, C. Tang, M. Wartmann, J. Wood & G. Caravatti: AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res*, 64, 4931-41(2004)
178. Dias, S., K. Hattori, Z. Zhu, B. Heissig, M. Choy, W. Lane, Y. Wu, A. Chadburn, E. Hyjek, M. Gill, D. J. Hicklin, L. Witte, M. A. Moore & S. Rafii: Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. *J Clin Invest*, 106, 511-21(2000)
179. Fiedler, W., U. Graeven, S. Ergun, S. Verago, N. Kilic, M. Stockschlader & D. K. Hossfeld: Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. *Blood*, 89, 1870-5(1997)
180. Graeven, U., W. Fiedler, S. Karpinski, S. Ergun, N. Kilic, U. Rodeck, W. Schmiegel & D. K. Hossfeld: Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR. *J Cancer Res Clin Oncol*, 125, 621-9(1999)
181. Ratajczak, M. Z., J. Ratajczak, B. Machalinski, M. Majka, W. Marlicz, A. Carter, Z. Pietrzkowski & A. M. Gewirtz: Role of vascular endothelial growth factor (VEGF) and placenta-derived growth factor (PlGF) in regulating human haemopoietic cell growth. *Br J Haematol*, 103, 969-79(1998)
182. Dias, S., K. Hattori, B. Heissig, Z. Zhu, Y. Wu, L. Witte, D. J. Hicklin, M. Tateno, P. Bohlen, M. A. Moore & S. Rafii: Inhibition of both paracrine and autocrine VEGF/ VEGFR-2 signaling pathways is essential to induce long-term remission of xenotransplanted human leukemias. *Proc Natl Acad Sci U S A*, 98, 10857-62(2001)
183. Zhang, H., Y. Li, H. Li, R. Bassi, X. Jimenez, L. Witte, P. Bohlen, D. Hicklin & Z. Zhu: Inhibition of both the autocrine and the paracrine growth of human leukemia with a fully human antibody directed against vascular endothelial growth factor receptor 2. *Leuk Lymphoma*, 45, 1887-97(2004)
184. Ria, R., A. Vacca, F. Russo, T. Cirulli, M. Massaia, P. Tosi, M. Cavo, D. Guidolin, D. Ribatti & F. Dammacco: A VEGF-dependent autocrine loop mediates proliferation and capillarogenesis in bone marrow endothelial cells of patients with multiple myeloma. *Thromb Haemost*, 92, 1438-45(2004)
185. Rockwell, P., G. Neufeld, A. Glassman, D. Caron & N. Goldstein: In vitro Neutralization of Vascular Endothelial Growth Factor Activation of Flk-1 by A Monoclonal Antibody. *Mol. Cell Differ.*, 3, 91-109(1995)
186. Witte, L., D. J. Hicklin, Z. Zhu, B. Pytowski, H. Kotanides, P. Rockwell & P. Bohlen: Monoclonal antibodies targeting the VEGF receptor-2 (Flk1/KDR) as an anti-angiogenic therapeutic strategy. *Cancer Metastasis Rev*, 17, 155-61(1998)
187. Prewett, M., J. Huber, Y. Li, A. Santiago, W. O'Connor, K. King, J. Overholser, A. Hooper, B. Pytowski, L. Witte, P. Bohlen & D. J. Hicklin: Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res*, 59, 5209-18(1999)
188. Bruns, C. J., M. Shrader, M. T. Harbison, C. Portera, C. C. Solorzano, K. W. Jauch, D. J. Hicklin, R. Radinsky & L. M. Ellis: Effect of the vascular endothelial growth factor receptor-2 antibody DC101 plus gemcitabine on growth, metastasis and angiogenesis of human pancreatic cancer growing orthotopically in nude mice. *Int J Cancer*, 102, 101-8(2002)
189. Hansen-Algenstaedt, N., B. R. Stoll, T. P. Padera, D. E. Dolmans, D. J. Hicklin, D. Fukumura & R. K. Jain: Tumor oxygenation in hormone-dependent tumors during vascular endothelial growth factor receptor-2 blockade, hormone ablation, and chemotherapy. *Cancer Res*, 60, 4556-60(2000)
190. Inoue, K., J. W. Slaton, D. W. Davis, D. J. Hicklin, D. J. McConkey, T. Karashima, R. Radinsky & C. P. Dinney: Treatment of human metastatic transitional cell carcinoma of the bladder in a murine model with the anti-vascular endothelial growth factor receptor monoclonal antibody DC101 and paclitaxel. *Clin Cancer Res*, 6, 2635-43(2000)
191. Bruns, C. J., W. Liu, D. W. Davis, R. M. Shaheen, D. J. McConkey, M. R. Wilson, C. D. Bucana, D. J. Hicklin & L. M. Ellis: Vascular endothelial growth factor is an in vivo survival factor for tumor endothelium in a murine model of colorectal carcinoma liver metastases. *Cancer*, 89, 488-499.(2000)
192. Raymond, M., R. M. Shaheen, W. W. Tseng, R. Vellagas, W. Liu, S. A. Ahmad, Y. D. Jung, N. Reinmuth, K. E. Drazan, C. D. Bucana, D. J. Hicklin & L. M. Ellis: Effects of DC101, an Antibody to the Vascular Endothelial Growth Factor Receptor, on Survival, Tumor Vascularity, and Apoptosis in a Murine Model of Colon Carcinomatosis. *Int. J. Oncol.*, 18, 221-226.(2001)
193. Shaheen, R. M., S. A. Ahmad, W. Liu, N. Reinmuth, Y. D. Jung, W. W. Tseng, K. E. Drazan, C. D. Bucana, D. J. Hicklin & L. M. Ellis: Inhibited growth of colon cancer carcinomatosis by antibodies to vascular endothelial and epidermal growth factor receptors. *Br J Cancer*, 85, 584-9(2001)
194. Shaheen, R. M., W. W. Tseng, R. Vellagas, W. Liu, S. A. Ahmad, Y. D. Jung, N. Reinmuth, K. E. Drazan, C. D. Bucana, D. J. Hicklin & L. M. Ellis: Effects of an antibody to vascular endothelial growth factor receptor-2 on survival, tumor vascularity, and apoptosis in a murine model of colon carcinomatosis. *Int J Oncol*, 18, 221-6(2001)
195. Skobe, M., P. Rockwell, N. Goldstein, S. Vosseler & N. E. Fusenig: Halting angiogenesis suppresses carcinoma cell invasion. *Nat Med*, 3, 1222-7(1997)
196. Stoelcker, B., B. Echtenacher, H. A. Weich, H. Sztajer, D. J. Hicklin & D. N. Mannel: VEGF/Flk-1 interaction, a requirement for malignant ascites recurrence. *J Interferon Cytokine Res*, 20, 511-7(2000)
197. Sweeney, P., T. Karashima, S. J. Kim, D. Kedar, B. Mian, S. Huang, C. Baker, Z. Fan, D. J. Hicklin, C. A. Pettaway & C. P. Dinney: Anti-vascular endothelial growth factor receptor 2 antibody reduces tumorigenicity and metastasis in orthotopic prostate cancer xenografts via induction of endothelial cell apoptosis and reduction of

endothelial cell matrix metalloproteinase type 9 production. *Clin Cancer Res*, 8, 2714-24(2002)
198. Yoshiji, H., S. Kuriyama, D. J. Hicklin, J. Huber, J. Yoshii, Y. Ikenaka, R. Noguchi, T. Nakatani, H. Tsujinoue & H. Fukui: The vascular endothelial growth factor receptor KDR/Flk-1 is a major regulator of malignant ascites formation in the mouse hepatocellular carcinoma model. *Hepatology*, 33, 841-7(2001)
199. Yoshiji, H., S. Kuriyama, D. J. Hicklin, J. Huber, J. Yoshii, Y. Miyamoto, M. Kawata, Y. Ikenaka, T. Nakatani, H. Tsujinoue & H. Fukui: KDR/Flk-1 is a major regulator of vascular endothelial growth factor-induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. *Hepatology*, 30, 1179-86(1999)
200. Kozin, S. V., Y. Boucher, D. J. Hicklin, P. Bohlen, R. K. Jain & H. D. Suit: Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. *Cancer Res*, 61, 39-44(2001)
201. Hanahan, D., G. Bergers & E. Bergsland: Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. *J Clin Invest*, 105, 1045-7(2000)
202. Klement, G., S. Baruchel, J. Rak, S. Man, K. Clark, D. J. Hicklin, P. Bohlen & R. S. Kerbel: Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest*, 105, R15-24(2000)
203. Klement, G., P. Huang, B. Mayer, S. K. Green, S. Man, P. Bohlen, D. Hicklin & R. S. Kerbel: Differences in therapeutic indexes of combination metronomic chemotherapy and an anti-VEGFR-2 antibody in multidrug-resistant human breast cancer xenografts. *Clin Cancer Res*, 8, 221-32(2002)
204. Man, S., G. Bocci, G. Francia, S. K. Green, S. Jothy, D. Hanahan, P. Bohlen, D. J. Hicklin, G. Bergers & R. S. Kerbel: Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res*, 62, 2731-5(2002)
205. Zhang, L., D. Yu, D. J. Hicklin, J. A. Hannay, L. M. Ellis & R. E. Pollock: Combined anti-fetal liver kinase 1 monoclonal antibody and continuous low-dose doxorubicin inhibits angiogenesis and growth of human soft tissue sarcoma xenografts by induction of endothelial cell apoptosis. *Cancer Res*, 62, 2034-42(2002)
206. Ferrara, N.: Molecular and biological properties of vascular endothelial growth factor. *J Mol Med*, 77, 527-43(1999)
207. Zimmermann, R. C., T. Hartman, P. Bohlen, M. V. Sauer & J. Kitajewski: Preovulatory treatment of mice with anti-VEGF receptor 2 antibody inhibits angiogenesis in corpora lutea. *Microvasc Res*, 62, 15-25(2001)
208. Zhu, Z., D. Lu, H. Kotanides, A. Santiago, X. Jimenez, T. Simcox, D. J. Hicklin, P. Bohlen & L. Witte: Inhibition of vascular endothelial growth factor induced mitogenesis of human endothelial cells by a chimeric anti-kinase insert domain-containing receptor antibody. *Cancer Lett*, 136, 203-13(1999)
209. Zhu, Z., P. Rockwell, D. Lu, H. Kotanides, B. Pytowski, D. J. Hicklin, P. Bohlen & L. Witte: Inhibition of vascular endothelial growth factor-induced receptor activation with anti-kinase insert domain-containing receptor single-chain antibodies from a phage display library. *Cancer Res*, 58, 3209-14(1998)
210. Zhu, Z. & L. Witte: Inhibition of tumor growth and metastasis by targeting tumor-associated angiogenesis with antagonists to the receptors of vascular endothelial growth factor. *Invest New Drugs*, 17, 195-212(1999)
211. Hunt, S.: Technology evaluation: IMC-1C11, ImClone Systems. *Curr Opin Mol Ther*, 3, 418-24(2001)
212. McLeod, D. S., M. Taomoto, J. Cao, Z. Zhu, L. Witte & G. A. Lutty: Localization of VEGF receptor-2 (KDR/Flk-1) and effects of blocking it in oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci*, 43, 474-82(2002)
213. Zimmermann, R. C., E. Xiao, P. Bohlen & M. Ferin: Administration of antivascular endothelial growth factor receptor 2 antibody in the early follicular phase delays follicular selection and development in the rhesus monkey. *Endocrinology*, 143, 2496-502(2002)
214. Traxler, P., G. Bold, E. Buchdunger, G. Caravatti, P. Furet, P. Manley, T. O'Reilly, J. Wood & J. Zimmermann: Tyrosine kinase inhibitors: from rational design to clinical trials. *Med Res Rev*, 21, 499-512(2001)
215. Underiner, T. L., B. Ruggeri & D. E. Gingrich: Development of vascular endothelial growth factor receptor (VEGFR) kinase inhibitors as anti-angiogenic agents in cancer therapy. *Curr Med Chem*, 11, 731-45(2004)
216. Bayes, M., X. Rabasseda & J. R. Prous: Gateways to clinical trials. *Methods Find Exp Clin Pharmacol*, 25, 483-506(2003)
217. Bold, G., K. H. Altmann, J. Frei, M. Lang, P. W. Manley, P. Traxler, B. Wietfeld, J. Bruggen, E. Buchdunger, R. Cozens, S. Ferrari, P. Furet, F. Hofmann, G. Martiny-Baron, J. Mestan, J. Rosel, M. Sills, D. Stover, F. Acemoglu, E. Boss, R. Emmenegger, L. Lasser, E. Masso, R. Roth, C. Schlachter & W. Vetterli: New anilinophthalazines as potent and orally well absorbed inhibitors of the VEGF receptor tyrosine kinases useful as antagonists of tumor-driven angiogenesis. *J Med Chem*, 43, 2310-23(2000)
218. Drevs, J., I. Hofmann, H. Hugenschmidt, C. Wittig, H. Madjar, M. Muller, J. Wood, G. Martiny-Baron, C. Unger & D. Marme: Effects of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor receptor tyrosine kinases, on primary tumor, metastasis, vessel density, and blood flow in a murine renal cell carcinoma model. *Cancer Res*, 60, 4819-24(2000)
219. Furet, P., G. Bold, F. Hofmann, P. Manley, T. Meyer & K. H. Altmann: Identification of a new chemical class of potent angiogenesis inhibitors based on conformational considerations and database searching. *Bioorg Med Chem Lett*, 13, 2967-71(2003)
220. Morgan, B., A. L. Thomas, J. Drevs, J. Hennig, M. Buchert, A. Jivan, M. A. Horsfield, K. Mross, H. A. Ball, L. Lee, W. Mietlowski, S. Fuxuis, C. Unger, K. O'Byrne, A. Henry, G. R. Cherryman, D. Laurent, M. Dugan, D. Marme & W. P. Steward: Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: results from two phase I studies. *J Clin Oncol*, 21, 3955-64(2003)
221. Thomas, A. L., B. Morgan, J. Drevs, C. Unger, B. Wiedenmann, U. Vanhoefer, D. Laurent, M. Dugan & W.

P. Steward: Vascular endothelial growth factor receptor tyrosine kinase inhibitors: PTK787/ZK 222584. *Semin Oncol*, 30, 32-8(2003)
222. Wood, J. M., G. Bold, E. Buchdunger, R. Cozens, S. Ferrari, J. Frei, F. Hofmann, J. Mestan, H. Mett, T. O'Reilly, E. Persohn, J. Rosel, C. Schnell, D. Stover, A. Theuer, H. Towbin, F. Wenger, K. Woods-Cook, A. Menrad, G. Siemeister, M. Schirner, K. H. Thierauch, M. R. Schneider, J. Drevs, G. Martiny-Baron & F. Totzke: PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res*, 60, 2178-89(2000)
223. Gourley, M. & J. S. Williamson: Angiogenesis: new targets for the development of anticancer chemotherapies. *Curr Pharm Des*, 6, 417-39(2000)
224. Hennequin, L. F., E. S. Stokes, A. P. Thomas, C. Johnstone, P. A. Ple, D. J. Ogilvie, M. Dukes, S. R. Wedge, J. Kendrew & J. O. Curwen: Novel 4-anilinoquinazolines with C-7 basic side chains: design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *J Med Chem*, 45, 1300-12(2002)
225. Wedge, S. R., D. J. Ogilvie, M. Dukes, J. Kendrew, J. O. Curwen, L. F. Hennequin, A. P. Thomas, E. S. Stokes, B. Curry, G. H. Richmond & P. F. Wadsworth: ZD4190: an orally active inhibitor of vascular endothelial growth factor signaling with broad-spectrum antitumor efficacy. *Cancer Res*, 60, 970-5(2000)
226. Checkley, D., J. J. Tessier, S. R. Wedge, M. Dukes, J. Kendrew, B. Curry, B. Middleton & J. C. Waterton: Dynamic contrast-enhanced MRI of vascular changes induced by the VEGF-signalling inhibitor ZD4190 in human tumour xenografts. *Magn Reson Imaging*, 21, 475-82(2003)
227. Pradel, C., N. Siauve, G. Bruneteau, O. Clement, C. de Bazelaire, F. Frouin, S. R. Wedge, J. L. Tessier, P. H. Robert, G. Frija & C. A. Cuenod: Reduced capillary perfusion and permeability in human tumour xenografts treated with the VEGF signalling inhibitor ZD4190: an in vivo assessment using dynamic MR imaging and macromolecular contrast media. *Magn Reson Imaging*, 21, 845-51(2003)
228. Carlomagno, F., D. Vitagliano, T. Guida, F. Ciardiello, G. Tortora, G. Vecchio, A. J. Ryan, G. Fontanini, A. Fusco & M. Santoro: ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res*, 62, 7284-90(2002)
229. Wedge, S. R., D. J. Ogilvie, M. Dukes, J. Kendrew, R. Chester, J. A. Jackson, S. J. Boffey, P. J. Valentine, J. O. Curwen, H. L. Musgrove, G. A. Graham, G. D. Hughes, A. P. Thomas, E. S. Stokes, B. Curry, G. H. Richmond, P. F. Wadsworth, A. L. Bigley & L. F. Hennequin: ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res*, 62, 4645-55(2002)
230. McCarty, M. F., J. Wey, O. Stoeltzing, W. Liu, F. Fan, C. Bucana, P. F. Mansfield, A. J. Ryan & L. M. Ellis: ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor with additional activity against epidermal growth factor receptor tyrosine kinase, inhibits orthotopic growth and angiogenesis of gastric cancer. *Mol Cancer Ther*, 3, 1041-8(2004)
231. Sandstrom, M., M. Johansson, U. Andersson, A. Bergh, A. T. Bergenheim & R. Henriksson: The tyrosine kinase inhibitor ZD6474 inhibits tumour growth in an intracerebral rat glioma model. *Br J Cancer*, 91, 1174-80(2004)
232. Ciardiello, F., R. Caputo, V. Damiano, R. Caputo, T. Troiani, D. Vitagliano, F. Carlomagno, B. M. Veneziani, G. Fontanini, A. R. Bianco & G. Tortora: Antitumor effects of ZD6474, a small molecule vascular endothelial growth factor receptor tyrosine kinase inhibitor, with additional activity against epidermal growth factor receptor tyrosine kinase. *Clin Cancer Res*, 9, 1546-56(2003)
233. Checkley, D., J. J. Tessier, J. Kendrew, J. C. Waterton & S. R. Wedge: Use of dynamic contrast-enhanced MRI to evaluate acute treatment with ZD6474, a VEGF signalling inhibitor, in PC-3 prostate tumours. *Br J Cancer*, 89, 1889-95(2003)
234. Heffelfinger, S. C., M. Yan, R. B. Gear, J. Schneider, K. LaDow & D. Warshawsky: Inhibition of VEGFR2 prevents DMBA-induced mammary tumor formation. *Lab Invest*, 84, 989-98(2004)
235. Sridhar, S. S. & F. A. Shepherd: Targeting angiogenesis: a review of angiogenesis inhibitors in the treatment of lung cancer. *Lung Cancer*, 42 Suppl 1, S81-91(2003)
236. Antonian, L., H. Zhang, C. Yang, G. Wagner, L. K. Shawver, M. Shet, B. Ogilvie, A. Madan & A. Parkinson: Biotransformation of the anti-angiogenic compound SU5416. *Drug Metab Dispos*, 28, 1505-12(2000)
237. Bischof, M., A. Abdollahi, P. Gong, C. Stoffregen, K. E. Lipson, J. U. Debus, K. J. Weber & P. E. Huber: Triple combination of irradiation, chemotherapy (pemetrexed), and VEGFR inhibition (SU5416) in human endothelial and tumor cells. *Int J Radiat Oncol Biol Phys* , 60, 1220-32(2004)
238. Haspel, H. C., G. M. Scicli, G. McMahon & A. G. Scicli: Inhibition of vascular endothelial growth factor-associated tyrosine kinase activity with SU5416 blocks sprouting in the microvascular endothelial cell spheroid model of angiogenesis. *Microvasc Res*, 63, 304-15(2002)
239. Huss, W. J., R. J. Barrios & N. M. Greenberg: SU5416 selectively impairs angiogenesis to induce prostate cancer-specific apoptosis. *Mol Cancer Ther*, 2, 611-6(2003)
240. Mendel, D. B., A. D. Laird, B. D. Smolich, R. A. Blake, C. Liang, A. L. Hannah, R. M. Shaheen, L. M. Ellis, S. Weitman, L. K. Shawver & J. M. Cherrington: Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent. *Anticancer Drug Des*, 15, 29-41(2000)
241. Vajkoczy, P., M. D. Menger, B. Vollmar, L. Schilling, P. Schmiedek, K. P. Hirth, A. Ullrich & T. A. Fong: Inhibition of tumor growth, angiogenesis, and microcirculation by the novel Flk-1 inhibitor SU5416 as assessed by intravital multi-fluorescence videomicroscopy. *Neoplasia*, 1, 31-41(1999)
242. Zhao, Y., C. Y. Yang, J. Haznedar & L. Antonian: Simultaneous determination of SU5416 and its phase I and phase II metabolites in rat and dog plasma by LC/MS/MS. *J Pharm Biomed Anal*, 25, 821-32(2001)
243. Giles, F. J., A. T. Stopeck, L. R. Silverman, J. E. Lancet, M. A. Cooper, A. L. Hannah, J. M. Cherrington, A. M. O'Farrell, H. A. Yuen, S. G. Louie, W. Hong, J. E.

Cortes, S. Verstovsek, M. Albitar, S. M. O'Brien, H. M. Kantarjian & J. E. Karp: SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes. *Blood*, 102, 795-801(2003)

244. Kuenen, B. C., L. Rosen, E. F. Smit, M. R. Parson, M. Levi, R. Ruijter, H. Huisman, M. A. Kedde, P. Noordhuis, W. J. van der Vijgh, G. J. Peters, G. F. Cropp, P. Scigalla, K. Hoekman, H. M. Pinedo & G. Giaccone: Dose-finding and pharmacokinetic study of cisplatin, gemcitabine, and SU5416 in patients with solid tumors. *J Clin Oncol*, 20, 1657-67(2002)

245. Stopeck, A., M. Sheldon, M. Vahedian, G. Cropp, R. Gosalia & A. Hannah: Results of a Phase I dose-escalating study of the antiangiogenic agent, SU5416, in patients with advanced malignancies. *Clin Cancer Res*, 8, 2798-805(2002)

246. Fiedler, W., R. Mesters, H. Tinnefeld, S. Loges, P. Staib, U. Duhrsen, M. Flasshove, O. G. Ottmann, W. Jung, F. Cavalli, R. Kuse, J. Thomalla, H. Serve, A. M. O'Farrell, M. Jacobs, N. M. Brega, P. Scigalla, D. K. Hossfeld & W. E. Berdel: A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. *Blood*, 102, 2763-7(2003)

247. Giles, F. J., M. A. Cooper, L. Silverman, J. E. Karp, J. E. Lancet, M. Zangari, P. J. Shami, K. D. Khan, A. L. Hannah, J. M. Cherrington, D. A. Thomas, G. Garcia-Manero, M. Albitar, H. M. Kantarjian & A. T. Stopeck: Phase II study of SU5416--a small-molecule, vascular endothelial growth factor tyrosine-kinase receptor inhibitor--in patients with refractory myeloproliferative diseases. *Cancer*, 97, 1920-8(2003)

248. Heymach, J. V., J. Desai, J. Manola, D. W. Davis, D. J. McConkey, D. Harmon, D. P. Ryan, G. Goss, T. Quigley, A. D. Van den Abbeele, S. G. Silverman, S. Connors, J. Folkman, C. D. Fletcher & G. D. Demetri: Phase II study of the antiangiogenic agent SU5416 in patients with advanced soft tissue sarcomas. *Clin Cancer Res*, 10, 5732-40(2004)

249. Mesters, R. M., T. Padro, R. Bieker, M. Steins, M. Kreuter, M. Goner, S. Kelsey, P. Scigalla, W. Fiedler, T. Buchner & W. E. Berdel: Stable remission after administration of the receptor tyrosine kinase inhibitor SU5416 in a patient with refractory acute myeloid leukemia. *Blood*, 98, 241-3(2001)

250. Peterson, A. C., S. Swiger, W. M. Stadler, M. Medved, G. Karczmar & T. F. Gajewski: Phase II study of the Flk-1 tyrosine kinase inhibitor SU5416 in advanced melanoma. *Clin Cancer Res*, 10, 4048-54(2004)

251. Stadler, W. M., D. Cao, N. J. Vogelzang, C. W. Ryan, K. Hoving, R. Wright, T. Karrison & E. E. Vokes: A randomized Phase II trial of the antiangiogenic agent SU5416 in hormone-refractory prostate cancer. *Clin Cancer Res*, 10, 3365-70(2004)

252. Zangari, M., E. Anaissie, A. Stopeck, A. Morimoto, N. Tan, J. Lancet, M. Cooper, A. Hannah, G. Garcia-Manero, S. Faderl, H. Kantarjian, J. Cherrington, M. Albitar & F. J. Giles: Phase II study of SU5416, a small molecule vascular endothelial growth factor tyrosine kinase receptor inhibitor, in patients with refractory multiple myeloma. *Clin Cancer Res*, 10, 88-95(2004)

253. Pharmacia's SU5416 not effective. *Expert Rev Anticancer Ther*, 2, 5(2002)

254. Laird, A. D., J. G. Christensen, G. Li, J. Carver, K. Smith, X. Xin, K. G. Moss, S. G. Louie, D. B. Mendel & J. M. Cherrington: SU6668 inhibits Flk-1/KDR and PDGFRbeta in vivo, resulting in rapid apoptosis of tumor vasculature and tumor regression in mice. *Faseb J*, 16, 681-90(2002)

255. Laird, A. D., P. Vajkoczy, L. K. Shawver, A. Thurnher, C. Liang, M. Mohammadi, J. Schlessinger, A. Ullrich, S. R. Hubbard, R. A. Blake, T. A. Fong, L. M. Strawn, L. Sun, C. Tang, R. Hawtin, F. Tang, N. Shenoy, K. P. Hirth, G. McMahon & Cherrington: SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res*, 60, 4152-60(2000)

256. Hoekman, K.: SU6668, a multitargeted angiogenesis inhibitor. *Cancer J*, 7 Suppl 3, S134-8(2001)

257. Shaheen, R. M., D. W. Davis, W. Liu, B. K. Zebrowski, M. R. Wilson, C. D. Bucana, D. J. McConkey, G. McMahon & L. M. Ellis: Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. *Cancer Res*, 59, 5412-6(1999)

258. Marzola, P., A. Degrassi, L. Calderan, P. Farace, C. Crescimanno, E. Nicolato, A. Giusti, E. Pesenti, A. Terron, A. Sbarbati, T. Abrams, L. Murray & F. Osculati: In vivo assessment of antiangiogenic activity of SU6668 in an experimental colon carcinoma model. *Clin Cancer Res*, 10, 739-50(2004)

259. Garofalo, A., E. Naumova, L. Manenti, C. Ghilardi, G. Ghisleni, M. Caniatti, T. Colombo, J. M. Cherrington, E. Scanziani, M. I. Nicoletti & R. Giavazzi: The combination of the tyrosine kinase receptor inhibitor SU6668 with paclitaxel affects ascites formation and tumor spread in ovarian carcinoma xenografts growing orthotopically. *Clin Cancer Res*, 9, 3476-85(2003)

260. Abdollahi, A., K. E. Lipson, X. Han, R. Krempien, T. Trinh, K. J. Weber, P. Hahnfeldt, L. Hlatky, J. Debus, A. R. Howlett & P. E. Huber: SU5416 and SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor production and amplify the direct anti-endothelial action of radiation in vitro. *Cancer Res*, 63, 3755-63(2003)

261. Lu, B., L. Geng, A. Musiek, J. Tan, C. Cao, E. Donnelly, G. McMahon, H. Choy & D. E. Hallahan: Broad spectrum receptor tyrosine kinase inhibitor, SU6668, sensitizes radiation via targeting survival pathway of vascular endothelium. *Int J Radiat Oncol Biol Phys*, 58, 844-50(2004)

262. Xiong, H. Q., R. Herbst, S. C. Faria, C. Scholz, D. Davis, E. F. Jackson, T. Madden, D. McConkey, M. Hicks, K. Hess, C. A. Charnsangavej & J. L. Abbruzzese: A phase I surrogate endpoint study of SU6668 in patients with solid tumors. *Invest New Drugs*, 22, 459-66(2004)

263. Sistla, A., A. Sunga, K. Phung, A. Koparkar & N. Shenoy: Powder-in-bottle formulation of SU011248. Enabling rapid progression into human clinical trials. *Drug Dev Ind Pharm*, 30, 19-25(2004)

264. Abrams, T. J., L. B. Lee, L. J. Murray, N. K. Pryer & J. M. Cherrington: SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol Cancer Ther*, 2, 471-8(2003)

265. Baratte, S., S. Sarati, E. Frigerio, C. A. James, C. Ye & Q. Zhang: Quantitation of SU1 1248, an oral multi-target tyrosine

kinase inhibitor, and its metabolite in monkey tissues by liquid chromatograph with tandem mass spectrometry following semi-automated liquid-liquid extraction. *J Chromatogr A*, 1024, 87-94(2004)
266. Bayes, M., X. Rabasseda & J. R. Prous: Gateways to clinical trials. *Methods Find Exp Clin Pharmacol*, 26, 473-503(2004)
267. Mendel, D. B., A. D. Laird, X. Xin, S. G. Louie, J. G. Christensen, G. Li, R. E. Schreck, T. J. Abrams, T. J. Ngai, L. B. Lee, L. J. Murray, J. Carver, E. Chan, K. G. Moss, J. O. Haznedar, J. Sukbuntherng, R. A. Blake, L. Sun, C. Tang, T. Miller, S. Shirazian, G. McMahon & J. M. Cherrington: In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res*, 9, 327-37(2003)
268. O'Farrell, A. M., T. J. Abrams, H. A. Yuen, T. J. Ngai, S. G. Louie, K. W. Yee, L. M. Wong, W. Hong, L. B. Lee, A. Town, B. D. Smolich, W. C. Manning, L. J. Murray, M. C. Heinrich & J. M. Cherrington: SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood*, 101, 3597-605(2003)
269. Sun, L., C. Liang, S. Shirazian, Y. Zhou, T. Miller, J. Cui, J. Y. Fukuda, J. Y. Chu, A. Nematalla, X. Wang, H. Chen, A. Sistla, T. C. Luu, F. Tang, J. Wei & C. Tang: Discovery of 5-[5-fluoro-2-oxo-1,2- dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J Med Chem*, 46, 1116-9(2003)
270. von Mehren, M.: New therapeutic strategies for soft tissue sarcomas. *Curr Treat Options Oncol*, 4, 441-51(2003)
271. Abrams, T. J., L. J. Murray, E. Pesenti, V. W. Holway, T. Colombo, L. B. Lee, J. M. Cherrington & N. K. Pryer: Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. *Mol Cancer Ther*, 2, 1011-21(2003)
272. O'Farrell, A. M., J. M. Foran, W. Fiedler, H. Serve, R. L. Paquette, M. A. Cooper, H. A. Yuen, S. G. Louie, H. Kim, S. Nicholas, M. C. Heinrich, W. E. Berdel, C. Bello, M. Jacobs, P. Scigalla, W. C. Manning, S. Kelsey & J. M. Cherrington: An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. *Clin Cancer Res*, 9, 5465-76(2003)
273. AE 941--Neovastat. *Drugs R D*, 1, 135-6(1999)
274. Beliveau, R., D. Gingras, E. A. Kruger, S. Lamy, P. Sirois, B. Simard, M. G. Sirois, L. Tranqui, F. Baffert, E. Beaulieu, V. Dimitriadou, M. C. Pepin, F. Courjal, I. Ricard, P. Poyet, P. Falardeau, W. D. Figg & E. Dupont: The antiangiogenic agent neovastat (AE-941) inhibits vascular endothelial growth factor-mediated biological effects. *Clin Cancer Res*, 8, 1242-50(2002)
275. Dupont, E., P. Falardeau, S. A. Mousa, V. Dimitriadou, M. C. Pepin, T. Wang & M. A. Alaoui-Jamali: Antiangiogenic and antimetastatic properties of Neovastat (AE-941), an orally active extract derived from cartilage tissue. *Clin Exp Metastasis*, 19, 145-53(2002)
276. Dupont, E., B. Wang, A. J. Mamelak, B. G. Howell, G. Shivji, L. Zhuang, V. Dimitriadou, P. Falardeau & D. N. Sauder: Modulation of the Contact Hypersensitivity Response by AE-941 (Neovastat), a Novel Antiangiogenic Agent. *J Cutan Med Surg*(2003)
277. Gingras, D., G. Batist & R. Beliveau: AE-941 (Neovastat): a novel multifunctional antiangiogenic compound. *Expert Rev Anticancer Ther*, 1, 341-7(2001)
278. Gingras, D., D. Boivin, C. Deckers, S. Gendron, C. Barthomeuf & R. Beliveau: Neovastat--a novel antiangiogenic drug for cancer therapy. *Anticancer Drugs*, 14, 91-6(2003)
279. Boivin, D., S. Gendron, E. Beaulieu, D. Gingras & R. Beliveau: The antiangiogenic agent Neovastat (AE-941) induces endothelial cell apoptosis. *Mol Cancer Ther*, 1, 795-802(2002)
280. Latreille, J., G. Batist, F. Laberge, P. Champagne, D. Croteau, P. Falardeau, C. Levinton, C. Hariton, W. K. Evans & E. Dupont: Phase I/II trial of the safety and efficacy of AE-941 (Neovastat) in the treatment of non-small-cell lung cancer. *Clin Lung Cancer*, 4, 231-6(2003)
281. Shepherd, F. A.: Angiogenesis inhibitors in the treatment of lung cancer. *Lung Cancer*, 34 Suppl 3, S81-9(2001)
282. Sauder, D. N., J. Dekoven, P. Champagne, D. Croteau & E. Dupont: Neovastat (AE-941), an inhibitor of angiogenesis: Randomized phase I/II clinical trial results in patients with plaque psoriasis. *J Am Acad Dermatol*, 47, 535-41(2002)
283. AEterna reports on its phase II trial of neovastat. *Expert Rev Anticancer Ther*, 1, 3-4(2001)
284. Batist, G., F. Patenaude, P. Champagne, D. Croteau, C. Levinton, C. Hariton, B. Escudier & E. Dupont: Neovastat (AE-941) in refractory renal cell carcinoma patients: report of a phase II trial with two dose levels. *Ann Oncol*, 13, 1259-63(2002)
285. Weber, M. H., J. Lee & F. W. Orr: The effect of Neovastat (AE-941) on an experimental metastatic bone tumor model. *Int J Oncol*, 20, 299-303(2002)
286. Dev, I. K., R. E. Dornsife, T. M. Hopper, J. A. Onori, C. G. Miller, L. E. Harrington, K. M. Dold, R. J. Mullin, J. H. Johnson, R. M. Crosby, A. T. Truesdale, A. H. Epperly, K. W. Hinkle, M. Cheung, J. A. Stafford, D. K. Luttrell & R. Kumar: Antitumour efficacy of VEGFR2 tyrosine kinase inhibitor correlates with expression of VEGF and its receptor VEGFR2 in tumour models. *Br J Cancer*, 91, 1391-8(2004)
287. Ruggeri, B., J. Singh, D. Gingrich, T. Angeles, M. Albom, S. Yang, H. Chang, C. Robinson, K. Hunter, P. Dobrzanski, S. Jones-Bolin, S. Pritchard, L. Aimone, A. Klein-Szanto, J. M. Herbert, F. Bono, P. Schaeffer, P. Casellas, B. Bourie, R. Pili, J. Isaacs, M. Ator, R. Hudkins, J. Vaught, J. Mallamo & C. Dionne: CEP-7055: a novel, orally active pan inhibitor of vascular endothelial growth factor receptor tyrosine kinases with potent antiangiogenic activity and antitumor efficacy in preclinical models. *Cancer Res*, 63, 5978-91(2003)
288. Yigitbasi, O. G., M. N. Younes, D. Doan, S. A. Jasser, B. A. Schiff, C. D. Bucana, B. N. Bekele, I. J. Fidler & J. N. Myers: Tumor cell and endothelial cell therapy of oral cancer by dual tyrosine kinase receptor blockade. *Cancer Res*, 64, 7977-84(2004)
289. Fraley, M. E., W. F. Hoffman, R. S. Rubino, R. W. Hungate, A. J. Tebben, R. Z. Rutledge, R. C. McFall, W. R. Huckle, R. L. Kendall, K. E. Coll & K. A. Thomas: Synthesis and initial SAR studies of 3,6-disubstituted pyrazolo[1,5-a]pyrimidines: a new class of KDR kinase inhibitors. *Bioorg Med Chem Lett*, 12, 2767-70(2002)
290. Manley, P. J., A. E. Balitza, M. T. Bilodeau, K. E. Coll, G. D. Hartman, R. C. McFall, K. W. Rickert, L. D. Rodman &

K. A. Thomas: 2,4-disubstituted pyrimidines: a novel class of KDR kinase inhibitors. *Bioorg Med Chem Lett*, 13, 1673-7(2003)
291. Bevacizumab. Anti-VEGF monoclonal antibody, avastin, rhumab-VEGF. *Drugs R D*, 3, 28-30(2002)
292. Adding a humanized antibody to vascular endothelial growth factor (Bevacizumab, Avastin) to chemotherapy improves survival in metastatic colorectal cancer. *Clin Colorectal Cancer*, 3, 85-8(2003)
293. Bevacizumab (Avastin). *Med Lett Drugs Ther*, 46, 47-8(2004)
294. Ratner, M.: Genentech discloses safety concerns over Avastin. *Nat Biotechnol*, 22, 1198(2004)
295. Holash, J., S. Davis, N. Papadopoulos, S. D. Croll, L. Ho, M. Russell, P. Boland, R. Leidich, D. Hylton, E. Burova, E. Ioffe, T. Huang, C. Radziejewski, K. Bailey, J. P. Fandl, T. Daly, S. J. Wiegand, G. D. Yancopoulos & J. S. Rudge: VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci U S A*, 99, 11393-8(2002)
296. Konner, J. & J. Dupont: Use of soluble recombinant decoy receptor vascular endothelial growth factor trap (VEGF Trap) to inhibit vascular endothelial growth factor activity. *Clin Colorectal Cancer*, 4 Suppl 2, S81-5(2004)
297. Saishin, Y., Y. Saishin, K. Takahashi, R. Lima e Silva, D. Hylton, J. S. Rudge, S. J. Wiegand & P. A. Campochiaro: VEGF-TRAP(R1R2) suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier. *J Cell Physiol*, 195, 241-8(2003)
298. Forster, Y., A. Meye, S. Krause & B. Schwenzer: Antisense-mediated VEGF suppression in bladder and breast cancer cells. *Cancer Lett*, 212, 95-103(2004)
299. He, R., B. Liu, C. Yang, R. C. Yang, G. Tobelem & Z. C. Han: Inhibition of K562 leukemia angiogenesis and growth by expression of antisense vascular endothelial growth factor (VEGF) sequence. *Cancer Gene Ther*, 10, 879-86(2003)
300. Ruan, G. R., Y. R. Liu, S. S. Chen, J. Y. Fu, Y. Chang, Y. Z. Qin, J. L. Li, H. Yu & H. Wang: Effect of antisense VEGF cDNA transfection on the growth of chronic myeloid leukemia K562 cells in vitro and in nude mice. *Leuk Res*, 28, 763-9(2004)
301. Jain, R. K.: Tumor angiogenesis and accessibility: role of vascular endothelial growth factor. *Semin Oncol*, 29, 3-9(2002)
302. Munn, L. L.: Aberrant vascular architecture in tumors and its importance in drug-based therapies. *Drug Discov Today*, 8, 396-403(2003)
303. Boehm, T., J. Folkman, T. Browder & M. S. O'Reilly: Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature*, 390, 404-7(1997)
304. Geng, L., E. Donnelly, G. McMahon, P. C. Lin, E. Sierra-Rivera, H. Oshinka & D. E. Hallahan: Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. *Cancer Res*, 61, 2413-9(2001)
305. Kerbel, R. S.: A cancer therapy resistant to resistance. *Nature*, 390, 335-6(1997)
306. Kerbel, R. S.: Molecular and physiologic mechanisms of drug resistance in cancer: an overview. *Cancer Metastasis Rev*, 20, 1-2(2001)
307. Yu, J. L., J. W. Rak, B. L. Coomber, D. J. Hicklin & R. S. Kerbel: Effect of p53 status on tumor response to antiangiogenic therapy. *Science*, 295, 1526-8(2002)
308. Jung, Y. D., P. F. Mansfield, M. Akagi, A. Takeda, W. Liu, C. D. Bucana, D. J. Hicklin & L. M. Ellis: Effects of combination anti-vascular endothelial growth factor receptor and anti-epidermal growth factor receptor therapies on the growth of gastric cancer in a nude mouse model. *Eur J Cancer*, 38, 1133-40(2002)
309. Huang, X., G. Molema, S. King, L. Watkins, T. S. Edgington & P. E. Thorpe: Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science*, 275, 547-50(1997)
310. Jain, R. K.: Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med*, 7, 987-9(2001)
311. Jain, R. K. & P. F. Carmeliet: Vessels of death or life. *Sci Am*, 285, 38-45(2001)
312. Tong, R. T., Y. Boucher, S. V. Kozin, F. Winkler, D. J. Hicklin & R. K. Jain: Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res*, 64, 3731-6(2004)
313. Lambin, P. & W. Landuyt: Vascular targeting: a potential additional anti-cancer treatment. *Verh K Acad Geneeskd Belg*, 65, 29-46(2003)
314. Lee, C. G., M. Heijn, E. di Tomaso, G. Griffon-Etienne, M. Ancukiewicz, C. Koike, K. R. Park, N. Ferrara, R. K. Jain, H. D. Suit & Y. Boucher: Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. *Cancer Res*, 60, 5565-70(2000)
315. Huang, S., E. A. Armstrong, S. Benavente, P. Chinnaiyan & P. M. Harari: Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. *Cancer Res*, 64, 5355-62(2004)
316. Matar, P., F. Rojo, R. Cassia, G. Moreno-Bueno, S. Di Cosimo, J. Tabernero, M. Guzman, S. Rodriguez, J. Arribas, J. Palacios & J. Baselga: Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. *Clin Cancer Res*, 10, 6487-501(2004)

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