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VEGF Inhibitors for Cancer Therapy

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ABSTRACT

Despite significant advances in systemic therapies, radiation oncology, and surgical techniques, many patients with cancer are still incurable. A novel therapeutic approach has been to target the vascular endothelial growth factors (VEGFs) which are often mutated and/or over-expressed in many tumors. The ligands and receptors of VEGF family are well established as key regulators of angiogenesis and vasculogenesis processes. VEGF is a homodimeric, basic, 45 kDa glycoprotein specific for vascular endothelial cells. Specifically, VEGF participates in regulation of the female reproductive cycle, wound healing, inflammation, vascular permeability, vascular tone, hematopoiesis and also contributes to pathological angiogenesis disorders such as cancer, rheumatoid arthritis, diabetic retinopathy and the neovascular form of macular degeneration. Thus, the role of VEGF has been extensively studied in the pathogenesis and angiogenesis of human cancers. Clinical trials have anti-VEGF therapies are effective in reducing tumor size, metastasis and blood vessel formation. Clinically, this may result in increased progression free survival, overall patient survival rate and will expand the potential for combinatorial therapies. The aim of present review is on the cellular responses of VEGF inhibitors and their implications for cancer therapy.

Keywords: Vascular Endothelial Growth Factor, Tyrosine Kinase, Kinase Inhibitors.

INTRODUCTION

Vascular endothelial growth factor (VEGF) a sub-family of growth factors, to be more specific, of platelet derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). VEGF is a homodimeric, basic, 45 kDa glycoprotein specific for vascular endothelial cells. [1-2] VEGF was first described as vascular permeability factor (VPF) by Dvorak and colleagues after it were discovered to increase the permeability of tumor blood vessels. [3] Currently, the VEGF family consists of seven members - VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and PlGF. Each isoform is distinct in its composition of 121, 145, 165, 183, 189 and 206 amino acids by monomer (respectively $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$) and $VEGF_{165}$ is the predominant protein among the major splice variants.

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Each isomer is the result of alternative splicing of mRNA from a common gene composed of eight-cysteine residues. [4] These VEGF isoforms have different physical and biological properties and act through three specific tyrosine kinase receptors - Fms-like tyrosine kinase Flt-1 (VEGFR- 1/Flt-1), the kinase domain region, also referred to as fetal liver kinase (VEGFR-2/KDR/Flk-1), and Flt-4 (VEGFR-3). Each receptor has seven immunoglobulin-like domains in the extracellular domain, a single trans-membrane region, and a consensus tyrosine kinase sequence interrupted by a kinase insert domain. [5] The complete function of each receptor has not been fully determined; however certain VEGFRs have been targeted by cancer therapeutics due to their known roles in angiogenesis. [6]

The broad term 'VEGF' covers a number of proteins from two families that result from alternate splicing of mRNA from a single, 8-exon, and VEGF gene. [7] The gene encoding VEGF is located on the short arm of chromosome 6 (6p21.1) in humans and on chromosome 17 (24.20 cM) in the mouse. [8] The two different families are referred to according to their terminal exon (exon 8) splice site - the proximal splice site (denoted $VEGF_{xxx}$) or distal splice site ($VEGF_{xxx}b$). In addition, alternate splicing of exon 6 and 7 alters their heparin binding affinity, and amino acid number (in humans: $VEGF_{121}$, $VEGF_{121}b$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165}b$,

$VEGF_{189}$, $VEGF_{206}$; the rodent orthologs of these proteins contain one fewer amino acid). These domains have important functional consequences for the VEGF splice variants, as the terminal (exon 8) splice site determines whether the proteins are pro-angiogenic (proximal splice site, expressed during angiogenesis) or anti-angiogenic (distal splice site, expressed in normal tissues). In addition, inclusion or exclusion of exons 6 and 7 mediate interactions with HSPGs and neuropilin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors. [7] In addition to its role in developmental angiogenesis, VEGF also modulates adult physiological angiogenesis and vessel function in numerous pathologies. In the adult, VEGF participates in regulation of the female reproductive cycle, wound healing, inflammation, vascular permeability, vascular tone, and hematopoiesis. [9] VEGF function also contributes to pathological angiogenesis in disorders such as cancer, rheumatoid arthritis, diabetic retinopathy and the neovascular form of macular degeneration. [10]

The most important member is VEGF-A. Other members are PlGF, VEGF-B, VEGF-C and VEGF-D. The latter ones were discovered later than VEGF-A, and, before their discovery, VEGF-A was called just VEGF. A number of VEGF-related proteins have also been discovered encoded by viruses (VEGF-E) and in the venom of some snakes (VEGF-F). [11] Activity of VEGF-A, as its name implies, has been studied mostly on cells of the vascular endothelium, although it does have effects on a number of other cell types (e.g., stimulation monocyte/macrophage migration, neurons, cancer cells, kidney epithelial cells). *In vitro*, VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor. [7]

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, and become activated through transphosphorylation, although to different sites, times and extents. [7] VEGF-A binds to VEGFR-1 (Flt- 1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF. The function of VEGFR-1 is less well-defined, although it is thought to modulate VEGFR-2 signalling. Another function of VEGFR-1 may be to act as a dummy/decoy receptor, sequestering VEGF from VEGFR-2 binding (this appears to be particularly important during vasculogenesis in the embryo). VEGF-C and VEGF-D, but not VEGF-A, are ligands for a third receptor (VEGFR-3), which mediates lymphangiogenesis. [11]

The role of VEGFR-1 in blood vessel development and vascular permeability remains unclear. VEGFR-1 has been shown to be weaker in kinase activity and is thus incapable of provoking endothelial cell proliferation when stimulated with VEGF. [12] The role of VEGFR-1 in post-fetal blood vessel formation has yet to be proven and pre-clinical experiments have continued to reveal VEGFR-2 as a more potent mediator of post-embryonic vascular formation. [13] It is currently understood that the major mediator of endothelial cell proliferation, angiogenesis, and vessel permeability as caused by VEGF signaling is VEGFR-2. The key role of this receptor in developmental vasculogenesis and blood island formation is evidenced by the failure of VEGFR-2 knockout mice to develop organized blood vessels and typical vasculature resulting in death inutero. [19]

The third VEGF receptor, VEGFR-3, displays slightly different signaling characteristics. Contrary to the previously described mechanisms, VEGFR-3 undergoes proteolytic cleavage in the extracellular domain into two disulfide linked peptides. While this receptor is capable of stimulating cell migration, differentiation, and mitogenesis, VEGFR-3 is predominantly localized to the surface of lymphatic endothelial cells. [15-16] Thus, VEGF-C, VEGF-D, and their receptor, present a strong molecular signaling system with VEGFR-3 for tumor lymphangiogenesis and another possible avenue for anti-angiogenic and anti-metastatic therapeutics. [17] VEGF has a multitude of different functions both on endothelial cells and on non-endothelial cells, dependent both on developmental stages as well as tissue and cell specificity. $VEGF_{xxx}$ has been implicated with poor prognosis in breast cancer. Numerous studies show a decreased overall survival and disease-free survival in those tumors over-expressing VEGF. Although $VEGF_{xxx}$ has been correlated with poor survival, its exact mechanism of action in the progression of tumors remains unclear. [18]

$VEGF_{xxx}$ is also released in rheumatoid arthritis in response to TNF-α, increasing endothelial permeability and swelling and also stimulating angiogenesis. $VEGF_{xxx}$ is also important in diabetic retinopathy. The microcirculatory problems in the retina of people with diabetes can cause retinal ischaemia, which results in the release of $VEGF_{xxx}$ and a switch in the balance of pro-angiogenic $VEGF_{xxx}$ isoforms over the normally expressed $VEGF_{xxx}$b isoforms. [18] $VEGF_{xxx}$ plays a role in the disease pathology of the wet form AMD, which is the leading cause of blindness for the elderly of the industrialized world. The vascular pathology of AMD shares certain similarities with diabetic retinopathy, although the cause of disease and the typical source of neovascularization differes between the two diseases. VEGF-D serum levels are significantly elevated in patients with angiosarcoma. Once released, $VEGF_{xxx}$ may elicit several responses. It may cause a cell to survive, move, or further differentiate. [14] Besides its role as an essential regulator of physiological endothelial cell growth, permeability, and migration in vitro and in vivo, VEGF is a pivotal factor in hematopoiesis which affects the differentiation of multiple hematopoietic lineages. VEGF triggers the differentiation of hematopoietic and endothelial lineages from a common potential precursor cell within the blood islands, the hemangioblasts. [19-22]

VEGF SIGNALING PATHWAY

VEGF is a heparin-binding homodimeric glycoprotein that acts via endothelial-specific receptor tyrosine kinases, VEGFR1 (Flt1), VEGFR2 (KDR/Flk1), and VEGFR3 (Flt4). [23] Besides VEGFA, the VEGF family of growth factors currently contains five other known members, namely PlGF (Placenta Growth Factor), VEGFB, VEGFC, VEGF-D and orf viral VEGF homologs. Additional novel VEGF-like heparin-binding proteins have been isolated recently from snake venom. Disruption of the genes encoding either VEGF or any of the three receptors of the VEGF family, results in embryonic lethality because of failure of blood vessel development. [24] VEGF signal transduction in endothelial cells is initiated by binding and activating three related transmembrane receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), and VEGFR-3 (Flt-4).

Table 1: Types and Functions of VEGF Family

Type	Function
VEGF-A	➢ Angiogenesis ✓ ↑ Migration of endothelial cells ✓ ↑ Mitosis of endothelial cells ✓ ↑ Methane monooxygenase activity ✓ ↑ αvβ3 Activity ✓ Creation of blood vessel lumen ✓ Creates fenestrations ➢ Chemotactic for macrophages and granulocytes. ➢ Vasodilation (indirectly by NO release).
VEGF-B	Embryonic angiogenesis.
VEGF-C	Lymphangiogenesis.
VEGF-D	Needed for the development of lymphatic vasculature surrounding lung bronchioles.
PlGF	Important for Vasculogenesis, Also needed for angiogenesis during ischemia, inflammation, wound healing, and cancer.

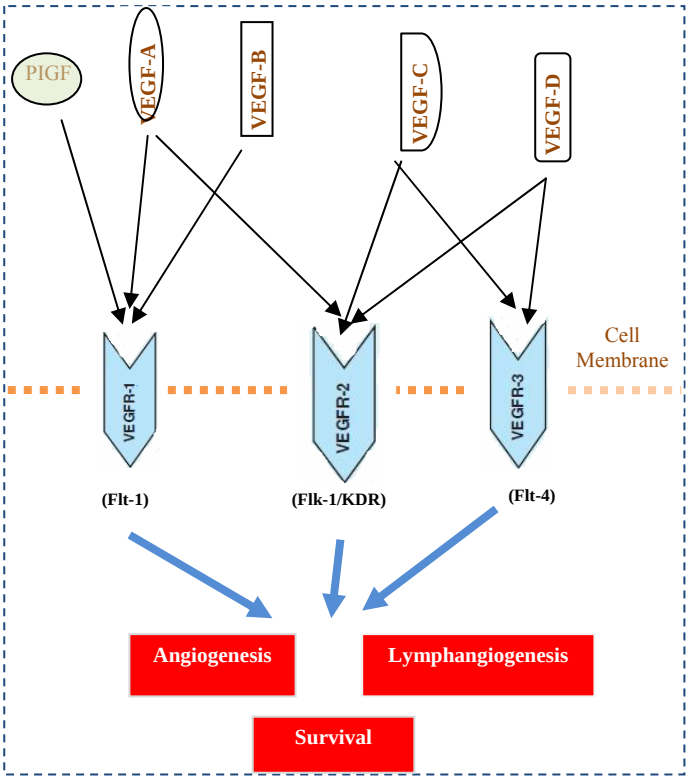

Fig. 1: VEGF Family and its receptor binding

All VEGFRs bear structural resemblance to the type III or PDGF/Fms/Kit family of receptors that contain a split intracellular kinase domain. [25] VEGFR2 is the main signal transducing VEGF receptor for angiogenesis and mitogenesis of endothelial cells. [24] The interaction across the interface of two receptor subunits triggers ligand-induced receptor dimerization thus initiating VEGF-induced signal transduction. Receptor dimerization is essential for both high affinities binding to VEGF and receptor activation. An engineered VEGF mutant which is able to bind but not dimerize VEGFR subunits is completely inactive and antagonizes the activity of wild type VEGF. While VEGFRs are thought to signal mainly as homodimers, some evidence exists for heterodimerization of VEGFR-1 and -2 subunits upon binding to VEGFA. Upon stimulation by ligand, transmembrane receptor tyrosine kinases appear to share a common signalling paradigm. Ligand binding induces receptor dimerization, which results in activation of intrinsic tyrosine kinase activity. The activated receptor undergoes auto-phosphorylation which promotes receptor association with, and activation of, intracellular signal relay proteins. Most of these proteins contain modules such as SH2 and PTB domains that promote binding to phosphorylated tyrosine residues on the activated receptor. [25] After receptor dimerization and auto-phosphorylation, several SH2 domain-containing signal transduction molecules are activated either directly such as PLC-γ, VRAP and Sck, or by indirect mechanisms, such as Src and PI3K. Activation of PKC plays a crucial role in VEGFA mitogenic signaling via the Raf1-MEK-ERK pathway. Cell survival signal is mainly mediated through PI3K-mediated activation of Akt/PKB. Activation of PI3K results in accumulation of PIP3, which in turn mediates membrane targeting and phosphorylation of Akt/PKB by binding to its PH (Pleckstrin Homology) domain. Downstream targets for Akt/PKB pathway include the proapototic proteins BAD, FKHR1 and Caspase-9, whose phosphorylation inhibits apoptosis. Moreover, VEGFA induces expression of the antiapoptotic protein BCL2 and the IAP family members XIAP and Survivin in HUVEC, suggesting that these proteins also play an important role in endothelial cell survival. [24]

PLC-γ catalyzes the hydrolysis of PIP2, creating IP3 and DAG, which stimulate the release of Ca^{2+} from internal stores and activate PKC. VEGF-A-induced Ca^{2+} mobilization is involved in short-term production of Nitric Oxide and Ptg. SHC phosphorylation promotes formation of SHC-GRB2-SOS complexes and induces PKC-dependent and Ras-independent induction of the Raf1-MEK-ERK1/2 pathway in sinusoidal endothelial cells and in HUVEC cells. Negative feedback for the mitogenic effects of VEGF is provided by cPLA, activation and biosynthesis of Prostaglandin. p38 pathway conveys the VEGF signal to microfilaments inducing rearrangements of the Actin cytoskeleton that regulate endothelial cell migration by modulating the activation of MAPKAPK2/3 and phosphorylation of the F-Actin polymerization modulator, HSP27. The activation of FAK and Paxillin by VEGF-A in HUVE cells through VEGFR2 leads to recruitment of Actin-anchoring proteins such as Talin and Vinculin to the focal adhesion plaque, which are essential for VEGFA-induced actin reorganization. VEGFA also stimulates tyrosine phosphorylation of the FAK-related cytoplasmic tyrosine kinase PYK2 (also termed RAFTK) in a bone marrow endothelial cell line. [24]

Although VEGF is known to be a powerful growth factor for therapeutic angiogenesis/ vascularization in the ischemic hind limb and myocardium, it has other activities that can increase the proliferation and permeability of capillary endothelial cells. These activities may produce unwanted side effects, such as tumor angiogenesis, vascular leakage, edema, and inflammation. [26] Different cytokines including VEGFA have been reported to modulate Kaposi's sarcoma, a major vascular tumor commonly associated with HIV1 and HHV-8. [27-28] In endothelial cells, the VEGF-Flk1/KDR signal system is a very important generator of NO through the activation of its downstream effectors PI3K, Akt kinase and eNOS. NO regulates hematopoiesis and modulates AML cell growth. [29] VEGF also plays major roles in the progression of ovarian cancer and colon cancer by modulating tumor proliferation through its promotion of tumor angiogenesis. In a variety of human pathological situations that are associated with aberrant endothelial proliferation and aberrant neovascularization VEGF-based therapy to treat diseases include the use of neutralizing antibodies against VEGFA or VEGFR2, antisense oligonucleotides, negative regulatory peptides, soluble receptors, and ATP analogs to inhibit the

kinase activity of VEGFR. [24] However, inhibition of VEGF function may result in infertility by blockade of Corpus Luteum function. Interference with VEGF function has therefore become of major interest for drug development to block angiogenesis and targeting the VEGF signaling pathway may be of therapeutic importance for many diseases.

THERAPEUTIC VEGF INHIBITORS FOR CANCER THERAPY

Vascular endothelial growth factor and its cognate receptors, VEGFRs, play an essential role in angiogenesis process. Inhibition of the VEGF pathway has become the focus of angiogenesis research as approximately 60 % of malignant tumours express high concentrations of VEGF. As tumors grow beyond a certain size, simple diffusion of nutrients and oxygen becomes insufficient, necessitating the de novo establishment of a blood supply. Inhibition of tumor angiogenesis, by blocking the action of VEGF, would therefore be predicted to starve the tumors to death. It has been suggested that VEGF inhibitors would be most effective in a minimal disease state; however the ability of VEGF, also known as vascular permeability factor, to regulate vessel permeability suggests that its inhibition could also decrease the ascitic fluid formation and edema observed in tumors with established vascularization. [30-31] Confirmation of this hypothesis has been provided by DCE-MRI studies of tumors treated with two VEGFR inhibitors vatalanib or bevacizumab. Strategies to inhibit the VEGF pathway include antibodies directed against VEGF or VEGFR, soluble VEGFR/VEGFR hybrids, soluble analogues of the VEGFR (VEGF-Trap) and tyrosine kinase inhibitors. One of the earliest strategies to inhibit VEGF activity involved the use of antibodies directed against VEGFRs. For example, preclinical data with anti-VEGFR-2 antibodies demonstrated decreased VEGF-induced signaling, decreased angiogenesis and decreased primary and metastatic growth in a variety of tumour systems. [32]

Since VEGFR2 is generally considered the most important transducer of VEGF-dependent angiogenesis, this receptor represents a major target within the angiogenesis-related kinases. However, it must be kept in mind that VEGF and VEGFR2 are involved in the regulation of physiologic processes such as lung function, pulmonary hypertension, liver regeneration, and neurogenesis. VEGF plays an important role in neuronal protection from hypoxic and ischemic injury. Recent findings also suggested a previously unsuspected connection between VEGF and ALS, a major neurodegenerative disease of unknown origin. [34] VEGF inhibitors therapies are also used other than cancers like AMD and diabetic retinopathy, the most significant causes of blindness in the developed world, are characterized by the growth of leaky, fragile blood vessels. VEGF has been demonstrated to be a key regulator of ocular neovascularization and current trials are addressing the effects of anti-VEGF therapy in patients with AMD. Pegaptanib, an anti-VEGF pegylated aptamer, and Lucentis/Ranibizumab, an anti-VEGF humanized antibody, have both shown promising results in the clinic. Neovascularization also accompanies chronic inflammation in conditions such as psoriasis, rheumatoid arthritis, and atherosclerosis. Neovastat, a naturally occurring antiangiogenic product that inhibits both VEGF signaling and matrix metalloproteinase activation, has shown clinical activity in phase I/II trials of psoriasis patients. [33]

Bevacizumab: Bevacizumab (Avastin™) was the first U.S. FDA approved drug, designed to block the formation of new blood vessels to tumors. It is manufactured by Genentech. National Institutes of Health has been involved in the clinical development of bevacizumab in several tumor types under a CRADA with Genentech. Bevacizumab was approved by the FDA in February 2004 for use in metastatic colorectal cancer when used with standard chemotherapy treatment (as first-line treatment) and with 5-fluorouracil-based therapy for second-line metastatic colorectal cancer.

Avastin is a monoclonal antibody, a type of genetically engineered protein. It is a recombinant humanized IgG monoclonal antibody directed against VEGF, which acts by blocking the binding of VEGF to its cognate receptors, prolonged time to progression of disease in patients with metastatic renal cancer, metastatic breast cancer, and NSCLC in phase I and II clinical trials. Bevacizumab was developed to inhibit VEGF. It was designed to cause the destruction of the blood vessel networks that nourish cancer cells, as the lack of a constant source of blood may slow tumor growth. Bevacizumab is an antibody—a type of targeting device produced by the immune system that can locate and bind to a specific protein. In the case of bevacizumab, it is a monoclonal (cells derived from a single common ancestor) antibody that binds to and inhibits VEGF. Genetic engineering produces a 93 % human and 7 % murine protein sequence. The molecule has the same biochemical and pharmacologic properties as the natural antibody, but with reduced immunogenicity and a longer biological half-life. By binding to VEGF-A bevacizumab prevents it from binding with its receptors. [32] It has recently been approved by the FDA as a first line treatment, in combination with 5-FU/leucovorin/CPT-11 therapy (Salz regimen), for treatment of patients with metastatic colorectal cancer. [35] Clinical studies are underway in non-metastatic breast cancer, renal cell carcinoma, glioblastoma multiforme, ovarian cancer, castrate-resistant prostate cancer, non-metastatic unresectable liver cancer and metastatic or unresectable locally advanced pancreatic cancer. Several studies revealed in April 2009 that bevacizumab is not effective at preventing recurrences of non-metastatic colon cancer. In May 2009, it received FDA approval for treatment of reoccurring GM, while treatment for initial growth is still in phase III clinical trial. Bevacizumab is usually given intravenously through the arm every 14 days. In colon cancer, it is given in combination with the chemotherapy drug 5-FU, leucovorin, and oxaliplatin or irinotecan. In 2009, the FDA approved Bevacizumab for use in metastatic renal cell cancer which is the drug's sixth indication, following earlier reports of activity and EU approval in 2007. [36] Also in 2009, an FDA advisory committee recommended Bevacizumab for treatment of GM.

For colorectal cancer, Meyer wrote that bevacizumab extended life by 4.7 months in the initial study, at a cost of $42,800 to $55,000. The addition of bevacizumab to standard treatment can prolong the lives of breast and lung cancer patients by several months, at a cost of $100,000 a year. [37]

Many diseases of the eye, such as age-related macular degeneration (AMD) and diabetic retinopathy, damage the retina and cause blindness when blood vessels around the retina grow abnormally and leak fluid, causing the layers of

the retina to separate. This abnormal growth is caused by VEGF, so bevacizumab has been successfully used to inhibit VEGF and slow this growth. [38] The main side effects are hypertension and heightened risk of bleeding. Bowel perforation has been reported. In advanced lung cancer, less than half of patients qualify for treatment. [39] Common toxicities associated with bevacizumab include hypertension, proteinuria, bleeding episodes, thrombotic events and general toxicities includes bleeding, arterial clots (which could lead to stroke and heart attack), bowel perforation, wound healing difficulties, and hypertension. [40]

Vatalanib: Vatalanib (PTK787 or PTK/ZK) is an oral phthalazine derivative and low molecular-weight competitive inhibitor (small molecule protein Kinase inhibitor) that inhibits angiogenesis. Vatalanib is being developed by Bayer Schering and Novartis. It inhibits all known VEGF receptors, as well as platelet-derived growth factor receptor-beta and c-kit, but is most selective for VEGFR-2. [41-43] Actually, it is a multi-VEGFR inhibitor designed to block angiogenesis and lymphangiogenesis by binding the intracellular kinase domain of all three VEGFRs, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4).

Vatalanib was discovered through high-throughput screening. [45] It has been extensively investigated in Phase I, II and III clinical trials. [42-43] Two large, randomized controlled Phase III trials have studied the effect of adding vatalanib to the FOLFOX chemotherapy regimen in people with metastatic colorectal cancer: CONFIRM-1, whose participants had not yet received any treatment for their cancer; and CONFIRM-2, in which participants had received first-line treatment with irinotecan and fluoropyrimidines. Vatalanib produced no significant improvement in overall survival (the primary endpoint of the studies), although it did significantly increase progression-free survival in CONFIRM-2. [43] Both trials found that progression-free survival was improved in people with high levels of lactate dehydrogenase, an enzyme used as a marker of tissue breakdown; the reasons for and implications of this difference are still unclear. [43-44] PTK787 is now going into large phase III clinical trials either as single agent or in combination with conventional anticancer drugs. It is currently being evaluated in breast, lung, and prostate cancers and multiple myeloma. [46] The adverse effects of vatalanib appear similar to those of other VEGF inhibitors. In the CONFIRM trials, the most common side effects were high blood pressure, gastrointestinal upset (diarrhea, nausea, and vomiting), fatigue, and dizziness. [43]

Semaxanib: One of the initial, and probably the most extensively studied KDR inhibitor, semaxanib (SU5416) was the first specific synthesized inhibitor of VEGF-RTK activity and showed a growth inhibition in mouse xenotransplants of human tumors; also demonstrated promising antiangiogenic and anti-tumor effects in animal models; however poor solubility and the lack of any survival advantage when administered in combination with chemotherapy led to the drug being discontinued. [47] Semaxanib is a drug intended for use in the treatment of cancer. It is still at an experimental stage and as such has not yet received a licence for use on human patients (except in the setting of a clinical trial). Semaxanib is a potent and selective synthetic inhibitor of the Flk-1/KDR VEGF receptor tyrosine kinase. It targets the VEGF pathway, and both in vivo and in vitro studies have demonstrated antiangiogenic potential. In advanced or metastatic soft tissue sarcomas at a dose of 145 mg/m^2 twice weekly, semaxanib was relatively well tolerated but did not demonstrate significant antitumor activity. [48]

The most common toxicities were headache, thrombosis, fatigue, nausea, and abdominal pain. The drug is lipophilic, highly protein-bound, and needs to be formulated with Cremophor and administered intravenously. Because of the toxicity of drug administration, the lack of efficacy in combination with chemotherapy in patients with metastatic colon cancer, and the promise of newer agents, the development of semaxanib was terminated. [49] On February 2002, Pharmacia, the developer of semaxanib, ended Phase III clinical trials due to discouraging results for drug's effectiveness in the treatment of advanced colorectal cancer. Other studies, at earlier phases, have since been conducted. However, due to the prospect of next-generation tyrosine kinase inhibitors and the ineffaciousness of semaxanib in clinic trials, further development of the drug has been discontinued. [49]

Ranibizumab: Ranibizumab (Lucentis), a humanized monoclonal antibody fragment derived from the same parent murine antibody as bevacizumab (Avastin). It is much smaller than the parent molecule and has been affinity matured to provide stronger binding to VEGF-A. It is an anti-angiogenic approved to treat the "wet" type of AMD, a common form of age-related vision loss. Ranibizumab was developed by Genentech and is marketed in the US by Genentech and elsewhere by Novartis, under the brand name of Lucentis.

Ranibizumab binds and inhibits all subtypes of VEGF-A. VEGF may trigger the growth of new vessels, which may leak blood and fluid into the eye. This causes macular edema and choroidal neovascularization, resulting in the wet type of AMD. By blocking VEGF-A in the eye, ranibizumab may prevent and reverse vision loss caused by wet macular degeneration. [71] The most common side effects in clinical trials were conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation. [71]

IMC-IC11: ImClone is developing IMC-IC11, an anti-angiogenesis chimeric monoclonal antibody VEGFR-2 (also known Flk-1 in mice) specific, for the potential treatment of cancer; it in phase I trials for the treatment of colorectal carcinoma. It blocks the ligand receptor binding and preventing phosphorylation. A phase I trial with 14 patients suffering from metastasized colon carcinoma showed a prolonged stabilization. Of the cohort, 7 patients developed antibodies against the chimeric IgG1 molecule; 2 patients had neutralizing antibodies. [50]

***Pegaptanib*:** Pegaptanib (Macugen) is an anti-angiogenic agent (selective VEGF antagonist) for the treatment of neovascular (wet) AMD. It was discovered by Gilead Sciences and licensed in 2000 to EyeTech Pharmaceuticals, now OSI Pharmaceuticals, for late stage development and marketing in the United States. Outside the U.S.A. Macugen is marketed by Pfizer. Approval was granted by the U.S. FDA in December 2004. Pegaptanib is a pegylated anti-VEGF aptamer, a single strand of nucleic acid that binds with specificity to a particular target. Pegaptanib specifically binds to VEGF 165, a protein that plays a critical role in angiogenesis and increased permeability, two of the primary pathological processes responsible for the vision loss associated with neovascular AMD. Drug interaction studies have not been conducted with Macugen. Pegaptanib is

metabolized by nucleases and is generally not affected by the cytochrome P450 system. Pegaptanib is administered in a 0.3 mg dose once every six weeks by intravitreal injection into the eye. [51]

Pegaptanib sodium is a covalent conjugate of an oligonucleotide of twenty-eight nucleotides in length that terminates in a pentylamino linker, to which two 20-kDa mono-methoxy PEG units are covalently attached via the two amino groups on a lysine residue. Adverse events associated with the use of Macugen may include Ocular Discomfort, Eye Pain, bronchitis, Endophthalmitis, Reduced Visual Acuity, Visual Disturbance, Corneal Edema, Blurred Vision, Urinary tract infection and Dizziness. [51]

Pegaptanib produced no maternal toxicity and no evidence of teratogenicity or foetal mortality in mice at intravenous doses of up to 40 mg/kg/day (about 7,000 times the recommended human monocular ophthalmic dose of 0.3 mg/eye). Pegaptanib crosses the placenta in mice. There are no studies in pregnant women were done. The potential risk to humans is still unknown. Macugen should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the foetus.

Sunitinib: Sunitinib (marketed as Sutent, and previously known as SU11248) is an oral, small-molecule, multi-targeted RTK inhibitor, based on an indolinone backbone and has a wider spectrum of activity than either SU5416 or SU-6668 that was approved by the FDA for the treatment of RCC and imatinib-resistant GIST on January 26, 2006.

Sunitinib is a primary choice in the first-line treatment of metastatic RCC; other therapeutic options in this setting are sorafenib (Nexavar), temsirolimus (Torisel) and interleukin-2 (Proleukin).

Sunitinib inhibits cellular signaling by targeting multiple RTKs. These include all receptors for PDGFRs and VEGFRs, which play a role in both tumor angiogenesis and tumor cell proliferation. The simultaneous inhibition of these targets therefore leads to both reduced tumor vascularization and cancer cell death, and ultimately tumor shrinkage. Sunitinib also inhibits KIT (CD117), [52] the RTK that (when improperly activated by mutation) drives the majority of GIST.

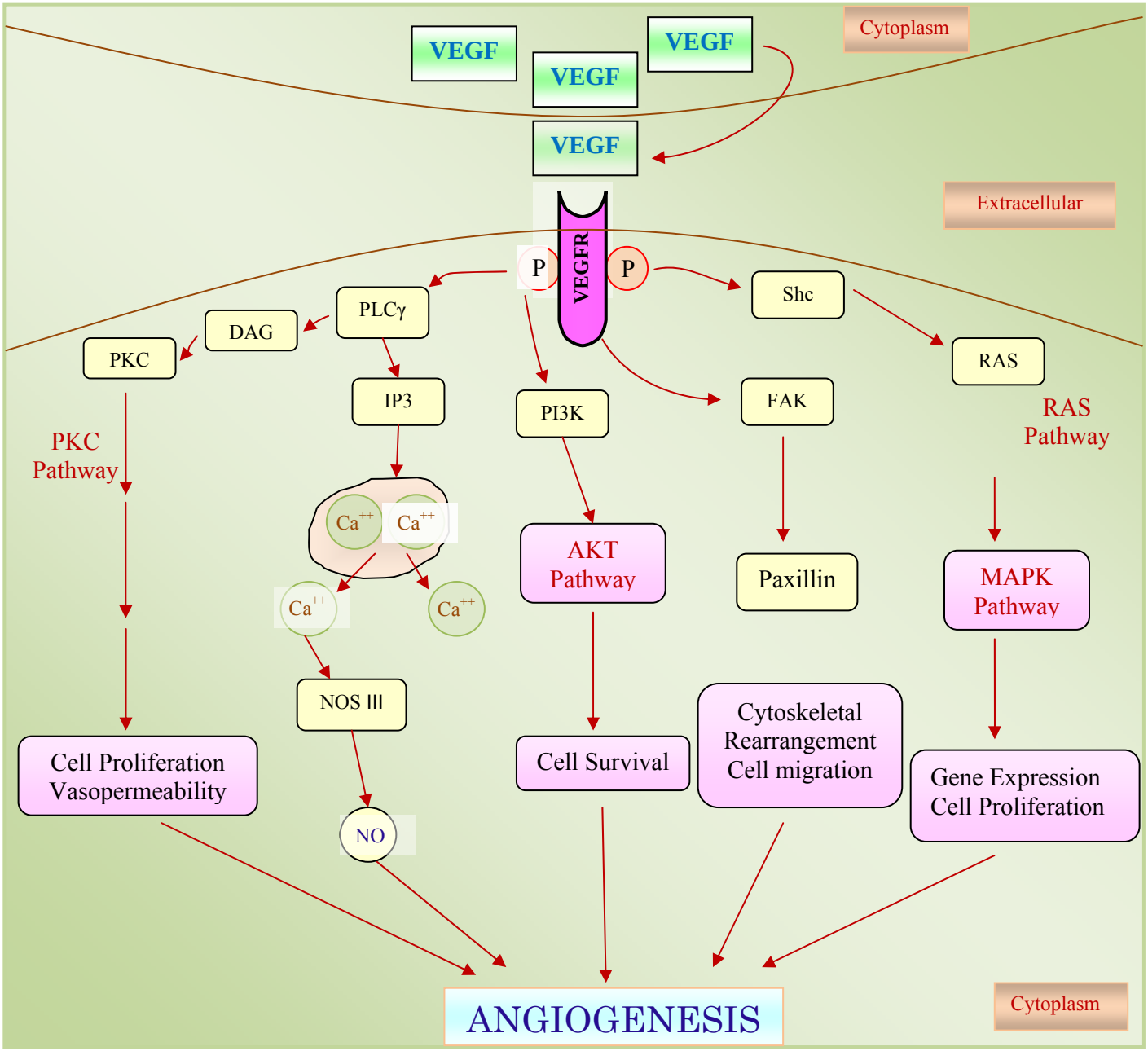

Fig. 2: VEGF signaling pathway

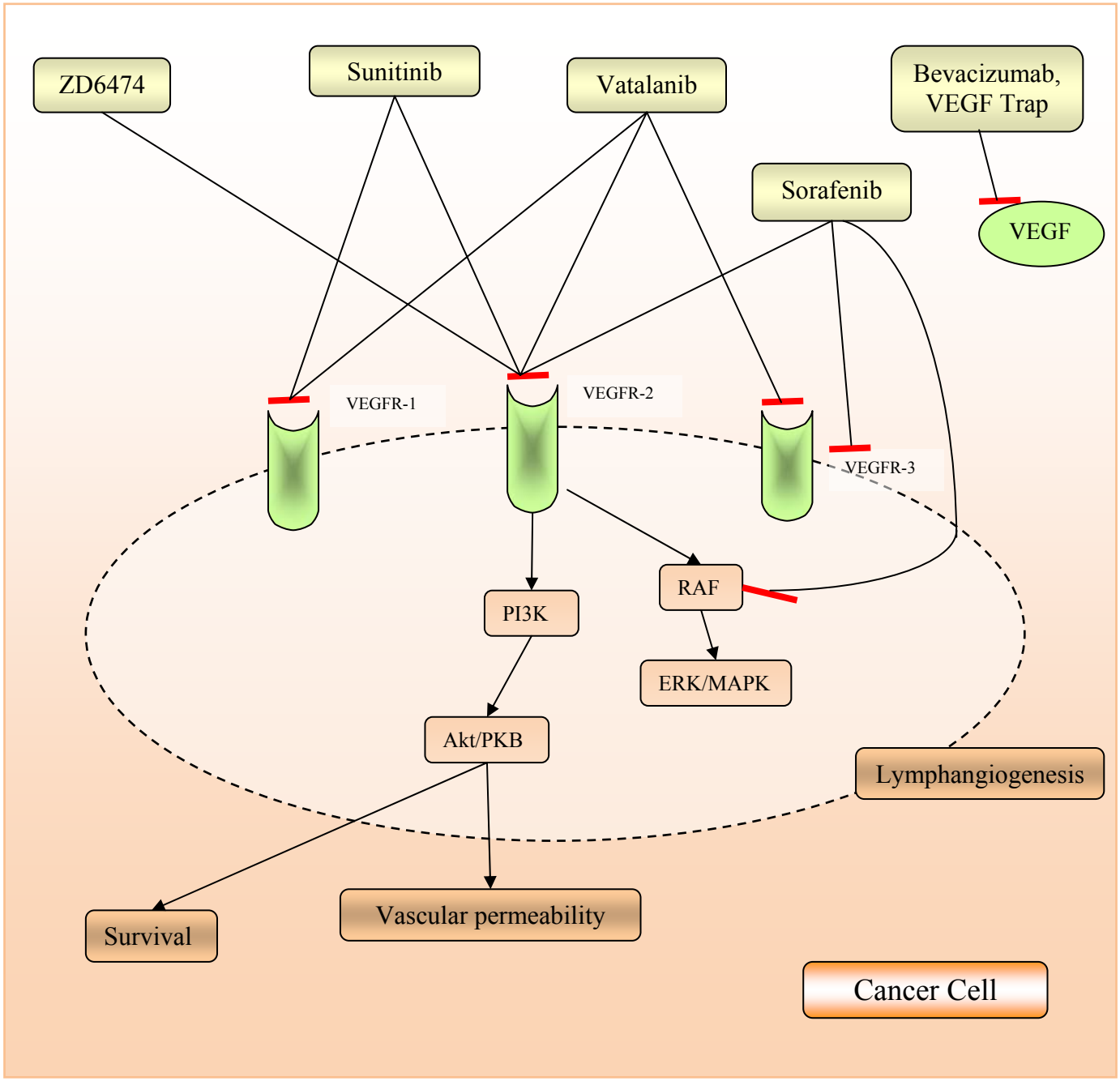

Fig. 3: Blocking sites of novel VEGF inhibitors

It has been recommended as a second-line therapy for patients whose tumors develop mutations in KIT that make them resistant to imatinib, or who become intolerant to the drug. In addition, sunitinib inhibits other RTKs. These includes RET, CSF-1R, flt-3. The fact that sunitinib targets many different receptors, leads too many of its side effects such as sore mouth, classic hand-foot syndrome and other dermatologic toxicities. The most common adverse events associated with sunitinib therapy are fatigue, vomiting, diarrhea, nausea, anorexia, hypertension, a yellow skin discoloration, hand-foot skin reaction, and stomatitis. Hypothyroidism has also been sometime associated with sunitinib. [52] Sunitinib is marketed by Pfizer as Sutent, and is subject to patents and market exclusivity as a new chemical entity until February 15, 2021. Sunitinib is one of several new smart cancer drugs called TKIs that target specific signalling molecules inside cancer cells that aid cancer spread; which boost up the market for the other kinase research activity.

Sorafenib: Another novel agent that indirectly inhibits VEGFR is the orally available Raf kinase inhibitor, sorafenib (Nexavar), which primarily acts through the oncogenic Raf/MEK/ERK pathway. It is a novel oral bis-aryl urea compound and small molecule TKI of c-Raf, B-Raf, PDGFR and VEGFR. In 2005, the FDA approved sorafenib to treat adults with advanced RCC. BAY32-9006 was first developed by Onyx Pharmaceuticals. Onyx subsequently collaborated with Bayer (Bayer is the "BAY" in BAY43-9006) to complete development of the drug.

BAY 43-9006 is a "targeted drug" specifically engineered to inhibit RAF kinase within the cancer cells. RAF in turn is part of the RAS oncogene pathway. RAS is a gene which drives cell division and is over-expressed in many cancers, including RCC. It turns out that in addition to targeting RAF Kinase, BAY 43-9006 also inhibits the VEGF and PDGF

receptors on blood vessel cells. Clear cell kidney cancer is well known for vastly over-producing VEGF, which in turn stimulates blood vessel growth which supplies the tumor with oxygen and nutrients. Most clear cell RCC has a defect in what is known as the VHL gene which causes the cell to think it is short on oxygen and to pump out huge amounts of VEGF. BAY 43-9006 interrupts this signal on the blood vessel cells. This inhibition of VEGF is probably the main reason BAY 43-9006 has had much greater effect in kidney cancer than in other cancers. [53]

Drug-related toxicities (all grades) included rash, hand-foot-skin reaction, fatigue, diarrhea, and hypertension. Phase I studies of sorafenib alone or in combination with chemotherapy have shown encouraging results in melanoma, pancreatic and ovarian cancers. Studies in Kaposi's sarcoma and in lung, prostate, and hepatocellular cancers are underway. [53-54]

VEGF Trap: VEGF Trap, a soluble decoy receptor for VEGF, also called aflibercept and vascular endothelial growth factor trap; target VEGFR: Flt (1–3) IgG and consists of parts of VEGFR-1, VEGFR-2 and IgG. The molecule binds to VEGF-A before it can reach its normal receptors. [32] A hybrid Fc construct in which domain 2 of VEGFR-1 is joined to domain 3 of the VEGFR-2 (VEGF-trap) causes regression of coopted vessels in a model of neuroblastoma. Importantly, ongoing clinical studies are evaluating the efficacy of the VEGF-trap in patients with incurable relapsed or refractory solid tumors or NHL. [55]

It is a potent angiogenesis inhibitor and binds to VEGF-A up to 1000-fold more tightly than monoclonal antibodies and inactivates all circulating and tissue VEGF-A isoforms plus placental growth factor. Blockade of VEGF by VEGF Trap leads to increased VEGF and perlecan expression by tumor cells. Perlecan/VEGF migrates to the vasculature where it binds the basement membrane collagen IV. Perivascular HPA1 locally releases VEGF, which then binds to VEGFR2, inducing phosphorylation of the receptor and downstream phosphorylation of Akt. [56] VEGF-Trap-mediated blockade may be superior to that achieved by other agents, such as monoclonal antibodies targeted against the VEGF receptor. [57]

Sanofi-aventis and Regeneron currently are enrolling approximately 4000 patients in the U.S., Europe, and other countries around the world, for Phase 3 studies that combine aflibercept with standard chemotherapy regimens. Aflibercept has a relatively long half-life of approximately two weeks. In phase I trials in solid tumors and lymphoma, fatigue, pain, proteinuria, and constipation were the most common adverse events. No response has been reported. [60] This VEGF-Trap effectively suppresses tumor growth and vascularization in vivo, resulting in stunted and almost completely avascular tumors and also inhibits tumor growth in a murine rhabdomyosarcoma xenograft model. [57-58] Finally, clinical trials results indicate that the clinical success of VEGF Trap may depend on a prolonged treatment in combined therapy aiming to simultaneously inhibit angiogenesis and tumor invasion. [59]

***Vandetanib*:** Vandetanib (Zactima, ZD6474) is an orally available Tyrosine Kinase Inhibitor (TKI) under development by AstraZeneca for the treatment of solid tumours. ZD6474 is an oral inhibitor of VEGFR-2 and the EGFR-TK. Safety and tolerability of ZD6474 have been evaluated in two phase I studies with refractory tumors including a large proportion of NSCLC cancer patients. An oral administration of doses of 300 mg or less was generally safe and well tolerated. Concurrent side effects are diarrhea, rash and asymptomatic corrected QT interval prolongation. The characteristics of this side-effect profile (rash) is attributable to the EGFR-inhibiting properties, whereas the dominant side-effects caused by VEGF inhibition known from other compounds such as hypertension are not reported in high frequency. This leads to the hypothesis that the clinical activity of ZD6474 has a predominant component of EGFR inhibition. [70]

In addition to NSCLC, Zactima is also being investigated as a treatment for thyroid cancers. It was granted orphan drug status by the FDA for the treatment of follicular, medullary, anaplastic, and locally advanced and metastatic papillary thyroid cancers. In July 2005, AstraZeneca announced plans for phase III development of Zactima in NSCLC. This followed successful outcome in a phase II trial in which Zactima was combined with docetaxel in patients with locally-advanced or advanced NSCLC. The preliminary results of the Phase II trial were showed that Zactima in combination with docetaxel, increased progression-free survival in patient population. Zactima is a once-daily, oral TKI that combines the action of Iressa and Tarceva with an additional ability to deprive tumours of their blood supply via VEGFR-2-mediated antiangiogenic effects. A broader spectrum of anti-tumour activity may confer treatment advantages but this awaits confirmation in large-scale trials. [70]

Axitinib: Axitinib (AG013736) is an imidazole derivative, an oral anti-angiogenesis agent with activity against RTK, including VEGFR-1, VEGFR-2, VEGFR-3, c-kit, and PDGFR-β at low nanomolar concentrations under development by Pfizer. [61-62] Patients with AML and MDS, AG013736 grade 3 or 4 toxicities included hypertension, mucositis, and deep venous thrombosis. No objective responses occurred; 2 patients with MDS had stable disease. Adverse events in solid tumors included hypertension, hemoptysis, and stomatitis. [63] A Phase II clinical trial showed good response in combination chemotherapy with Gemcitabine for advanced pancreatic cancer. However, Pfizer reported that Phase III clinical trials of the drug when used in combination with Gemcitabine showed no evidence of improved survival rates over treatments using Gemcitabine alone for advanced pancreatic cancer and halted the trial. [64]

RPI.4610: RPI.4610 (ANGIOZYME) is a chemically stabilized ribozyme targeting vascular endothelial growth factor receptor 1. [65] Ribozymes function by cleaving RNA phosphodiester bonds at specific sites and in doing so destroy the ability of targeted mRNA to direct synthesis of an encoded protein. While single ribozyme molecules can degrade multiple mRNA strands, they are limited by their susceptibility to nuclease degradation that results in poor serum stability. Preclinical studies of an anti-VEGF hairpin ribozyme compound has shown efficacy in significantly inhibiting the growth and proliferation of ovarian cancer SKOV3 cells. [13]

Chiron Corporation/Ribozyme Pharmaceuticals Inc. (RPI) holds the rights to more than 100 worldwide patents encompassing ribozyme design, synthesis, chemical modification, delivery, and production. Their signature anti-angiogenic product is an anti-Flt-1 ribozyme known as Angiozyme. In a study conducted by Pavco et al, ribozymes

targeting either VEGFR-1 or VEGFR-2 significantly inhibited primary tumor growth in a highly metastatic variant of Lewis lung carcinoma and significantly inhibited liver metastasis in a xenograft colorectal cancer model. Further combinatorial studies have revealed that RPI.4610, carboplatin, and paclitaxel can be administered safely in combination without substantial pharmacokinetic interactions. [13]

Table 2: Representative Examples of VEGF inhibitors with Clinical Status

Drug	Target	Clinical Status	Company
	Monoclonal Antibodies		
Bevacizumab (Avastin)	VEGF-A	Approved (2004)	Genentech
Ranibizumab (Lucentis)	VEGF-A	Approved (2006)	Genentech
IMC-1C11	VEGFR-2	Phase I	ImClone Systems
IMC-1121B	VEGFR-2	Phase II/III	ImClone Systems
2C3	VEGF-A	Preclinical	Peregrine Pharmaceuticals
CDP-791	VEGFR-2	Phase II	UCB
IMC-18F1	VEGFR-1	Phase I	ImClone Systems
2C5	VEGFR-3	Preclinical	ImClone Systems
HuMV833	VEGFR-2	Phase I	EORTC
	Receptor Tyrosine Kinase Inhibitors		
PTK-787/ZK222584 (Vatalanib)	VEGFR-1, -2	Phase III	Novartis Pharma AG/ Schering AG
AEE788	VEGFR-2, EGFR	Preclinical	-
ZD6474 (Vandetanib)	VEGFR-1, -2, -3, EGFR	Phase II	AstraZeneca
AZD2171 (Cediranib)	VEGFR-1, -2	Phase I	AstraZeneca
SU11248 (Sunitinib)	VEGFR-1, -2, PDGFR, KIT, FLT3	Approved (2006)	SUGEN Inc./ Pharmacia
CP-547,632	VEGFR-1, -2	Phase I/II	Pfizer
GW786034 (Pazopanib)	VEGFR-1, -2, -3	Phase I/II	GlaxoSmithKline
BAY439006 (Sorafenib)	VEGFR-1, -2, PDGFR	Approved (2005)	Bayer Pharmaceuticals
CHR-200131	VEGFR, PDGFR (Flt-4), FGFR	Phase I/II	Chiron
CEP-7055	VEGFR, PDGFR, Flt-3	Phase I/II	Cephalon
AMG706 (Motesanib)	VEGFR-1, -2, -3, PDGFR, c-Kit	Phase II	Amgen
AE-941 (Neovastat)	VEGF, MMP	Phase II	AEterna Zentaris
SU5416 (Semaxanib)	VEGFR-2	Phase II/III	SUGEN Inc.
SU6668	VEGFR-2, PDGF, FGFR	Phase I/II	SUGEN Inc./ Pharmacia
AV-951	VEGFR-1, -2, -3	Phase I/II	AVEO Pharmaceuticals
AG013736 (Axitinib)	VEGFR-1, VEGFR-2, VEGFR-3, c-kit, PDGFR-β	Phase II/III	Pfizer/Agouron
Telatinib	VEGFR, PDGFR	Phase II	Act Biotech
YM359445	VEGFR-2	Preclinical	-
Midostaurin (PKC412)	VEGF, PDGF, and c-KIT	Phase II	Novartis
KRN-633	VEGFR, c-kit	Phase I/II	Kirin Brewery
XL 647	EGFR, VEGFR, HER2	Phase I	Exelixis
XL 880	VEGFR-2, Met	Phase I	Exelixis
XL184	VEGFR2, Met, c-Kit, FLT3, Tie2	Phase I	Exelixis
XL999	VEGFR, FGFR, PDGFR, FLT3	Phase I	Symphony Evolution Inc.
OSI930	VEGFR2, c-Kit	Phase I/II	OSI Pharmaceuticals
	Soluble receptor chimeric protein		
VEGF-Trap	VEGF-A, PlGF	Phase III	Sanofi-aventis and Regeneron Pharmaceuticals Inc.
	Others		
RPI.4610 (ANGIOZYME)	VEGFR-1	Phase III	Ribozyme Pharmaceuticals Inc.
VEGF-AS (antisense oligonucleotide, Veglin)	VEGF, VEGF-C, VEGF-D	Phase I /II	USC
Pegaptanib (Macugen)	VEGF	Approved (2004)	EyeTech/OSI Pharmaceuticals

YM359445: YM359445 ((3Z)-3-quinolin-2(1H)-ylidene-1,3-dihydro-2H-indol-2-one derivative), [66] an orally VEGFR-2 tyrosine kinase inhibitor, has a greater antitumor activity against established tumors in preclinical studies compared with other VEGFR2 tyrosine kinase inhibitors. [67] It is also known as (3Z)-3-(6-((4-methylpiperazin-1-yl) methyl) quinolin-2(1H)-ylidene)-2-oxoindoline-6-carbaldehyde-O-(1, 3-thiazol-4-ylmethyl)oxime mono-L-tartrate.

SU6668: An orally bioavailable RTK inhibitor, SU6668 binds to and inhibits the auto-phosphorylation of VEGFR2, PDGFR and FGFR and their cognate RTKs are strongly implicated in angiogenesis associated with solid tumors; thereby inhibiting angiogenesis and cell proliferation. SU6668 also inhibits the phosphorylation of the stem cell factor RTK c-kit, often expressed in acute myelogenous leukemia cells. It possesses a similar selectivity profile to SU5416 but greater solubility, also entered clinical trials, but was discontinued for the treatment of solid tumors as adverse events, including severe pain and serositis, necessitated reducing the dosing regimen below that predicted to be required to achieve pharmacologically active levels. [68] Its preclinical antitumor activity has been documented in many tumors including ovarian, glioma, melanoma, lung, and colon. A phase I trial in solid tumors showed a high rate of toxicity with no objective responses. Thrombocytopenia, pericarditis, pleuritic chest pain, nausea, abdominal pain, fatigue, headache, constipation, diarrhea, and abnormal liver function tests were reported as treatment side effects. The development of this drug has been discontinued due to unacceptable toxicity. [69]

DISCUSSION

VEGF family has crucial role in signaling proteins involved in both vasculogenesis and pathological angiogenesis in disorders like Cancer, Rheumatoid arthritis, Diabetic retinopathy and the Neovascular form of macular degeneration. The past studies shows that VEGF receptors play a pivotal role in cell transformation and progression of many carcinomas, including breast, ovarian, renal, NSCLC, Head and neck, Colorectal, Pancreas, Brain (glioma), Bladder, Esophagus, Stomach, Prostate, Melanoma, Thyroid, and Endometrial cancers thereby been a major focal point in cancer drug discovery. VEGF blockade has been shown to

have a direct and rapid anti-vascular effect in both animal and human tumors, through deprivation of tumor vascular supply and inhibition of endothelial proliferation. Several new tyrosine kinase inhibitors targeting the VEGF pathway are currently in advanced clinical development for NSCLC and offer several possible advantages compared with monoclonal antibodies, including oral administration, more flexible dosing, a broader spectrum of target inhibition, and different toxicity profiles. Among these agents, vandetanib (ZD6474), an inhibitor of the VEGF receptor (VEGFR-2) has been the most extensively studied. To date, the FDA has approved two monoclonal antibodies for clinical use, Bevacizumab (Avastin) and Ranibizumab (Lucentis); two TKIs, Sunitinib (SU11248) and Sorafenib (BAY439006); anti-VEGF pegylated aptamer named Pegaptanib (Macugen) as VEGF inhibitors. The addition of bevacizumab to standard treatment can prolong the lives of breast and lung cancer patients by several months, at a cost of $100,000 a year. It appears likely that VEGF inhibitors (and other rationally designed molecular growth inhibitors) will play a meaningful role in cancer therapy in the years to come.

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