Angiogenesis

An Integrative Approach From Science to Medicine

Edited by

William D. Figg
Medical Oncology Branch, Center for Cancer Research,
National Cancer Institute, Bethesda, Maryland, USA

Judah Folkman
Department of Surgery, Harvard Medical School and
Vascular Biology Program, Children's Hospital Boston,
Boston, Massachusetts, USA

Springer

Editors
William D. Figg
Medical Oncology Branch
Center for Cancer Research
National Cancer Institute
Bethesda, Maryland
USA

Judah Folkman
Department of Surgery
Harvard Medical School and
Vascular Biology Program
Children's Hospital Boston
Boston, Massachusetts
USA

ISBN: 978-0-387-71517-9 e-ISBN: 978-0-387-71518-6
DOI: 10.1007/978-0-387-71518-6

Library of Congress Control Number: 2007934647

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Chapter 36
Clincal Development of VEGF Trap

John S. Rudge, Ella Ioffe, Jingtai Cao, Nick Papadopoulos, Gavin Thurston, Stanley J. Wiegand, and George D. Yancopoulos

Keywords: VEGF Trap, vascular eye disease, kinase inhibitors

Abstract: The inhibition of angiogenesis is proving to be an effective strategy in treating diseases involving pathological angiogenesis such as cancer and ocular vascular diseases. Since its discovery in the 1980s, vascular endothelial cell growth factor (VEGF) has been shown to play a vital role in both physiological and pathological angiogenesis, resulting in the development of numerous approaches to block VEGF and VEGF signaling, ranging from small molecule tyrosine kinase inhibitors to protein-based and RNA-based therapeutic candidates. VEGF Trap is one such protein-based agent that has been engineered to bind and sequester VEGF, as well as placental growth factor (PlGF), with high affinity. VEGF Trap has been shown to effectively inhibit pathological angiogenesis in numerous preclinical models of cancer and eye disease, and is now being evaluated in clinical trials in several types of cancer, as well as the 'wet' or neovascular form of age-related macular degeneration (AMD). This chapter will summarize the basic biology of VEGF and the progress of the VEGF Trap from the bench to the clinic.

Introduction

Angiogenesis is a vital process not only during development, but also in the adult in settings of wound repair and reproduction [1, 2]. However, in diseases characterized by uncontrolled, pathological angiogenesis, such as solid tumors or vascular diseases of the eye, inhibition of angiogenesis would be of therapeutic benefit. While diverse factors might be expected to regulate angiogenesis in different settings, a clear consensus is emerging that a single growth factor, VEGF is the critical requisite driver of both physiological and pathological angiogenesis in most settings [3]. Thus, while blocking VEGF signaling during early development can lead to severe growth retardation, it can also produce highly beneficial effects when applied to disease states characterized by pathological neovascularization [3]. Recent clinical studies have validated VEGF as a bona fide target for therapeutic intervention in cancer as well as in vascular diseases affecting the eye, such as wet AMD. These studies have led to approval by the U.S. Food and Drug Administration (FDA) of drugs that target the VEGF pathway, specifically antibodies directed against VEGF, or kinase inhibitors which block activation of the VEGF receptors [4–7]. Emerging therapeutic candidates that otherwise target VEGF signaling have also reported encouraging results [8].

Regeneron Pharmaceuticals, 777 Old Saw Mill River Road
Tarrytown, NY 10591, USA
E-mail: John.Rudge@regeneron.com

Biology of VEGF and Its Receptors

VEGF is widely known to promote angiogenesis, by stimulating vascular endothelial cell proliferation, migration and tube formation, and it can also markedly increase vascular permeability [9]. VEGF-A is the prototypical member of a family of factors that also consists of VEGF-B, VEGF-C, VEGF-D and PlGF, which bind differentially to VEGF receptors 1, 2 and 3 and neuropilin with different specificities [10,11]. In addition, alternative exon splicing results in the production of four major isoforms of human VEGF-A – $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$ differentiated by their heparin binding affinity [12]. $VEGF_{121}$ differs from the other isoforms in that it does not bind heparin and thus is freely diffusible. As the isoforms increase in molecular weight, they show increasing heparin binding affinity and consequently bind extracellular matrix such that they tend to be sequestered near their site of production [13–15]. Matrix metalloproteases (MMPs) appear to play an important role in regulating the release of these isoforms from matrix [16]. Although endothelial cells are the primary targets of VEGF actions, more recently it has been shown that other cell types, including monocytes, hematopoietic stem cells and neurons, can also respond to VEGF [17–19].

The VEGF receptor 2 (VEGFR2) appears to be the major mediator of the mitogenic, angiogenic and pro-permeability actions of VEGF-A. Indeed, genetic deletion of VEGFR2 results in profound defects in embryonic vasculogenesis, including a failure to develop blood islands and organized blood vessels [20]. In contrast, the role of VEGFR1, which was the first VEGF receptor identified, is more difficult to discern [21]. To date, VEGFR1 has been implicated in MMP9 induction, hematopoiesis, and monocyte chemotaxis, and also appears to act as a 'biological sink' which sequesters VEGF from binding to the lower affinity receptor, VEGFR2 [11]. A further level of complexity was revealed when VEGF was shown to bind neuropilin, a molecule previously identified as a receptor for the collapsin/semaphorin family of ligands involved in axon guidance. The binding of VEGF to neuropilin is thought to result in presentation of VEGF to VEGFR2, augmenting VEGFR2 signal transduction [22].

The Evidence for VEGF as a Key Player in Tumor Angiogenesis

In situ hybridization studies have revealed that VEGF is highly expressed in a number of human tumors [23–27]. This is most apparent in renal cell carcinoma where mutations in the von Hippel-Lindau (VHL) tumor suppressor gene result in increased transcription of hypoxia-inducible factor (HIF1) genes [28,29].

Numerous preclinical studies have shown that the growth of many different tumor types can be inhibited using agents that variously inhibit VEGF signaling (small molecule inhibitors, VEGFR2 antibodies, soluble VEGF receptors including the VEGF Trap) [30–35]. These studies have also shown that VEGF derived from the stromal compartment, as well as the tumor itself, plays an important role in mediating the angiogenesis which supports tumor growth [32,36].

Not surprisingly, antiangiogenic therapy results in a cytostatic effect in many tumor types rather than frank regression. Thus, in many models, combination of VEGF blockade with chemotherapy or radiation therapy results in greater efficacy than either approach alone [37,38].

VEGF Pathway Inhibitors Approved for the Treatment of Cancer

Bevacizumab (Avastin®)

The positive results from preclinical studies have led to the testing of several VEGF inhibitors in clinical trials. These include the humanized anti-VEGF-A monoclonal antibody (bevacizumab, Avastin®), an anti-VEGFR antibody [39], small molecules that inhibit VEGFR signaling and VEGF Trap [4,40–42]. The first clinical validation of the anti-VEGF approach came with FDA approval of bevacizumab in 2004, based on the results of a randomized double-blind phase III trial in which bevacizumab was combined with bolus IFL (irinotecan, 5FU, leucovorin) chemotherapy as first line therapy for metatstatic colorectal cancer. Median survival increased from 15.6 months in the IFL alone arm to 20.3 months in the IFL + bevacizumab arm. Severe hypertension was observed in about 10% of bevacizumab treated patients, and gastrointestinal perforation was noted in ~2% of patients. In addition, the incidence of arterial thromboembolic complications (stroke, myocardial infarction, transient ischemic attacks, unstable angina) was double the incidence seen with chemotherapy alone [43].

Kinase Inhibitors

An alternative approach to using antibodies that bind and neutralize VEGF is the use of tyrosine kinase inhibitors that target the VEGF and other receptors. To date, two such kinase inhibitors have been approved by the FDA: SU11248 (sunitinib; Sutent®) and Bay 43–9006 (sorafenib; Nexavar®). Sunitinib inhibits tyrosine phosphorylation of VEGFR1, VEGFR2, platelet derived growth factor receptor (PDGFR), c-kit and Flt3, and has been approved for the treatment of Gleevec-resistant gastrointestinal stromal tumors (GIST) and metastatic renal cell carcinoma [7]. Sorafenib, which inhibits tyrosine phosphorylation of raf, VEGFR2/3, PDGFR, kit and Flt3, was approved in 2006 for the treatment of metastatic renal cell carcinoma [5,6]. It should be pointed out that these kinase inhibitors do not block VEGF signaling selectively or completely, and much of their clinical benefit could derive from the inhibition of other kinase pathways.

Development and Application of VEGF Trap in Preclinical Animal Models

Despite the important benefits in patient care provided by the currently approved VEGF pathway blockers, current evidence suggests that optimal VEGF blockade may not have yet been achieved (e.g., dose response studies do not indicate that saturation of benefit has been reached) [44,45], raising the possibility that more potent VEGF blockade could provide even more benefit for patients. The VEGF Trap may provide the opportunity to test this hypothesis, as it was designed to bind VEGF with exceedingly high-affinity. The VEGF Trap is a soluble chimeric receptor in which key domains of VEGFR1 (domain 2) and VEGFR2 (domain 3) are fused to the constant region (Fc portion) of human IgG1 [34]. This fully human protein is capable of binding all isoforms of VEGF-A with very high affinity (KD <0.5 pM for $hVEGF_{165}$). Moreover, and in contrast to antibodies directed against VEGF-A, the VEGF Trap also binds PlGF, a VEGF family member also implicated in pathological angiogenesis [46] (KD ~25 pM for hPlGF2). In addition, the VEGF Trap was engineered to exhibit excellent pharmacokinetic properties, and has a circulating half-life in humans of approximately 2–3 weeks, allowing for bi-weekly or even less frequent dosing.

Once VEGF Trap had been optimized, it was tested and shown to have significant antiangiogenic and anti-tumor efficacy in

numerous preclinical tumor models [34,37,38,47–50]. When administered in combination with chemotherapy or radiation, VEGF Trap also produced a significant additive impact on tumor growth. Moreover, in an ovarian cancer model, VEGF Trap potently inhibited ascites formation [37,38,50]. The impressive efficacy profile of VEGF Trap in preclinical animal models justified its progression into clinical trials.

Interestingly, VEGF Trap also has an additional property that distinguishes it from antibodies: while antibodies form multimeric complexes with their antigens that are rapidly cleared from the systemic circulation, VEGF Trap forms an inert 1:1 complex with VEGF that remains in the circulation and can thus be readily measured. Thus, when given in doses that saturate VEGF binding, this unique property of the VEGF:VEGF Trap complex allows for the accurate determination of tumor and whole body VEGF production rates [51]. Somewhat surprisingly, when non-tumor bearing animals and humans are given the VEGF Trap, we find that total body production rates of VEGF are quite high, challenging previous claims that systemic VEGF levels can be used as a sensitive measure of tumor burden [51]. This finding has the important corollary that agents designed to bind and inactivate tumor-derived VEGF must be provided in amounts sufficient to ensure that they are not effectively consumed by the large amounts of VEGF normally produced by the body. Building on this finding, we have determined that measurement of the levels of free and bound VEGF Trap provides a clear index of the doses required to capture all available VEGF, offering a useful guide for dosing of this antiangiogenic agent. Based on these assays, the doses currently being used in the clinic for cancer appear to be in the therapeutic range.

VEGF Trap in Clinical Trials for Cancer

Multiple Phase 1 trials have been carried out using the VEGF Trap as a single agent, or in combination with various chemotherapeutic regimens, in patients with advanced cancers, with more than 300 patients treated to date. Numerous objective responses as well as cases of prolonged stable disease have been noted in these trials. Interestingly, in addition to anti-tumor responses, improvements in tumor-associated co-morbidities have been observed: in particular, substantial or complete resolution of tumor-associated ascites. The major adverse events noted in these trials are consistent with those seen with other anti-VEGF agents, and include hypertension and proteinuria. Importantly, no antibodies to VEGF Trap were observed in any patients.

VEGF Trap is currently in Phase II clinical trials and is about to initiate multiple large Phase 3 trials. Preliminary results from two of these studies were recently reported at the American Society of Clinical Oncology 2007 annual meeting [41,42] Results were reported from an interim analysis of a randomized, double-blind, multi-center Phase 2 trial comparing two doses of VEGF Trap in patients with recurrent, platinum-resistant epithelial ovarian cancer. The patients selected for this study were heavily pre-treated and had failed several other treatment regimens. While the study remains blinded with respect to dose, the pooled preliminary results from both the high and low dose groups demonstrated anti-tumor activity, as evidenced by an 8% objective tumor response rate, with a 13% response rate as measured by a 50% reduction in circulating levels of the tumor marker CA-125. Disease was judged to be stable in 77% of patients at 4 weeks, and in 41% of patients at 14 weeks. In addition, of 24 patients in the study who had tumor-associated ascites, 29% experienced complete resolution of their ascites while 54% experienced no increase in ascites during treatment. Tolerability was similar to other molecules in this class with hypertension being the most common grade 3/4 event (16%). Two patients (1.2%) experienced bowel perforation, both of whom recovered. In a collaboration involving Sanofi-Aventis and Regeneron Pharmaceuticals, the ongoing single-agent studies of VEGF Trap will be complemented by a large Phase 3 program combining VEGF Trap with standard chemotherapy regimens in at least 5 different advanced solid tumors: colorectal, non-small cell lung, prostate, pancreas and gastric cancer, with the first of these studies initiating in 2007.

Preclinical Studies with VEGF Trap in Vascular Eye Diseases

The finding that VEGF-A was up-regulated in the aqueous and vitreous humor of patients with proliferative diabetic retinopathy extended the focus of antiangiogenic agents from cancer to vascular eye disease [52,53]. Wet age-related macular degeneration (AMD) is the most common cause of vision loss in the elderly [53,54]. In this disease, VEGF is believed to mediate the abnormal growth of choroidal vessels that, together with the associated vascular leak and edema, disrupts the normal retinal architecture. VEGF Trap has also been tested in a number of rodent models of ocular vascular disease, where it has been shown to inhibit choroidal [55] and corneal [56] neovascularization in addition to suppressing vascular leak in the retina [57]. VEGF Trap has also been shown to improve the survival of corneal transplants [58]. In a primate model of AMD, in which a laser was used to induce choroidal vascular lesions, intravitreal administration of VEGF Trap was found not only to prevent the development of vascular leak and neovascularization when administered before the time of injury but also completely resolved vascular leak when administration was delayed until after the lesions had fully developed [59].

VEGF Trap in Clinical Trials for Vascular Eye Disease

The promising results in preclinical models supported the introduction of VEGF Trap into the clinic for treatment of both wet AMD and diabetic macular edema, using a version of VEGF Trap specifically formulated for intra-ocular administration,

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.