Title

VEGF in Signaling and Disease: Beyond Discovery and Development

Journal

Cell, 176(6)

ISSN

0092-8674

Authors

Apte, Rajendra S
Chen, Daniel S
Ferrara, Napoleone

Publication Date

2019-03-01

DOI

10.1016/j.cell.2019.01.021

Peer reviewed

Cell

Leading Edge
Review

VEGF in Signaling and Disease: Beyond Discovery and Development

Rajendra S. Apte,[1,2,3,*] Daniel S. Chen,[4] and Napoleone Ferrara[5,6,7]
[1]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA
[2]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
[3]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA
[4]IGM Biosciences, Mountain View, CA, USA
[5]Department of Pathology, University of California, San Diego, CA, USA
[6]Department of Ophthalmology, University of California, San Diego, CA, USA
[7]The Moores Cancer Center, University of California, San Diego, CA, USA
*Correspondence: apte@wustl.edu
https://doi.org/10.1016/j.cell.2019.01.021

The discovery of vascular endothelial-derived growth factor (VEGF) has revolutionized our understanding of vasculogenesis and angiogenesis during development and physiological homeostasis. Over a short span of two decades, our understanding of the molecular mechanisms by which VEGF coordinates neurovascular homeostasis has become more sophisticated. The central role of VEGF in the pathogenesis of diverse cancers and blinding eye diseases has also become evident. Elucidation of the molecular regulation of VEGF and the transformative development of multiple therapeutic pathways targeting VEGF directly or indirectly is a powerful case study of how fundamental research can guide innovation and translation. It is also an elegant example of how agnostic discovery and can transform our understanding of human disease. This review will highlight critical nodal points in VEGF biology, including recent developments in immunotherapy for cancer and multitarget approaches in neovascular eye disease.

Introduction

The development of a neovascular supply, or angiogenesis, serves crucial homeostatic roles, since blood vessels carry nutrients to tissues and organs and remove catabolic products. However, uncontrolled growth of blood vessels can promote or facilitate numerous disease processes, including tumors and intraocular vascular disorders. Over 70 years ago, it was hypothesized that the ability to induce new vessel growth through release of ''blood-vessel growth-stimulating factors'' confers on tumor cells a growth advantage (Ide et al., 1939; Algire et al., 1945). At about the same time, it was proposed that a diffusible factor may be responsible not only for the development of the normal retinal vasculature, but also for pathological neovascularization in proliferative diabetic retinopathy and other disorders (Michaelson, 1948). Judah Folkman's hypothesis that ''anti-angiogenesis'' could be a strategy to treat cancer and possibly other disorders (Folkman, 1971) generated a great deal of enthusiasm and gave a major boost to the field. However, harnessing such therapeutic potential required isolation, sequencing, and cDNA cloning of the mediators of angiogenesis—all major technological challenges at that time.

Vasculogenesis, the formation of blood vessels from *de novo* generation of endothelial cells, and angiogenesis, the process of new blood vessel formation, are critical during development and subsequent physiologic homeostasis but can be pathogenic in cancers and several ophthalmic diseases. Vascular endothelial-derived growth factor (VEGF), important in vasculogenesis and angiogenesis, was identified, isolated, and cloned over 25 years ago (Ferrara and Adamis, 2016). While VEGF mainly targets endothelial cells, it has been shown that this factor has multiple effects on additional cell types. Although there are several related genes, including VEGF-B, VEGF-C, and placental growth factor (PlGF), most attention is focused on VEGF-A due to its key role in regulating angiogenesis during homeostasis and disease. Although VEGF is essential for physiologic vascular homeostasis in diverse cells and tissues, it has been demonstrated to be important in the molecular pathogenesis of tumor growth and metastasis and in retinopathy associated with several blinding eye diseases, including age-related macular degeneration (AMD) and diabetic and hypertensive retinopathy (Adamis and Shima, 2005; Ferrara, 2016). VEGF-mediated pathogenic effects are primarily due to its effects on vascular permeability and neoangiogenesis (neovascularization). A number of therapeutic approaches have since targeted one or more isoforms of VEGF, the VEGF receptors, or signaling pathways, and some have since led to approval of drugs by regulatory authorities around the world (summarized in Box 1; Ferrara and Adamis, 2016). The biology of VEGF is a unique illustration of how fundamental discovery at the bench has informed and transformed therapeutic discovery and development aimed at the bedside in a relatively short time span of less than 15 years. Targeting VEGF and associated pathways has prevented blindness in millions of patients with eye disease and increased survival/lifespan for patients suffering from a number of different cancer

Box 1. US FDA-Approved Drugs Targeting VEGF-Regulated Pathways in Oncology (in Combination with Other Therapies) and Indications

Adapted from Zirlik and Duyster (2018).

1. Bevacizumab (target: VEGF-A): locally, advanced, metastatic, or recurrent colorectal cancer (CRC); metastatic NSCLC; recurrent glioblastoma; cervical cancer; certain recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer; metastatic RCC
2. Ziv-aflibercept (targets: VEGF-A, VEGF-B, PlGF): metastatic CRC
3. Ramucirumab (target: VEGFR2): metastatic CRC, metastatic NSCLC, gastric or gastroesophageal adenocarcinoma
4. Multiple TKIs (sorafenib, sunitinib, regorafenib, pazopanib, axitinib, vandetanib, lenvatinib, cabozantinib): various cancers depending on the specific TKI, including RCC, hepatic cell carcinoma, thyroid cancer, pancreatic neuroendocrine tumors, gastrointestinal stromal tumors, soft tissue sarcoma, medullary thyroid cancer

types. In this review, we will focus on VEGF discovery and biology and its impact on cancer and eye disease therapies. It was initially demonstrated that tissue extracts stimulate cellular proliferation in explants (Carrel, 1913). This presaged the hypothesis in 1939 that biochemical factors increased tumor angiogenesis in animal models and that transplanted tumors induced significant neovascularization. A historical timeline extending from these initial hypotheses to VEGF discovery and cloning is illustrated in Figure 1.

Insights into the Complexity of VEGF Signaling

VEGF (now referred to as VEGF-A) is a member of a family of proteins including VEGF-B, VEGF-C, VEGF-D, VEGF-E (virally encoded), and PlGF (reviewed in Ferrara and Adamis, 2016). VEGF-C and VEGF-D are primarily implicated in regulation of lymphangiogenesis (Alitalo et al., 2005). Given the dominant role that VEGF-A plays in regulating angiogenesis and disease, it will be referred to as VEGF and will largely be the focus of this review. VEGF undergoes alternative exon splicing that leads to multiple isoforms. These include $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$. $VEGF_{165}$ ($VEGF_{164}$ in mice) is the most frequently expressed isoform in tissues. $VEGF_{165}$ is also the most physiologically relevant isoform, with characteristics in between that demonstrated by the highly diffusible $VEGF_{121}$ and the extracellular matrix (ECM)-bound $VEGF_{189}$. Less common isoforms, including $VEGF_{145}$ and $VEGF_{183}$, have since been described. A key feature that distinguishes these isoforms is their differential ability to bind heparin (Ferrara, 2010b; Houck et al., 1992; Poltorak et al., 1997). $VEGF_{121}$ has very little affinity for heparin, while $VEGF_{189}$ and $VEGF_{206}$ each have two heparin-binding domains (encoded by exons 6 and 7), which target the protein to the ECM or cell surface. $VEGF_{165}$ has a single heparin-binding domain, encoded by exon 7, and so is in part diffusible and in part ECM bound. $VEGF_{165}$ is the most physiologically relevant VEGF isoform (reviewed in Ferrara, 2010b). VEGF processing at the COOH terminus by proteases such as plasmin and MMP3 can turn ECM-bound peptides into non-heparin-binding, diffusible, molecular species (reviewed in Ferrara, 2010b). Several inhibitory isoforms of VEGF have also been recently described, including $VEGF_{165b}$ (Bates et al., 2002) and VEGF-Ax (Eswarappa et al., 2014), but there is some controversy regarding the mechanisms of inhibition, and VEGF-Ax has now been shown to actually have pro-angiogenic and pro-permeability features (Xin et al., 2016).

In 1992, VEGF receptor 1 (R1) was identified as a high-affinity tyrosine kinase VEGF receptor (de Vries et al., 1992), but it was since demonstrated that the lower-affinity, highly homologous VEGFR2 was the main signaling receptor for VEGF (Terman et al., 1992). Both VEGFR1 and VEGFR2 are predominantly expressed on endothelial cells. VEGF-A binds to both VEGFR1 and VEGFR2, VEGF-B and PlGF bind to VEGFR1, and VEGF-C and VEGF-D bind to VEGFR3 (implicated in lymphangiogenesis) but can bind to VEGFR2 after proteolytic cleavage (Pajusola et al., 1992) (Figure 2A). Heparin-binding VEGF-A or PlGF can also bind to neuropilin 1 (NRP-1), which increases their binding affinity to VEGFR2, but these molecules can also bind NRP-1 independent of VEGFR2 activation. NRP-2 performs a similar role in regulating lymphangiogenesis through its interactions with VEGFR3 (Olsson et al., 2006; Soker et al., 1998) (Figure 2B). As indicated above, VEGFR2 is the main signaling receptor whose activation promotes vascular endothelial cell mitogenesis and permeability. Two tyrosine residues in VEGFR2 have been shown to differentially regulate angiogenesis versus vascular permeability. Mice homozygous for the single substitution, tyrosine to phenylalanine, in position 1173 had defective vasculogenesis and angiogenesis and died *in utero* around day 8.5–9.5 (Sakurai et al., 2005). Phosphorylated Y949 interacts with the adaptor protein TSAd, an event that triggers formation of complexes between Src and VE-cadherin, leading to transient opening of interendothelial junctions (Li et al., 2016). Inactivating mutations in this pathway largely abolished the permeability-enhancing effects of VEGF in mice (Li et al., 2016). However, these permeability-deficient mice were normal and fertile, indicating that this function of VEGF does not play essential homeostatic roles (Li et al., 2016). However, a transient reduction in tumor edema and a decrease in the number of metastases was observed in the mutants, although primary tumor growth and blood vessel density were the same as in wild-type controls (Li et al., 2016). It has been hypothesized that VEGF-induced chronic hyper-permeability may be largely dependent on the growth of immature and structurally abnormal vessels that are inherently leaky rather than on direct stimulation of vascular leakage (reviewed in Ferrara and Adamis, 2016).

VEGFR1 displays weak ligand-dependent tyrosine autophosphorylation, and in some cases, it functions as a decoy receptor that binds PlGF and prevents VEGF binding to VEGFR2 (Park et al., 1994). Studies using receptor-selective VEGF mutants led to the conclusion that VEGFR1 cooperates with VEGFR2 in inducing gene expression in human umbilical vein endothelial cells (HUVECs), although no unique expression pattern was observed in response to VEGFR1 stimulation (Yang et al., 2002). However, other studies revealed that VEGFR1 may play a unique role in the tissue-specific release of growth factors such as HGF from liver sinusoidal endothelial cells, an effect that resulted in protecting hepatocytes from hepatotoxin-induced damage (LeCouter et al., 2003). VEGFR1 activation in monocytes and macrophages has been reported to mediate migration in response to VEGF or PlGF (Barleon et al., 1996). In

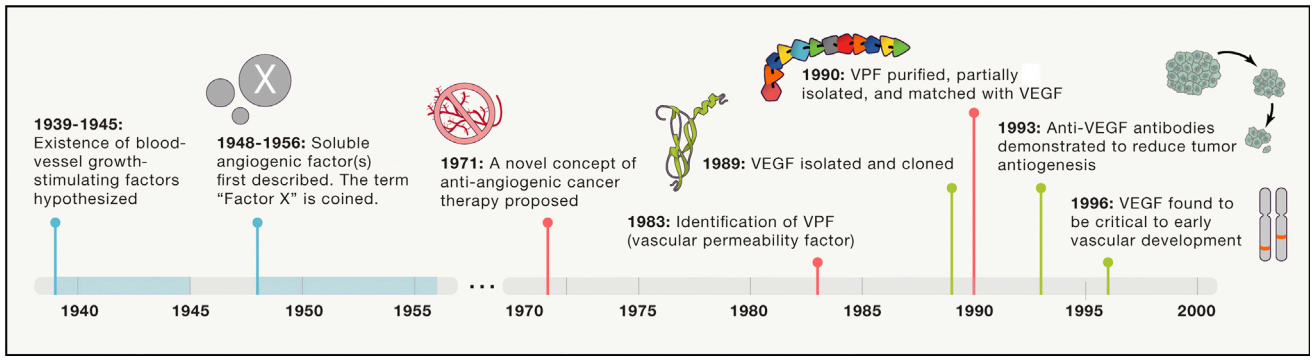

Figure 1. A Historical Timeline of VEGF Discovery

In diabetic retinopathy, it was proposed that diffuse angiogenic factor(s) were involved in neovascularization, and the term "factor X" was coined to describe such molecule(s) (Algire et al., 1945; Ashton, 1952; Carrel, 1913; Ide et al., 1939; Michaelson, 1948; Wise, 1956). In the early 1970s, it was suggested that an anti-angiogenic approach might be a unique and novel strategy to inhibit growth and proliferation of tumors (Folkman, 1971). Senger et al. reported the identification and initial biochemical characterization of VPF, a permeability-enhancing protein in the supernatant of a guinea pig tumor cell line (Senger et al., 1983, 1990). The Ferrara laboratory reported the isolation and cloning of a heparin-binding endothelial cell mitogen from medium conditioned by bovine pituitary follicular cells (Ferrara and Henzel, 1989; Leung et al., 1989), and the term VEGF was coined to describe this novel 45-kDa heparin-binding endothelial cell mitogen protein. Keck et al. (1989) reported the cloning of human VPF. Inactivation of a single allele of the VEGF gene in mice resulted in defective vascular development and early embryonic lethality (Carmeliet et al., 1996; Ferrara et al., 1996), highlighting the importance of VEGF during embryonic development. Neutralizing anti-VEGF antibodies dramatically reduced angiogenesis and growth of tumor cells implanted in immune-deficient mice (Kim et al., 1993), opening up novel therapeutic opportunities.

addition, expression of VEGFR1 in some tumor cell lines has been shown to mediate proliferation in response to VEGF or PlGF (Yao et al., 2011).

Hypoxia is a major regulator of VEGF expression via hypoxia-inducible factor (HIF). HIF and other hypoxia-regulated genes, factors in diverse contexts including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and oncogenic mutations (*vhl*, *ras*, *wnt-kras signaling pathway genes*) co-ordinate VEGF expression and, in turn, VEGF-driven signaling (Semenza, 2000a, 2000b). Canonical VEGF signaling through VEGFR1/R2 regulates the activities of several kinases and ultimately guides cell proliferation, migration, survival, and vascular permeability during vasculogenesis and angiogenesis. Endothelial cells, composed of tip and stalk cells, are at the leading edge of vascular proliferation. VEGF gradients induce tip cells and promote the formation of filopodia (De Bock et al., 2013; Eilken and Adams, 2010; Gerhardt et al., 2003; Gerhardt, 2008; Hellström et al., 2007; Potente et al., 2011; Ruhrberg et al., 2002). The molecular regulation of these events is via activation of notch signaling and by increased expression of notch ligands on endothelial cells, including but not limited to delta-like 4 (DLL4) (Wacker and Gerhardt, 2011). Increased notch signaling in neighboring cells then reduces VEGFR2 expression, completing a negative feedback loop. This canonical VEGF signaling is critical in physiologic homeostasis but can be hyperactivated in pathologic angiogenesis. In 2014, a non-canonical pathway for VEGFR2 signaling was characterized in neurons (Okabe et al., 2014). VEGFR2 was found to be highly expressed in retinal neurons but at much lower levels than in endothelial cells. Deletion of VEGFR2 in neurons caused abnormal angiogenesis toward neurons due to increased surrounding VEGF levels related to VEGFR2 deficiency. Of special interest, the aberrant juxta-neural angiogenesis was seen during physiological homeostasis and during the regenerative phase of ischemic retinopathy. These findings highlighted a role for neurons in titrating the amount of VEGF available for neuronal and tissue vascularization.

In 1993, the findings that anti-VEGF antibodies decreased the growth of tumor cells implanted in immune-deficient mice opened up translational possibilities for targeting VEGF-VEGFR signaling (Kim et al., 1993). In addition, it was also demonstrated that inactivation of a single allele of the *vegfa* gene in mice resulted in defective vascular development and early embryonic lethality (Carmeliet et al., 1996; Ferrara et al., 1996), highlighting the importance of VEGF during embryonic development. Inactivation of both copies of *vegfr2* largely pheno-copied *vegfa* single-allele deletion (Shalaby et al., 1995). The ability to delete VEGF in target tissues with the advent of *cre-lox* systems created the possibility of assessing the role of VEGF in individual tissues/cells (Gerber et al., 1999). Numerous studies employing this approach have documented the important role of VEGF in angiogenesis and homeostasis in a variety of pathophysiological circumstances (reviewed in Chung and Ferrara, 2011).

Understanding VEGF Biology in Cancer

VEGF secreted by tumor cells and surrounding stroma stimulates the proliferation and survival of endothelial cells, leading to the formation of new blood vessels, which may be structurally abnormal and leaky (Ferrara, 2010a, 2010b; Jain, 2003; Nagy et al., 2009). *VEGF* mRNA is overexpressed in the majority of human tumors and correlates with invasiveness, vascular density, metastasis, recurrence, and prognosis (Kerbel, 2008). Several strategies to inhibit the VEGF-VEGFR signaling pathway for the treatment of cancer have been devised (Ferrara and Adamis, 2016; Jayson et al., 2016).

Neutralizing monoclonal antibodies to VEGF were produced to further investigate the function of this growth factor (Kim et al.,

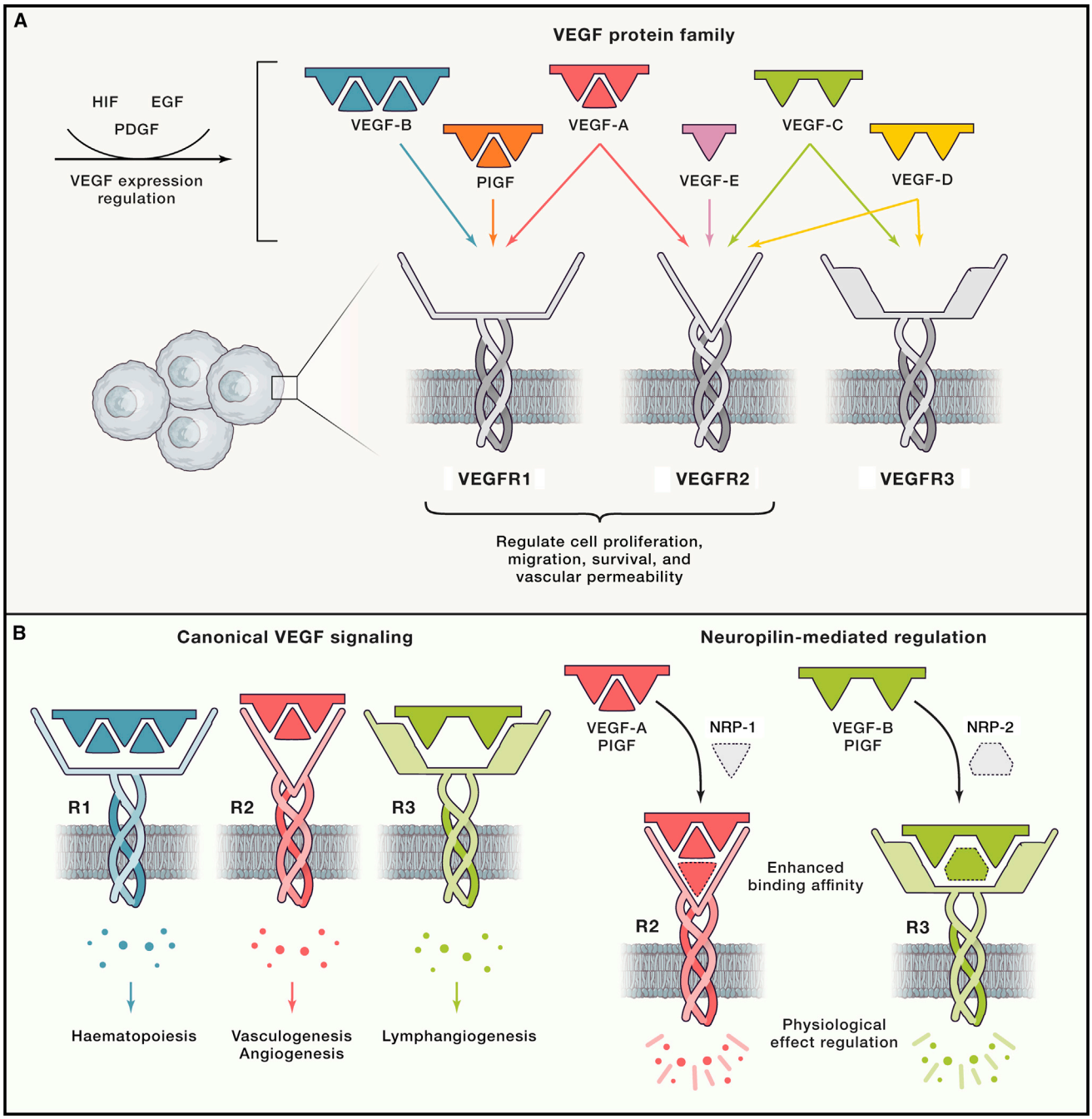

Figure 2. VEGF Activation and Signaling Pathways

(A) VEGF-A is a member of a family of proteins including VEGF-A, VEGF-B, VEGF-C, VEGF-D, virally encoded VEGF-E, and placental growth factor (PlGF). Hypoxia-inducible factor (HIF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) are among many hypoxia-/ischemia-induced genes that regulate VEGF expression. Canonical VEGF signaling through VEGF-R1/R2 (with R2 being the dominant signaling receptor) regulates the activities of several kinases and ultimately guides cell proliferation, migration, survival, and vascular permeability during vasculogenesis and angiogenesis.

(B) VEGF-A binds to both R1 and R2, VEGF-B and PlGF bind to VEGFR1, and VEGF-C and VEGF-D bind to VEGFR3, which may regulate lymphangiogenesis. VEGF-A or PlGF can also bind to neuropilin 1 (NRP-1) to increase their binding affinity to VEGFR2 or independent of this function. NRP-2 performs a similar role in regulating lymphangiogenesis through its interactions with VEGFR3 (adapted from Ferrara and Adamis, 2016).

1992). In 1993, the antibody A.4.6.1, which specifically recognized all bioactive isoforms of human VEGF, was reported to inhibit the growth of human tumor xenografts in mice in a dose-dependent manner (Kim et al., 1993). Further studies extended these findings to additional tumor models (Borgström et al., 1996; Warren et al., 1995).

To create an antibody suitable for clinical trials, murine antibody A.4.6.1 was humanized (Presta et al., 1997). The resulting recombinant antibody, known today as bevacizumab, retained the same binding characteristics and inhibitory potency of the original monoclonal antibody (Presta et al., 1997) and was assessed for use in human clinical trials (Ferrara et al., 2004).

Bevacizumab was approved by the US FDA for previously untreated metastatic colorectal cancer in February 2004. Subsequent clinical studies confirmed the benefits of bevacizumab in colorectal cancer and extended them to additional malignancies, including non-squamous non-small cell lung carcinoma (NSCLC), renal cell carcinoma (RCC), glioblastoma multiforme, ovarian cancer, and cervical cancer, resulting, as of today, in 10 FDA approvals for six different oncological indications in the United States and multiple regulatory approvals in other countries (Ferrara and Adamis, 2016). More than two million patients have been treated with bevacizumab, and today, this drug is one of the most widely used therapeutics in oncology. The approval of bevacizumab paved the way for the development of other VEGF pathway inhibitors, which include small-molecule VEGF receptor tyrosine kinase inhibitors (TKIs), an antibody targeting VEGFR2 (the major signaling VEGF receptor), and a chimeric soluble VEGF receptor (reviewed in Ferrara and Adamis, 2016).

Combination and Multitarget Therapies

Cytotoxic Agents. Anti-cancer therapy is rarely based on a single drug and almost always requires combinatorial approaches, since simultaneously attacking more than one target usually achieves greater efficacy. Preclinical studies have consistently shown additive or synergistic benefits from combinations of VEGF inhibitors with cytotoxic agents (Gerber and Ferrara, 2005). The mechanism of such benefit has been extensively debated. Several studies have shown that it stems, at least in part, from direct anti-vascular effects of the cytotoxic agents that amplify the pro-apoptotic effects of anti-VEGF agents on the vascular endothelium (Gerber and Ferrara, 2005; Klement et al., 2000; Sweeney et al., 2001). Another hypothesis postulated that "normalization" of the tumor vasculature by anti-VEGF agents plays a key role in such combinatorial benefits. According to this hypothesis, VEGF inhibition would result in pruning of endothelial cells not covered by pericytes and a reduction in the tortuosity and hyperpermeability of tumor vessels. These effects are expected to reduce tumor interstitial pressure and lead to enhanced uptake of cytotoxic agents and antibodies by the tumor (Jain, 2005; Willett et al., 2004). During a time window that varies depending on the model, tumor blood vessels could be transiently normalized, provided that a "judicious dose" of anti-angiogenic agent is employed. This dose is expected to be lower than anti-angiogenic or anti-vascular doses that would instead reduce drug uptake and lead to hypoxia, with detrimental effects and reduced clinical efficacy (Jain, 2014). A challenge in translating such concepts has been identifying the normalization window and normalizing doses. These appear to be dependent on the context and/or the tumor model. Doses of anti-VEGF or anti-VEGFR2 antibodies (5 mg/kg and 40mg/kg, respectively) that were initially reported to induce vascular normalization (as assessed by increased albumin fluxes or partial pressure of oxygen [pO2] levels in the tumor) in several mouse models (Lee et al., 2000; Tong et al., 2004; Winkler et al., 2004) were later found to have predominantly anti-vascular effects in other models (Arjaans et al., 2013; Huang et al., 2012, 2013), and considerably lower doses were needed to induce normalization (Chauhan et al., 2012). Indeed, efficacious doses of anti-VEGF agents that are commonly used in preclinical (Arjaans et al., 2013; Pastuskovas et al., 2012) or clinical (Van der Veldt et al., 2012; Zissen et al., 2011) studies have been unexpectedly reported to result in a sustained reduction in tumor uptake of cytotoxic agents and antibodies.

In a series of preclinical and clinical studies, Jain and colleagues sought to prospectively interrogate whether normalizing regimens of the VEGFR TKI cediranib result in a clinical benefit in glioblastoma multiforme (GBM) patients. Remarkably, treatment of GBM-implanted mice with doses of cediranib that did not inhibit tumor growth, but only normalized blood vessels and reduced edema, led to a significant increase in survival (Kamoun et al., 2009). A phase I (Batchelor et al., 2007) and a phase II (Batchelor et al., 2010) study showed rapid vascular normalization by MRI following cediranib administration, as well as preliminary evidence of patient benefit. However, a randomized placebo-controlled phase III study comparing cediranib plus lomustine to placebo plus lomustine failed to show any improvement in progression-free survival (PFS) or overall survival (OS) (Batchelor et al., 2013). Further studies are needed to precisely elucidate this complex relationship between combinations of anti-VEGF therapeutic approaches with tumor normalization strategies for delivery of cytotoxic agents.

Vascular Targets. Combining VEGF inhibitors with agents targeting other molecules or pathways involved in the assembly and/or survival of blood vessels holds the promise of improved efficacy outcomes. Indeed, a variety of preclinical studies supported this hypothesis (reviewed in Ferrara and Adamis, 2016; Jayson et al., 2016). These findings led to numerous clinical trials in multiple cancer types over the last decade (Ferrara and Adamis, 2016). Unfortunately, in spite of the aforementioned promising studies, attempts to improve outcomes of anti-VEGF therapy in various malignancies through combination strategies with agents targeting cMet (Wakelee et al., 2017), the PDGF signaling pathway (Hainsworth et al., 2007), vascular integrins (Weekes et al., 2018), the ECM protein EGFL7 (García-Carbonero et al., 2017), NRP-1 (Weekes et al., 2014), the Hedgehog pathway (Berlin et al., 2013), and the Tie-2 ligand Ang-2 so far have not been met with marked success. The lack of benefit was due in some cases to overt toxicity and in others to insufficient or unclear efficacy. Among the vascular targets, PlGF has received considerable attention. As already noted, PlGF is a member of the VEGF family that binds VEGFR1 but fails to bind to VEGFR2 (Park et al., 1994). The role of PlGF in angiogenesis and its significance as a therapeutic target remained largely unclear, with conflicting reports on the effects of anti-PlGF antibodies on tumor angiogenesis and growth (Bais et al., 2010; Fischer et al., 2007). A series of early-stage clinical trials in patients with multiple tumor types were conducted a few years ago with a humanized anti-PlGF antibody in combination with bevacizumab (reviewed in Ferrara and Adamis, 2016). A study in GBM patients has been published that indicates a lack of additional benefit from the combination anti-PlGF/bevacizumab compared to bevacizumab alone (Lassen et al., 2015). However,

the same anti-PlGF antibody is being evaluated in ongoing clinical trials in medulloblastoma patients, based on the hypothesis that PlGF, in this setting, promotes tumor growth by a non-angiogenic mechanism involving a direct stimulation of tumor cell growth through NRP-1 (Snuderl et al., 2013). In addition, this anti-PlGF antibody is being developed for ophthalmological indications, such as diabetic macular edema (DME) (Nguyen et al., 2018).

Clinical trials in multiple malignancies with aflibercept—a high-affinity chimeric soluble VEGF receptor that binds not only VEGF, but also PlGF and VEGF-B (Holash et al., 2002)—also provide indirect evidence that the role of PlGF in tumor angiogenesis may be relatively limited. Indeed, in spite of the promise that higher-affinity VEGF binding combined with the ability to bind two additional ligands may confer a substantial clinical advantage, the impact of aflibercept in cancer therapy has been somewhat more limited than bevacizumab, gaining FDA approval only for second-line therapy in colorectal cancer (reviewed in Ferrara and Adamis, 2016).

Even though combination therapies of anti-VEGF agents with conventional vascular targets have so far not achieved the expected benefits, several groups are exploring the possibility that combinations with various targets within the tumor microenvironment, including myeloid cell-derived angiogenesis mediators, may lead to better outcomes (De Palma et al., 2017; Negri and Ferrara, 2018) (Junttila and de Sauvage, 2013).

Immunotherapy. Over the last several years, cancer immunotherapy with immune checkpoint inhibitors has transformed cancer treatment, resulting in improvements in OS (Kelly, 2018). However, despite this benefit, patients who experience durable response and/or survival are only a subset of those treated, hence the need for identifying novel combinations (Chen and Mellman, 2013). One likely reason for this finding is that human cancer can utilize multiple immune inhibitory mechanisms, leading to primary or secondary immune escape (Chen and Mellman, 2017). One such mechanism relates to VEGF (Ott et al., 2015).

The biologic role of VEGF has, over the years, extended beyond its impact on neovascularization and angiogenesis. Numerous studies have suggested that VEGF is a central mediator of wound repair. Indeed, VEGF is generally associated with a gene expression signature associated with other wound repair genes, and this has been attributed to an impact on a multitude of cell types (Birkenhauer and Neethirajan, 2015). Physiologic wound repair generally follows on tissue damage, inflammation, and an immune response. However, to effectively engage in tissue repair, inflammation and active immune responses are necessarily downmodulated. This is consistent with the observed role of VEGF in downmodulating immunity (Motz and Coukos, 2013; Ohm and Carbone, 2001). Despite these considerations, the effects on wound repair could be mediated, directly or indirectly, by the vasculature, as wound healing and repair have been classically associated with angiogenesis (Folkman and Klagsbrun, 1987).

VEGF can have a direct effect on multiple cells involved in immunity, including dendritic cells, T cells, regulatory T cells, and myeloid-derived suppressor cells (Khan and Kerbel, 2018). Early observations suggested that the presence of VEGF could skew maturation of myeloid progenitors away from differentiation into dendritic cells and toward endothelial cells, impacting priming and activation of cancer-specific T cells (Gabrilovich et al., 1998). VEGF can also impact endothelial cell expression of immunologically important molecules, decreasing expression of vascular cell adhesion molecule-1 (VCAM-1), important for anti-cancer T cell adhesion and infiltration into tumors, and increasing expression of FASL, leading to apoptosis of anti-cancer T cells at the vascular border to cancer. VEGF inhibition has been shown to increase the number of tumor-infiltrating lymphocytes in animal models (Chung et al., 2013; Shrimali et al., 2010) and in humans (Wallin et al., 2016). Additionally, high levels of VEGF in the tumor microenvironment can further stimulate proliferation of myeloid-derived suppressor cells and regulatory T cells, both of which express VEGFR (Hegde et al., 2018) (Figure 3). Recent preclinical studies have also shown that anti-VEGF therapy can improve anti-PD-L1 (programmed death-ligand 1) treatment, specifically when it generates intratumoral high endothelial venules (HEVs) that facilitate enhanced cytotoxic T lymphocyte (CTL) infiltration and tumor cell destruction (Allen et al., 2017). Other studies have suggested that a dual anti-angiogenic approach with a bi-specific anti-VEGF/Ang2 antibody potentiated the activity of anti-PD-L1 treatment in multiple models (Schmittnaegel et al., 2017).

Recent clinical studies have further supported the role of VEGF in anti-cancer immunity (McDermott et al., 2018). Immunotherapy with PD-L1/PD-1 inhibitors has demonstrated clinical benefit across a wide range of cancer types. However, despite this benefit, patients who experience durable response and/or survival are only a subset of those treated, hence the need to identify novel combinations (Chen and Mellman, 2013). In a recently reported phase III study, the addition of bevacizumab (15 mg/kg) to cytotoxic chemotherapy (carboplatin, paclitaxel) and anti-PD-L1 antibody (atezolizumab) was confirmed to extend OS for patients with NSCLC (Socinski et al., 2018). In December 2018, the FDA approved atezolizumab, in combination with bevacizumab, paclitaxel, and carboplatin, for the first-line treatment of patients with metastatic, non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations.

It is noteworthy that, at the dose of 15mg/kg, bevacizumab has been shown to reduce tumor uptake of chemotherapy in NSCLC patients (Van der Veldt et al., 2012), suggesting that the above described combinatorial benefits are not related to vascular normalization. As already pointed out, normalization has been associated with significantly lower doses of anti-VEGF antibodies (Fukumura et al., 2018). As noted, the mechanism of the anti-VEGF/anti-PD-L1 interaction is likely multifactorial, but it is tempting to speculate that anti-vascular effects of VEGF inhibition, especially in combination with cytotoxic agents, may result in release of tumor antigens, thus facilitating immunotherapy. Interestingly, the survival benefit was particularly pronounced in high-VEGF settings, such as patients with EGFR mutant lung cancer following progression after an EGFR-targeted agent, ALK-rearranged lung cancer following progression after an ALK-targeted agent, and/or patients with liver metastases. In these settings, VEGF expression can be driven by EGFR signaling, ALK signaling, or the high vascular state present in the liver (Chen and Hurwitz, 2018). Additionally, a phase III study combining bevacizumab with atezolizumab in RCC has also confirmed benefit for this combination in this high-VEGF disease

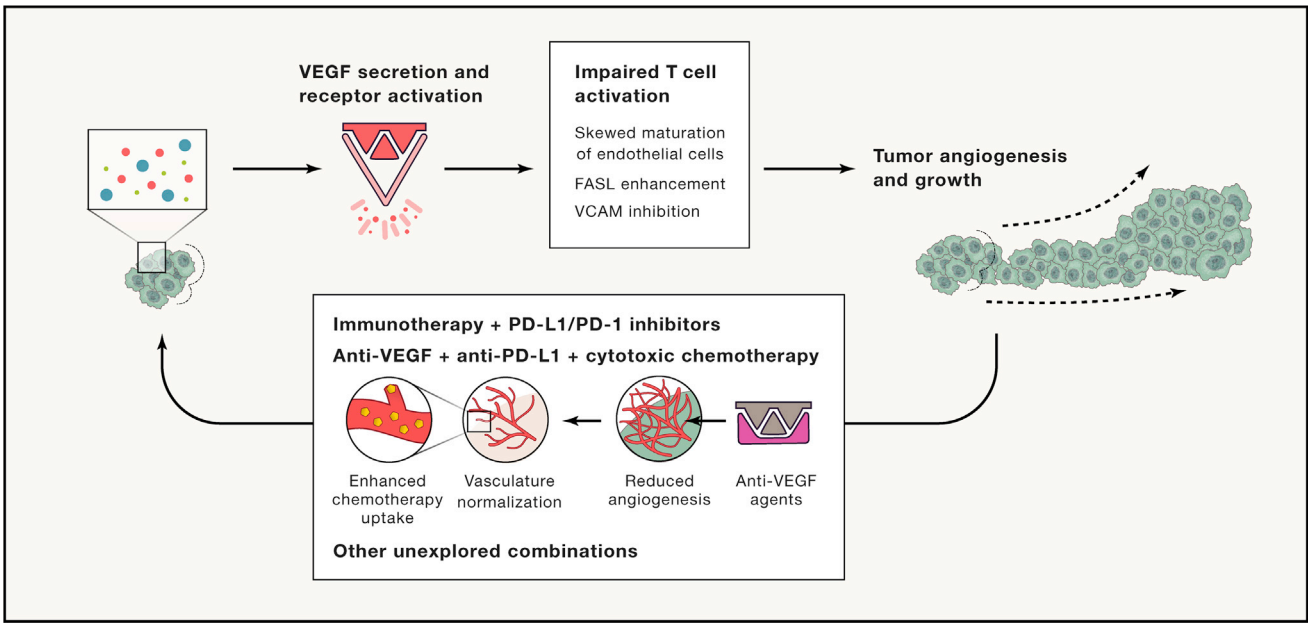

Figure 3. VEGF and Tumor Angiogenesis

VEGF secreted by cancer and stromal cells stimulates the proliferation and survival of endothelial cells, leading to the formation of new blood vessels, often with impaired tight junctions and increased permeability. Combining VEGF inhibitors with cytotoxic agents is synergistic and improves therapeutic outcomes. Although the mechanisms of synergy are debated, it has been suggested that the anti-vascular effects of the cytotoxic agents complement the pro-apoptotic effects of anti-VEGF agents on the vascular endothelium. Normalization of the vasculature by anti-VEGF agents that allows enhanced uptake of chemotherapeutic agents may also play a role but requires further investigation. VEGF may skew maturation of myeloid progenitors away from differentiation into dendritic cells and toward endothelial cells, impacting T cell activation. VEGF can also decrease expression of VCAM-1, important for anti-cancer T cell adhesion and infiltration into tumors, and increase expression of FASL, leading to apoptosis of anti-cancer T cells at the vascular border of the cancer. Clinical studies have recently supported a role for anti-VEGF agents in combination with in PD-L1/PD-1 inhibitors in anti-cancer immunity.

(Chen and Hurwitz, 2018). Furthermore, early-phase studies complete the clinical body of evidence supporting the role of VEGF inhibition in potentiating immunity. In phase I studies, the combination of VEGF inhibition and PD-L1/PD-1 blockade has reportedly led to increased response rates in hepatocellular carcinoma. VEGF inhibition with VEGF TKI and PD-L1/PD-1 blockade also appears to further enhance response rates in RCC in phase I studies (Atkins et al., 2017, 2018). Some of these anti-VEGF-based therapeutic approaches in cancer are summarized in Figure 3.

VEGF in the Eye: A Window to the Diverse Roles of VEGF

VEGF in Retinal Vascular Development

As mentioned previously, the retina is a complex neurovascular tissue made up of at least two types of glial cells, seven types of neurons, and a rich network of endothelial cells precisely layered in capillaries at various levels within the retina (Wechsler-Reya and Barres, 1997; Macosko et al., 2015). How endothelial cells that form capillaries within plexiform layers in the retina receive the appropriate cues was better understood once it was demonstrated that oxygen levels in the retina tightly regulated the formation of the retinal vasculature during development (Ashton, 1966). Retinal hyperoxia led to obliteration of the retinal vasculature, while hypoxia promoted vascular growth and proliferation (Miller, 1997; Smith, 2003). The discovery of VEGF and subsequent demonstration that VEGF production spatially and temporally localized to the areas of vascular development provided strong evidence that it was the driving force in developmental angiogenesis and in ischemic/hypoxic regulation of the retinal vasculature. It was also demonstrated that neurons such as retinal ganglion cells (RGCs) in conditions of metabolic hypoxia released growth factors such as basic fibroblast growth factor (bFGF) and PDGF. These in turn regulated secretion of VEGF by retinal microglia and Muller cells so as to stimulate blood vessel growth in order to meet the metabolic tissue demands and achieve homeostasis (Okabe et al., 2014; Wechsler-Reya and Barres, 1997). In elegant studies using animal models, it was also shown that when animals were exposed to high levels of oxygen during development, retinal VEGF production shut down dramatically (Penn et al., 1994; Smith et al., 1994). This led to capillary dropout and obliteration of the developing peripheral retinal vasculature. Upon return to normoxic conditions, there was a dramatic upregulation in local VEGF production that promoted massive neoangiogenesis. These new vessels are unfortunately abnormal, leaky, and often mislocalized to the preretinal space and lose the layered, plexiform intraretinal features. As such, they leak and can eventually cause intractable fibrosis that can lead to retinal detachment and blindness. These studies have led to a sophisticated understanding of retinopathy of prematurity (ROP), a blinding condition that affects premature infants. Retinal vascular development in these infants is incomplete and sensitive to the levels of VEGF in the eye. Unregulated

exposure to high levels of oxygen, a common practice several decades ago, leads to a phenotype of unbridled neovascularization as described above in animal models.

ROP is a leading cause of blindness in children around the world. The discovery of VEGF enhanced our understanding of the molecular pathogenesis of ROP (Smith, 2003). Retinal vascular development in humans begins at the fourth month of gestation and progresses from the central to the peripheral retina. In cases of premature birth, normal retinal vascular development stops. The avascular retina that lacks the oxygenation and nutrients becomes metabolically active and hypoxic around 32–34 weeks of development. In animal models of ROP and in humans, it has been conclusively demonstrated that retinal ischemia drives VEGF production prior to neovascularization, thus driving the neovascular phase of disease (Sonmez et al., 2008). In addition, randomized clinical trials that have enrolled infants with ROP have demonstrated that intraocular injections of agents that neutralize VEGF are efficacious in treating neovascularization and preventing complications associated with advanced stages of ROP (Mintz-Hittner et al., 2011). These studies also demonstrated that VEGF inhibition in ROP reduced the incidence of vision loss in these infants (Geloneck et al., 2014). This has opened the possibility of anti-VEGF pharmacotherapy in this disease in addition to the previously available option of laser or cryotherapy to ablate the non-vascularized retina in order to eliminate the release of VEGF from the ischemic peripheral retinal cells.

A Conceptual Vision for VEGF in Ocular Neovascularization

The role of VEGF in the development and molecular pathogenesis of ROP was established early on in our understanding of how VEGF influences the complex retinal neurovascular unit. In parallel, it became apparent that VEGF played a cardinal role after development both in the maintenance of physiologic homeostasis and in retinal vascular diseases such as diabetic retinopathy (DR) and AMD (Duh and Aiello, 1999; Jager et al., 2008). The neurovascular complex is best understood in the context of DR, a systemic disease that manifests as end-organ damage in the eye with cell death in both neuronal and vascular elements of the retina (Duh et al., 2017). Initial manifestations of DR were thought to be vascular with pericyte loss followed by apoptotic capillary cell death. Loss of retinal capillaries led to ischemia and VEGF-induced aberrant retinal neovascularization. It is now becoming increasingly clear that cellular dysfunction and death can manifest early in the life cycle of diabetes and that non-vascular elements of the retina, including neurons and glia, can be affected. It has been demonstrated both in animal models and in human patients with diabetes that damage to inner retinal neurons, including RGC and amacrine cells, can be seen prior to any vascular manifestations of disease (Fortune et al., 1999; Harrison et al., 2011; Rajagopal et al., 2016). In addition, activation of Muller glia that maintain VEGF homeostasis in the retina can also occur early in disease, a possible trigger for molecular inflammation, an important feature later in DR. In patients, these abnormalities in the neural and glial components of the retina manifest as impaired contrast sensitivity and dark adaptation.

In diseases such as AMD that affect the outer retina and the RPE, the role of VEGF in maintaining choroidal health in both aging and AMD is becoming increasingly appreciated. Although the role of VEGF in vascular endothelial cell homeostasis was highlighted above, nowhere is this function more important than in the choriocapillaris (Bhutto et al., 2006). The choroid is composed of a rich vascular plexus called choriocapillaris, lined by choroidal endothelial cells (CECs). This layer is anatomically juxtaposed underneath the RPE and separated from it by an acellular laminar layer called Bruch's membrane, partially formed from the basal lamina of the RPE. The RPE, a highly polarized monolayer of terminally differentiated cells, performs several important functions. As an anatomic monolayer underneath the outer retina, it contains key enzymes and substrates involved in recycling of chromophores essential to the photoreceptor-driven visual cycle (Apte, 2018). It is also responsible for phagocytosis of the diurnally shed photoreceptor outer segments. In addition to these functions performed at the apical aspect of the cell, the basolateral RPE secretes VEGF in a polarized manner that is indispensable to CEC health. In animal models, loss of VEGF secreted by the basolateral RPE leads to CEC atrophy and significant thinning of the choriocapillaris (Saint-Geniez et al., 2009). Although true during development, a number of other studies have failed to document such CEC atrophy in adult animals treated with anti-VEGF pharmacotherapy (Kim et al., 2006; Long et al., 2018). In addition, aging RPE cells lose their polarity and can secrete VEGF from the apical aspects of the cell surface that is thought to stimulate pathologic choroidal neovascularization (CNV), a blinding complication of AMD. VEGF is a critical signal for ocular neovascularization in DR and in neovascular AMD (Duh et al., 2017; Miller et al., 2013; Sene and Apte, 2014; Sene et al., 2015). Although the ischemic drive in AMD is not quite as profound as in diabetic retinopathy, molecular drivers such as monocytes/microglia and the RPE create a micromilieu that is prime for VEGF-driven CNV (reviewed in Sene et al., 2015).

Non-VEGF Factors that Drive Angiogenesis

Although it is now well established that VEGF plays a major role in initiating and sustaining pathologic angiogenesis in the eye, both animal and human studies have demonstrated that factors other than VEGF also contribute to these processes. Multiple large, placebo-controlled, randomized clinical trials have proven the efficacy and safety of intraocular anti-VEGF pharmacotherapy in AMD, DR, and retinal vascular diseases (Gross et al., 2015; Martin et al., 2011; Scott et al., 2017; Wells et al., 2015). What these studies have also shown is that VEGF-based pharmacotherapy requires chronic treatment, potentially over many years, and that a large proportion of patients treated with anti-VEGF medications can often be under-responsive and occasionally unresponsive to the treatment (Sene et al., 2015). These human studies highlight the possibility that there may be additional pathways independent of VEGF that regulate angiogenesis in disease states of the eye (reviewed in Sene and Apte, 2014; Sene et al., 2015). For example, semaphorins 3A (Joyal et al., 2012), 3F (Buehler et al., 2013), and 6A (Segarra et al., 2012) suppress normal revascularization in the ischemic retina but promote pathogenic vascularization, guiding vascular sprouts to the preretinal vitreous cavity. Other factors that

promote aberrant angiogenesis in and underneath the retina include PlGF (Nguyen et al., 2018), PDGF (Dong et al., 2014), erythropoietin (Caprara and Grimm, 2012), stromal-derived factor-1 (Lima e Silva et al., 2007), jak-stat3 hyper-activation (Nakamura et al., 2015), and factors associated with abnormal activation of monocyte derived macrophages and glial cells as they age (Apte et al., 2006; Ban et al., 2018; Combadière et al., 2007; Kelly et al., 2007; Ma and Wong, 2016; Sene et al., 2013).

The Vista for Multitarget, Synergistic Approaches in Clinical Pharmacotherapy

Although VEGF is key to the pathogenesis of neovascular eye disease, it has become clear, as in cancer treatment, that VEGF-targeted monotherapy has its limitations. For example, DME associated with increased vascular permeability and vision loss is exquisitely sensitive to anti-VEGF pharmacotherapy. Nevertheless, a large proportion of patients remain under-responsive to therapy with persistent edema at 3–5 years after therapy (Apte, 2016; Bressler et al., 2016). However, despite some discrepancies based on the disease and the individual trial examined (Maguire et al., 2016), the need for anti-VEGF therapy in general declined over time (Elman et al., 2011, 2012). In the RISE and RIDE trials, approximately a quarter of DME patients were able to discontinue therapy after 3 years, suggesting that anti-VEGF therapy may be disease modifying (Brown et al., 2013). In AMD, despite the successes of anti-VEGF therapy in preventing catastrophic vision loss, long-term studies demonstrate that patients who have been successfully treated may still continue to lose vision from atrophic retinal neurodegeneration rather than neovascularization (Maguire et al., 2016). Nevertheless, about half of neovascular AMD patients had good vision—i.e., visual acuity 20/40 or better on Snellen testing—after 5-year treatment with ranibizumab or bevacizumab, an outcome that would have not been possible before anti-VEGF agents were available (Maguire et al., 2016). In real-life clinical settings, it has been demonstrated that patients receive fewer anti-VEGF injections than in clinical trials, and it has been suggested that this may correlate with poor visual outcomes due, at least in part, to insufficient anti-VEGF treatment (Holz et al., 2015). There has also been renewed interest in sustained-release anti-VEGF formulations or delivery vehicles that may allow testing the hypothesis that continuous VEGF inhibition may result in better long-term visual outcomes (Ferrara and Adamis, 2016). Higher doses (2 mg) of the anti-VEGF agent ranibizumab did not provide additional visual acuity benefit over conventional dosing (0.5 mg), suggesting that a plateau had been reached. However, the higher-dose group required a reduced number of injections compared to the lower-dose group in the second year of the study in AMD patients (Ho et al., 2014; Sepah et al., 2016). Interestingly, a recent study comparing 6 mg of brolucizumab, a single-chain variable fragment (scFV) that binds VEGF with high affinity, to a standard dose of aflibercept (2 mg) in AMD suggested a need for fewer injections with brolucizumab, with comparable safety and efficacy profiles (Dugel et al., 2017). Considering the molecular masses, the brolucizumab dose was almost 10-fold higher than the aflibercept dose and ~20-fold higher than the standard dose of ranibizumab. The success of this approach seems almost counterintuitive in view of the already discussed hypothesis that high doses of VEGF-blocking agents may be associated with hypoxia and followed by tissue damage and other detrimental effects (Jain, 2014). As such, additional research is needed, but as the burden of intravitreal injections can reduce patient compliance, there is a need to develop more durable formulations/devices. A promising approach is a refillable slow-release device system that enables continuous delivery of ranibizumab in the vitreous cavity of the eye. According to a recently presented phase II study in neovascular AMD patients (LADDER, presented at the Retina Society Annual Meeting, 2018), at the highest concentration (100 mg/mL), 80% of patients went ≥6 months until the first required medication refill and had visual acuity comparable to that achieved with monthly injections with ranibizumab. A phase III trial is now underway. These studies should allow for testing the hypothesis that more steady VEGF inhibition than that achieved by periodic intravitreal injections may result in better long-term visual outcomes. Another potential approach is to use gene therapy to deliver VEGF antagonists to the eye in order to achieve durability and reduce therapeutic burden. Although non-human primate and human studies using recombinant adeno-associated vectors (rAAVs) to deliver a highly potent naturally occurring VEGF inhibitor, sFLT-1, or an anti-VEGF Fab fragment suggest that sub-retinal or intravitreal delivery of gene therapy is safe, larger trials need to be conducted to determine whether this approach is efficacious and durable (Heier et al., 2017; Maclachlan et al., 2011; Rakoczy et al., 2015). Box 2 summarizes the diversity of anti-VEGF compounds that have been tested in human clinical trials for ophthalmic diseases.

As such, therapeutic approaches that complement VEGF-based strategies are of high interest and relevance. The desired goals of multitarget approaches would be to find a combination that can reduce the high treatment burden of frequent intraocular injections, improve visual outcomes, and prevent continued vision loss from atrophic neurodegeneration. Unfortunately, several strategies that have targeted novel pathways have so far failed to demonstrate efficacy in clinical trials. Examples of such failures include an antibody fragment targeting complement factor D, a key activator of the complement pathway, strongly implicated in the retinal neovascularization and atrophic neurodegeneration associated with AMD. In spite of encouraging phase II data (Yaspan et al., 2017), two phase III studies failed to show any change in the progression of atrophy (Holz et al., 2018). In addition, a PDGF-B antagonist (an aptamer) and steroids (such as dexamethasone) both failed to meet primary efficacy endpoints in combination therapy trials of neovascular AMD with approved anti-VEGF agents despite initial promising results (Calvo et al., 2015; Chaudhary et al., 2016; Dunn et al., 2017; Jaffe et al., 2017). Considerable research is being devoted to the hypothesis that combining VEGF inhibitors with activators of the tyrosine kinase Tie2 may achieve greater efficacy than anti-VEGF monotherapy owing to the stabilizing role of Tie2 on the vasculature, with potential reduction in vascular leakage (Augustin et al., 2009; Campochiaro and Peters, 2016; Saharinen et al., 2017). Different approaches to activate Tie2 have been attempted, including the use of phosphatase inhibitors and antagonists of Ang2, a Tie2 ligand with antagonistic properties. A recent phase II study in DME patients reported that administration of AKB-9778, a small molecule

Box 2. Anti-VEGF Agents Investigated in Human Clinical Trials for Ophthalmic Diseases

1. Pegaptanib Na: anti-VEGF165 aptamer that was approved by the US FDA for treatment of neovascular AMD and DME
2. Bevacizumab: humanized whole antibody targeting all isoforms of VEGF-A initially approved by the FDA for intravenous therapy of several cancers but repurposed for intravitreal delivery in eye diseases, such as neovascular AMD, DME, neovascular glaucoma, and retinal vascular occlusions (RVO). This agent is widely used for eye disease, extensively studies in clinical trials, and covered by insurance payors including the Centers for Medicare and Medicaid Services (CMS) but did not go through the regulatory approval pathways for eye diseases.
3. Ranibizumab: humanized Fab antibody fragment targeting all isoforms of VEGF-A approved by the FDA for intravitreal delivery in the treatment of neovascular AMD, DR and DME, and RVO
4. Aflibercept: recombinant fusion protein consisting of the VEGF-binding portions from the extracellular domains of human VEGFR1 and VEGFR2 fused to the Fc portion of human immunoglobulin (Ig)G1. It is approved by the FDA for intravitreal administration in the treatment of neovacular AMD, DME, and RVO.
5. Ziv-aflibercept: the active aflibercept ingredient approved for the treatment of metastatic colorectal cancer that has been used off label by intravitreal injection for the treatment of retinal diseases
6. Brolucizumab: humanized scFV targeting VEGF-A designed for the treatment of neovascular AMD and recently tested in phase III human clinical trials for intravitreal delivery. It is currently making its way through the regulatory process for approval by the FDA. Additional studies are in the planning stages for testing its efficacy in DME and RVO.
7. DARPins: various formulations of recombinant-designed ankyrin repeat proteins with anti-VEGF-A activity are being investigated in phase II and phase III human clinical trials for neovascular AMD. These genetically engineered proteins mimic antibodies in their ability to bind specific proteins with high affinity.
8. Anti-VEGF biosimilars: a number of ranibizumab and aflibercept biosimilars targeting VEGF are also currently being investigated in human clinical trials to determine efficacy and safety in retinal diseases.
9. Gene-therapy-targeting VEGF or combination approaches: there are a number of approaches currently being investigated to target VEGF in eye diseases with gene therapy. These include use of different packaging vectors and either subretinal or intravitreal delivery to the eye. A summary of some of these agents being investigated in human clinical trials is listed below.
    a. RGX-314 is a recombinant NAV AAV8 gene-therapy vector carrying a coding sequence for a soluble anti-VEGF monoclonal antibody fragment administered by subretinal delivery currently in planning stages for phase II for patients with previously treated neovascular AMD.
    b. ADVM-022 is a recombinant AAV.7m8 gene-therapy vector capsid carrying an aflibercept coding sequence that expresses an anti-VEGF protein for intravitreal delivery currently in phase I for patients with neovascular AMD.
    c. AAV2-sFLT01 expresses soluble Flt-1 receptor and is being investigated in clinical trials for neovascular AMD.
    d. AAV2CAGsCD59 is being investigated in a human phase I trials in combination with an intravitreal anti-VEGF agent for the treatment of neovascular AMD. CD59 is a naturally occurring membrane bound inhibitor of the membrane attack complex.

inhibitor of the phosphatase VE-PTP that inactivates Tie2, potentiated the ability of ranibizumab to reduce DME (Campochiaro et al., 2016). In other studies, the benefits of such combination were less clear. Adding an anti-Ang2 antibody (nesvacumab) to aflibercept did not improve visual outcomes in DME and AMD patients (results presented at the Retina Society Annual Meeting, 2018 and the American Academy of Ophthalmology Annual Meeting, 2018). However, faricimab, a bispecific anti-VEGF/Ang2 antibody, was reported to have greater efficacy than anti-VEGF alone in a non-human primate laser-induced CNV model (Regula et al., 2016) and has been compared to ranibizumab in phase II studies. Initial data provide hints of improved efficacy at the higher dose of faricimab, at least in DME, and potentially increased durability in neovascular AMD (results presented at the Retina Society Annual Meeting, 2018 and the American Academy of Ophthalmology Annual Meeting, 2018). Phase III studies for evaluating faricimab in DME and AMD are being planned.

Resistance to Anti-VEGF Therapy and Forward-Looking Approaches

An important question is why VEGF-based approaches sometimes fail to achieve complete therapeutic response in cancers and eye disease. The answer is complex, and several possibilities have been examined. Interestingly, the mechanisms of reduced response/resistance to anti-VEGF in cancer are likely different from those in response to treatment with inhibitors of well-defined oncogenic pathways, as there is no evidence of selection of preexisting or acquired mutations in VEGF itself or in its signaling pathway (Wagle et al., 2011). The report that administration of bevacizumab, despite disease progression in metastatic colorectal cancer, still resulted in a small but significant OS benefit raised the possibility that resistance may be reversible, at least in some circumstances, and suggested that re-treating with the same or an alternate VEGF inhibitor may have clinical benefit (Bennouna et al., 2013). In retinal diseases, some clinical studies have reported an interesting positive therapeutic response upon switching from one anti-VEGF agent to another after a clinical determination of treatment resistance with the initial agent used for therapy (Bakall et al., 2013; Gasperini et al., 2012; Spooner et al., 2018; Yonekawa et al., 2013). Although intriguing, this finding has not been universally observed across all clinical studies (Stepien et al., 2009). It is conceivable that such plasticity may be mediated at least in part by the dynamic nature of the microenvironment (Chung et al., 2010). Preclinical studies have implicated the release of hematopoietic growth factors from tumor or host cells and the resulting tumor infiltration of myeloid and other inflammatory cell types in the induction of VEGF-independent angiogenesis and escape (Bergers and Hanahan, 2008; De Palma et al., 2017; Shojaei et al., 2007, 2009). In a recent study, it was

reported that human and mouse ovarian tumors implanted intraperitoneally in mice secreted microseminoprotein, prostate-associated (MSMP) under conditions of hypoxia. MSMP activated MAP kinase signaling leading to endothelial cell proliferation and tube formation. Serum levels of MSMP were also elevated in ovarian cancer patients treated with bevacizumab who were non-responsive to therapy (Mitamura et al., 2018). Obese breast cancer patients had elevated systemic concentrations of interleukin (IL)-6 and/or FGF-2 and a tumor vasculature that was more resistant to anti-VEGF agents. In mouse models, obesity neutralized the effects of VEGF blockade on tumor growth, angiogenesis, and metastasis. By neutralizing IL-6 or normalizing FGF-2 levels, tumor VEGF sensitivity in mice was restored (Incio et al., 2018). This is significant, as FGF signaling has also been reported to be important in promoting angiogenesis in tumors and the eye independent of VEGF (Batchelor et al., 2007; Casanovas et al., 2005; Itatani et al., 2018; Kopetz et al., 2010; Mitsuhashi et al., 2015; Oladipupo et al., 2014). Nevertheless, the role of FGF in angiogenesis/tumor escape remains to be clinically validated. Components of the immune system may also contribute to anti-VEGF resistance. Monocytes and macrophages promote resistance to anti-VEGF therapy by altering VEGF receptor expression (Dalton et al., 2017) or inhibiting adaptive immunity (Jung et al., 2017). Of interest, multitarget approaches that inhibit both Ang-2 and VEGF in an animal model of GBM are able to improve survival by re-programming tumor associated macrophages from an alternatively activated phenotype to a classically activated phenotype (Kloepper et al., 2016). It remains to be established whether these observations in preclinical models are clinically relevant. In eye disease, factors such as age, IL-10, and aberrant complement activation promote monocyte- and macrophage-driven proliferative neovascularization independent of VEGF (Apte et al., 2006; Calippe et al., 2017; Kelly et al., 2007; Sene et al., 2013).

Studies into VEGF biology have provided tremendous insights into physiologic homeostasis and the molecular mechanisms of cancers and eye disease. These channels of discovery have also illuminated our understanding of the complex interactions between VEGF and other signaling pathways and been an amazing demonstration of how molecular discovery can inform drug development and clinical trial design.

ACKNOWLEDGMENTS

This study was supported by NIH grants R01-EY019287-06A1 (R.S.A.), R21-EY026707-02 (R.S.A.), R21-EY028701-01 (N.F.), and P30-EY02687; Champalimaud Award (N.F.); Glenn Foundation for Medical Research Award (R.S.A.); Starr Foundation (R.S.A.); Jeffrey Fort Innovation Fund (R.S.A.); Edward N. and Della L. Thome Foundation Award (R.S.A.); and an unrestricted grant from Research to Prevent Blindness, New York, NY to the Department of Ophthalmology, Washington University School of Medicine, St. Louis, MO. The authors would like to thank Mr. Danyel Cavazos for assistance with the illustrations.

REFERENCES

Adamis, A.P., and Shima, D.T. (2005). The role of vascular endothelial growth factor in ocular health and disease. Retina *25*, 111–118.

Algire, G.H., Chalkley, H.W., Legallais, F.Y., and Park, H.D. (1945). Vasculae reactions of normal and malignant tissues in vivo. I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. J. Natl. Cancer Inst. *6*, 73–85.

Alitalo, K., Tammela, T., and Petrova, T.V. (2005). Lymphangiogenesis in development and human disease. Nature *438*, 946–953.

Allen, E., Jabouille, A., Rivera, L.B., Lodewijckx, I., Missiaen, R., Steri, V., Feyen, K., Tawney, J., Hanahan, D., Michael, I.P., and Bergers, G. (2017). Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Sci. Transl. Med. *9*.

Apte, R.S. (2016). What Is Chronic or Persistent Diabetic Macular Edema and How Should It Be Treated? JAMA Ophthalmol. *134*, 285–286.

Apte, R.S. (2018). Gene Therapy for Retinal Degeneration. Cell *173*, 5.

Apte, R.S., Richter, J., Herndon, J., and Ferguson, T.A. (2006). Macrophages inhibit neovascularization in a murine model of age-related macular degeneration. PLoS Med. *3*, e310.

Arjaans, M., Oosting, S.F., Schröder, C.P., and de Vries, E.G. (2013). Bevacizumab-induced vessel normalization hampers tumor uptake of antibodies–response. Cancer Res. *73*, 7147–7148.

Ashton, N. (1952). Observations on the choroidal circulation. Br. J. Ophthalmol. *36*, 465–481.

Ashton, N. (1966). Oxygen and the growth and development of retinal vessels. In vivo and in vitro studies. The XX Francis I. Proctor Lecture. Am. J. Ophthalmol. *62*, 412–435.

Atkins, M.B., Clark, J.I., and Quinn, D.I. (2017). Immune checkpoint inhibitors in advanced renal cell carcinoma: experience to date and future directions. Ann. Oncol. *28*, 1484–1494.

Atkins, M.B., Plimack, E.R., Puzanov, I., Fishman, M.N., McDermott, D.F., Cho, D.C., Vaishampayan, U., George, S., Olencki, T.E., Tarazi, J.C., et al. (2018). Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomised, open-label, dose-finding, and dose-expansion phase 1b trial. Lancet Oncol. *19*, 405–415.

Augustin, H.G., Koh, G.Y., Thurston, G., and Alitalo, K. (2009). Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat. Rev. Mol. Cell Biol. *10*, 165–177.

Bais, C., Wu, X., Yao, J., Yang, S., Crawford, Y., McCutcheon, K., Tan, C., Kolumam, G., Vernes, J.-M., Eastham-Anderson, J., et al. (2010). PlGF blockade does not inhibit angiogenesis during primary tumor growth. Cell *141*, 166–177.

Bakall, B., Folk, J.C., Boldt, H.C., Sohn, E.H., Stone, E.M., Russell, S.R., and Mahajan, V.B. (2013). Aflibercept therapy for exudative age-related macular degeneration resistant to bevacizumab and ranibizumab. Am. J. Ophthalmol. *156*, 15–22.

Ban, N., Lee, T.J., Sene, A., Choudhary, M., Lekwuwa, M., Dong, Z., Santeford, A., Lin, J.B., Malek, G., Ory, D.S., and Apte, R.S. (2018). Impaired monocyte cholesterol clearance initiates age-related retinal degeneration and vision loss. JCI Insight *3*.

Barleon, B., Sozzani, S., Zhou, D., Weich, H.A., Mantovani, A., and Marmé, D. (1996). Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood *87*, 3336–3343.

Batchelor, T.T., Sorensen, A.G., di Tomaso, E., Zhang, W.T., Duda, D.G., Cohen, K.S., Kozak, K.R., Cahill, D.P., Chen, P.J., Zhu, M., et al. (2007). AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell *11*, 83–95.

Batchelor, T.T., Duda, D.G., di Tomaso, E., Ancukiewicz, M., Plotkin, S.R., Gerstner, E., Eichler, A.F., Drappatz, J., Hochberg, F.H., Benner, T., et al. (2010). Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. J. Clin. Oncol. *28*, 2817–2823.

Batchelor, T.T., Mulholland, P., Neyns, B., Nabors, L.B., Campone, M., Wick, A., Mason, W., Mikkelsen, T., Phuphanich, S., Ashby, L.S., et al. (2013). Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. J. Clin. Oncol. *31*, 3212–3218.

Bates, D.O., Cui, T.G., Doughty, J.M., Winkler, M., Sugiono, M., Shields, J.D., Peat, D., Gillatt, D., and Harper, S.J. (2002). VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. Cancer Res. *62*, 4123–4131.

Bennouna, J., Sastre, J., Arnold, D., Österlund, P., Greil, R., Van Cutsem, E., von Moos, R., Viéitez, J.M., Bouché, O., Borg, C., et al.; ML18147 Study Investigators (2013). Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. Lancet Oncol. *14*, 29–37.

Bergers, G., and Hanahan, D. (2008). Modes of resistance to anti-angiogenic therapy. Nat. Rev. Cancer *8*, 592–603.

Berlin, J., Bendell, J.C., Hart, L.L., Firdaus, I., Gore, I., Hermann, R.C., Mulcahy, M.F., Zalupski, M.M., Mackey, H.M., Yauch, R.L., et al. (2013). A randomized phase II trial of vismodegib versus placebo with FOLFOX or FOLFIRI and bevacizumab in patients with previously untreated metastatic colorectal cancer. Clin. Cancer Res. *19*, 258–267.

Bhutto, I.A., McLeod, D.S., Hasegawa, T., Kim, S.Y., Merges, C., Tong, P., and Lutty, G.A. (2006). Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. Exp. Eye Res. *82*, 99–110.

Birkenhauer, E., and Neethirajan, S. (2015). A double-edged sword: the role of VEGF in wound repair and chemoattraction of opportunist pathogens. Int. J. Mol. Sci. *16*, 7159–7172.

Borgström, P., Hillan, K.J., Sriramarao, P., and Ferrara, N. (1996). Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. Cancer Res. *56*, 4032–4039.

Bressler, S.B., Ayala, A.R., Bressler, N.M., Melia, M., Qin, H., Ferris, F.L., 3rd, Flaxel, C.J., Friedman, S.M., Glassman, A.R., Jampol, L.M., and Rauser, M.E.; Diabetic Retinopathy Clinical Research Network (2016). Persistent Macular Thickening After Ranibizumab Treatment for Diabetic Macular Edema With Vision Impairment. JAMA Ophthalmol. *134*, 278–285.

Brown, D.M., Nguyen, Q.D., Marcus, D.M., Boyer, D.S., Patel, S., Feiner, L., Schlottmann, P.G., Rundle, A.C., Zhang, J., Rubio, R.G., et al.; RIDE and RISE Research Group (2013). Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. Ophthalmology *120*, 2013–2022.

Buehler, A., Sitaras, N., Favret, S., Bucher, F., Berger, S., Pielen, A., Joyal, J.S., Juan, A.M., Martin, G., Schlunck, G., et al. (2013). Semaphorin 3F forms an anti-angiogenic barrier in outer retina. FEBS Lett. *587*, 1650–1655.

Calippe, B., Augustin, S., Beguier, F., Charles-Messance, H., Poupel, L., Conart, J.B., Hu, S.J., Lavalette, S., Fauvet, A., Rayes, J., et al. (2017). Complement Factor H Inhibits CD47-Mediated Resolution of Inflammation. Immunity *46*, 261–272.

Calvo, P., Ferreras, A., Al Adel, F., Wang, Y., and Brent, M.H. (2015). Dexamethasone intravitreal implant as adjunct therapy for patients with wet age-related macular degeneration with incomplete response to ranibizumab. Br. J. Ophthalmol. *99*, 723–726.

Campochiaro, P.A., and Peters, K.G. (2016). Targeting Tie2 for Treatment of Diabetic Retinopathy and Diabetic Macular Edema. Curr. Diab. Rep. *16*, 126.

Campochiaro, P.A., Khanani, A., Singer, M., Patel, S., Boyer, D., Dugel, P., Kherani, S., Withers, B., Gambino, L., Peters, K., and Brigell, M.; TIME-2 Study Group (2016). Enhanced Benefit in Diabetic Macular Edema from AKB-9778 Tie2 Activation Combined with Vascular Endothelial Growth Factor Suppression. Ophthalmology *123*, 1722–1730.

Caprara, C., and Grimm, C. (2012). From oxygen to erythropoietin: relevance of hypoxia for retinal development, health and disease. Prog. Retin. Eye Res. *31*, 89–119.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., et al. (1996). Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature *380*, 435–439.

Carrel, A. (1913). Artificial Activation of the Growth in Vitro of Connective Tissue. J. Exp. Med. *17*, 14–19.

Casanovas, O., Hicklin, D.J., Bergers, G., and Hanahan, D. (2005). Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell *8*, 299–309.

Chaudhary, V., Barbosa, J., Lam, W.C., Mak, M., Mavrikakis, E., and Mohaghegh P, S.M. (2016). Ozurdex in age-related macular degeneration as adjunct to ranibizumab (The OARA Study). Can. J. Ophthalmol. *51*, 302–305.

Chauhan, V.P., Stylianopoulos, T., Martin, J.D., Popović, Z., Chen, O., Kamoun, W.S., Bawendi, M.G., Fukumura, D., and Jain, R.K. (2012). Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. Nat. Nanotechnol. *7*, 383–388.

Chen, D.S., and Hurwitz, H. (2018). Combinations of Bevacizumab With Cancer Immunotherapy. Cancer J. *24*, 193–204.

Chen, D.S., and Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. Immunity *39*, 1–10.

Chen, D.S., and Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. Nature *541*, 321–330.

Chung, A.S., and Ferrara, N. (2011). Developmental and pathological angiogenesis. Annu. Rev. Cell Dev. Biol. *27*, 563–584.

Chung, A.S., Lee, J., and Ferrara, N. (2010). Targeting the tumour vasculature: insights from physiological angiogenesis. Nat. Rev. Cancer *10*, 505–514.

Chung, A.S., Wu, X., Zhuang, G., Ngu, H., Kasman, I., Zhang, J., Vernes, J.M., Jiang, Z., Meng, Y.G., Peale, F.V., et al. (2013). An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. Nat. Med. *19*, 1114–1123.

Combadière, C., Feumi, C., Raoul, W., Keller, N., Rodéro, M., Pézard, A., Lavalette, S., Houssier, M., Jonet, L., Picard, E., et al. (2007). CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. J. Clin. Invest. *117*, 2920–2928.

Dalton, H.J., Pradeep, S., McGuire, M., Hailemichael, Y., Ma, S., Lyons, Y., Armaiz-Pena, G.N., Previs, R.A., Hansen, J.M., Rupaimoole, R., et al. (2017). Macrophages Facilitate Resistance to Anti-VEGF Therapy by Altered VEGFR Expression. Clin. Cancer Res. *23*, 7034–7046.

De Bock, K., Georgiadou, M., and Carmeliet, P. (2013). Role of endothelial cell metabolism in vessel sprouting. Cell Metab. *18*, 634–647.

De Palma, M., Biziato, D., and Petrova, T.V. (2017). Microenvironmental regulation of tumour angiogenesis. Nat. Rev. Cancer *17*, 457–474.

de Vries, C., Escobedo, J.A., Ueno, H., Houck, K., Ferrara, N., and Williams, L.T. (1992). The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science *255*, 989–991.

Dong, A., Seidel, C., Snell, D., Ekawardhani, S., Ahlskog, J.K., Baumann, M., Shen, J., Iwase, T., Tian, J., Stevens, R., et al. (2014). Antagonism of PDGF-BB suppresses subretinal neovascularization and enhances the effects of blocking VEGF-A. Angiogenesis *17*, 553–562.

Dugel, P.U., Jaffe, G.J., Sallstig, P., Warburton, J., Weichselberger, A., Wieland, M., and Singerman, L. (2017). Brolucizumab Versus Aflibercept in Participants with Neovascular Age-Related Macular Degeneration: A Randomized Trial. Ophthalmology *124*, 1296–1304.

Duh, E., and Aiello, L.P. (1999). Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. Diabetes *48*, 1899–1906.

Duh, E.J., Sun, J.K., and Stitt, A.W. (2017). Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight *2*.

Dunn, E.N., Hariprasad, S.M., and Sheth, V.S. (2017). An Overview of the Fovista and Rinucumab Trials and the Fate of Anti-PDGF Medications. Ophthalmic Surg. Lasers Imaging Retina *48*, 100–104.

Eilken, H.M., and Adams, R.H. (2010). Dynamics of endothelial cell behavior in sprouting angiogenesis. Curr. Opin. Cell Biol. *22*, 617–625.

Elman, M.J., Bressler, N.M., Qin, H., Beck, R.W., Ferris, F.L., 3rd, Friedman, S.M., Glassman, A.R., Scott, I.U., Stockdale, C.R., and Sun, J.K.; Diabetic Retinopathy Clinical Research Network (2011). Expanded 2-year follow-up

of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology *118*, 609–614.

Elman, M.J., Qin, H., Aiello, L.P., Beck, R.W., Bressler, N.M., Ferris, F.L., 3rd, Glassman, A.R., Maturi, R.K., and Melia, M.; Diabetic Retinopathy Clinical Research Network (2012). Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. Ophthalmology *119*, 2312–2318.

Eswarappa, S.M., Potdar, A.A., Koch, W.J., Fan, Y., Vasu, K., Lindner, D., Willard, B., Graham, L.M., DiCorleto, P.E., and Fox, P.L. (2014). Programmed translational readthrough generates antiangiogenic VEGF-Ax. Cell *157*, 1605–1618.

Ferrara, N. (2010a). Vascular endothelial growth factor and age-related macular degeneration: from basic science to therapy. Nat. Med. *16*, 1107–1111.

Ferrara, N. (2010b). Binding to the extracellular matrix and proteolytic processing: two key mechanisms regulating vascular endothelial growth factor action. Mol. Biol. Cell *21*, 687–690.

Ferrara, N. (2016). VEGF and Intraocular Neovascularization: From Discovery to Therapy. Transl. Vis. Sci. Technol. *5*, 10.

Ferrara, N., and Adamis, A.P. (2016). Ten years of anti-vascular endothelial growth factor therapy. Nat. Rev. Drug Discov. *15*, 385–403.

Ferrara, N., and Henzel, W.J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem. Biophys. Res. Commun. *161*, 851–858.

Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K.S., Powell-Braxton, L., Hillan, K.J., and Moore, M.W. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature *380*, 439–442.

Ferrara, N., Hillan, K.J., Gerber, H.P., and Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat. Rev. Drug Discov. *3*, 391–400.

Fischer, C., Jonckx, B., Mazzone, M., Zacchigna, S., Loges, S., Pattarini, L., Chorianopoulos, E., Liesenborghs, L., Koch, M., De Mol, M., et al. (2007). Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell *131*, 463–475.

Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. *285*, 1182–1186.

Folkman, J., and Klagsbrun, M. (1987). Angiogenic factors. Science *235*, 442–447.

Fortune, B., Schneck, M.E., and Adams, A.J. (1999). Multifocal electroretinogram delays reveal local retinal dysfunction in early diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. *40*, 2638–2651.

Fukumura, D., Kloepper, J., Amoozgar, Z., Duda, D.G., and Jain, R.K. (2018). Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat. Rev. Clin. Oncol. *15*, 325–340.

Gabrilovich, D., Ishida, T., Oyama, T., Ran, S., Kravtsov, V., Nadaf, S., and Carbone, D.P. (1998). Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood *92*, 4150–4166.

García-Carbonero, R., van Cutsem, E., Rivera, F., Jassem, J., Gore, I., Jr., Tebbutt, N., Braiteh, F., Argiles, G., Wainberg, Z.A., Funke, R., et al. (2017). Randomized Phase II Trial of Parsatuzumab (Anti-EGFL7) or Placebo in Combination with FOLFOX and Bevacizumab for First-Line Metastatic Colorectal Cancer. Oncologist *22*, 1281.

Gasperini, J.L., Fawzi, A.A., Khondkaryan, A., Lam, L., Chong, L.P., Eliott, D., Walsh, A.C., Hwang, J., and Sadda, S.R. (2012). Bevacizumab and ranibizumab tachyphylaxis in the treatment of choroidal neovascularisation. Br. J. Ophthalmol. *96*, 14–20.

Geloneck, M.M., Chuang, A.Z., Clark, W.L., Hunt, M.G., Norman, A.A., Packwood, E.A., Tawansy, K.A., and Mintz-Hittner, H.A.; BEAT-ROP Cooperative Group (2014). Refractive outcomes following bevacizumab monotherapy compared with conventional laser treatment: a randomized clinical trial. JAMA Ophthalmol. *132*, 1327–1333.

Gerber, H.P., and Ferrara, N. (2005). Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res. *65*, 671–680.

Gerber, H.P., Hillan, K.J., Ryan, A.M., Kowalski, J., Keller, G.-A., Rangell, L., Wright, B.D., Radtke, F., Aguet, M., and Ferrara, N. (1999). VEGF is required for growth and survival in neonatal mice. Development *126*, 1149–1159.

Gerhardt, H. (2008). VEGF and endothelial guidance in angiogenic sprouting. Organogenesis *4*, 241–246.

Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Alitalo, K., Shima, D., and Betsholtz, C. (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J. Cell Biol. *161*, 1163–1177.

Gross, J.G., Glassman, A.R., Jampol, L.M., Inusah, S., Aiello, L.P., Antoszyk, A.N., Baker, C.W., Berger, B.B., Bressler, N.M., Browning, D., et al.; Writing Committee for the Diabetic Retinopathy Clinical Research Network (2015). Panretinal Photocoagulation vs Intravitreous Ranibizumab for Proliferative Diabetic Retinopathy: A Randomized Clinical Trial. JAMA *314*, 2137–2146.

Hainsworth, J.D., Spigel, D.R., Sosman, J.A., Burris, H.A., 3rd, Farley, C., Cucullu, H., Yost, K., Hart, L.L., Sylvester, L., Waterhouse, D.M., and Greco, F.A. (2007). Treatment of advanced renal cell carcinoma with the combination bevacizumab/erlotinib/imatinib: a phase I/II trial. Clin. Genitourin. Cancer *5*, 427–432.

Harrison, W.W., Bearse, M.A., Jr., Ng, J.S., Jewell, N.P., Barez, S., Burger, D., Schneck, M.E., and Adams, A.J. (2011). Multifocal electroretinograms predict onset of diabetic retinopathy in adult patients with diabetes. Invest. Ophthalmol. Vis. Sci. *52*, 772–777.

Hegde, P.S., Wallin, J.J., and Mancao, C. (2018). Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. Semin. Cancer Biol. *52*, 117–124.

Heier, J.S., Kherani, S., Desai, S., Dugel, P., Kaushal, S., Cheng, S.H., Delacono, C., Purvis, A., Richards, S., Le-Halpere, A., et al. (2017). Intravitreous injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. Lancet *390*, 50–61.

Hellström, M., Phng, L.K., and Gerhardt, H. (2007). VEGF and Notch signaling: the yin and yang of angiogenic sprouting. Cell Adhes. Migr. *1*, 133–136.

Ho, A.C., Busbee, B.G., Regillo, C.D., Wieland, M.R., Van Everen, S.A., Li, Z., Rubio, R.G., and Lai, P.; HARBOR Study Group (2014). Twenty-four-month efficacy and safety of 0.5 mg or 2.0 mg ranibizumab in patients with subfoveal neovascular age-related macular degeneration. Ophthalmology *121*, 2181–2192.

Holash, J., Davis, S., Papadopoulos, N., Croll, S.D., Ho, L., Russell, M., Boland, P., Leidich, R., Hylton, D., Burova, E., et al. (2002). VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc. Natl. Acad. Sci. USA *99*, 11393–11398.

Holz, F.G., Tadayoni, R., Beatty, S., Berger, A., Cereda, M.G., Cortez, R., Hoyng, C.B., Hykin, P., Staurenghi, G., Heldner, S., et al. (2015). Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. Br. J. Ophthalmol. *99*, 220–226.

Holz, F.G., Sadda, S.R., Busbee, B., Chew, E.Y., Mitchell, P., Tufail, A., Brittain, C., Ferrara, D., Gray, S., Honigberg, L., et al.; Chroma and Spectri Study Investigators (2018). Efficacy and Safety of Lampalizumab for Geographic Atrophy Due to Age-Related Macular Degeneration: Chroma and Spectri Phase 3 Randomized Clinical Trials. JAMA Ophthalmol. *136*, 666–677.

Houck, K.A., Leung, D.W., Rowland, A.M., Winer, J., and Ferrara, N. (1992). Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. J. Biol. Chem. *267*, 26031–26037.

Huang, Y., Yuan, J., Righi, E., Kamoun, W.S., Ancukiewicz, M., Nezivar, J., Santosuosso, M., Martin, J.D., Martin, M.R., Vianello, F., et al. (2012). Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc. Natl. Acad. Sci. USA *109*, 17561–17566.

Huang, Y., Stylianopoulos, T., Duda, D.G., Fukumura, D., and Jain, R.K. (2013). Benefits of vascular normalization are dose and time dependent–letter. Cancer Res. *73*, 7144–7146.

Ide, A.G., Baker, N.H., and Warren, S.L. (1939). Vascularization of the Brown Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. Am. J. Roentgenol. *42*, 891–899.

Incio, J., Ligibel, J.A., McManus, D.T., Suboj, P., Jung, K., Kawaguchi, K., Pinter, M., Babykutty, S., Chin, S.M., Vardam, T.D., et al. (2018). Obesity promotes resistance to anti-VEGF therapy in breast cancer by up-regulating IL-6 and potentially FGF-2. Sci. Transl. Med. *10*.

Itatani, Y., Kawada, K., Yamamoto, T., and Sakai, Y. (2018). Resistance to Anti-Angiogenic Therapy in Cancer-Alterations to Anti-VEGF Pathway. Int. J. Mol. Sci. *19*.

Jaffe, G.J., Ciulla, T.A., Ciardella, A.P., Devin, F., Dugel, P.U., Eandi, C.M., Masonson, H., Monés, J., Pearlman, J.A., Quaranta-El Maftouhi, M., et al. (2017). Dual Antagonism of PDGF and VEGF in Neovascular Age-Related Macular Degeneration: A Phase IIb, Multicenter, Randomized Controlled Trial. Ophthalmology *124*, 224–234.

Jager, R.D., Mieler, W.F., and Miller, J.W. (2008). Age-related macular degeneration. N. Engl. J. Med. *358*, 2606–2617.

Jain, R.K. (2003). Molecular regulation of vessel maturation. Nat. Med. *9*, 685–693.

Jain, R.K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science *307*, 58–62.

Jain, R.K. (2014). Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. Cancer Cell *26*, 605–622.

Jayson, G.C., Kerbel, R., Ellis, L.M., and Harris, A.L. (2016). Antiangiogenic therapy in oncology: current status and future directions. Lancet *388*, 518–529.

Joyal, J.S., Omri, S., Sitaras, N., Rivera, J.C., Sapieha, P., and Chemtob, S. (2012). Neovascularization in retinopathy of prematurity: opposing actions of neuronal factors GPR91 and semaphorins 3A. Acta Paediatr. *101*, 819–826.

Jung, K., Heishi, T., Khan, O.F., Kowalski, P.S., Incio, J., Rahbari, N.N., Chung, E., Clark, J.W., Willett, C.G., Luster, A.D., et al. (2017). Ly6Clo monocytes drive immunosuppression and confer resistance to anti-VEGFR2 cancer therapy. J. Clin. Invest. *127*, 3039–3051.

Junttila, M.R., and de Sauvage, F.J. (2013). Influence of tumour micro-environment heterogeneity on therapeutic response. Nature *501*, 346–354.

Kamoun, W.S., Ley, C.D., Farrar, C.T., Duyverman, A.M., Lahdenranta, J., Lacorre, D.A., Batchelor, T.T., di Tomaso, E., Duda, D.G., Munn, L.L., et al. (2009). Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J. Clin. Oncol. *27*, 2542–2552.

Keck, P.J., Hauser, S.D., Krivi, G., Sanzo, K., Warren, T., Feder, J., and Connolly, D.T. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science *246*, 1309–1312.

Kelly, P.N. (2018). The Cancer Immunotherapy Revolution. Science *359*, 1344–1345.

Kelly, J., Ali Khan, A., Yin, J., Ferguson, T.A., and Apte, R.S. (2007). Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice. J. Clin. Invest. *117*, 3421–3426.

Kerbel, R.S. (2008). Tumor angiogenesis. N. Engl. J. Med. *358*, 2039–2049.

Khan, K.A., and Kerbel, R.S. (2018). Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. Nat. Rev. Clin. Oncol. *15*, 310–324.

Kim, K.J., Li, B., Houck, K., Winer, J., and Ferrara, N. (1992). The vascular endothelial growth factor proteins: identification of biologically relevant regions by neutralizing monoclonal antibodies. Growth Factors *7*, 53–64.

Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., and Ferrara, N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature *362*, 841–844.

Kim, I.K., Husain, D., Michaud, N., Connolly, E., Lane, A.M., Durrani, K., Hafezi-Moghadam, A., Gragoudas, E.S., O'Neill, C.A., Beyer, J.C., and Miller, J.W. (2006). Effect of intravitreal injection of ranibizumab in combination with verteporfin PDT on normal primate retina and choroid. Invest. Ophthalmol. Vis. Sci. *47*, 357–363.

Klement, G., Baruchel, S., Rak, J., Man, S., Clark, K., Hicklin, D.J., Bohlen, P., and Kerbel, R.S. (2000). Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J. Clin. Invest. *105*, R15–R24.

Kloepper, J., Riedemann, L., Amoozgar, Z., Seano, G., Susek, K., Yu, V., Dalvie, N., Amelung, R.L., Datta, M., Song, J.W., et al. (2016). Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. Proc. Natl. Acad. Sci. USA *113*, 4476–4481.

Kopetz, S., Hoff, P.M., Morris, J.S., Wolff, R.A., Eng, C., Glover, K.Y., Adinin, R., Overman, M.J., Valero, V., Wen, S., et al. (2010). Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. J. Clin. Oncol. *28*, 453–459.

Lassen, U., Chinot, O.L., McBain, C., Mau-Sørensen, M., Larsen, V.A., Barrie, M., Roth, P., Krieter, O., Wang, K., Habben, K., et al. (2015). Phase 1 dose-escalation study of the antiplacental growth factor monoclonal antibody RO5323441 combined with bevacizumab in patients with recurrent glioblastoma. Neuro-oncol. *17*, 1007–1015.

LeCouter, J., Moritz, D.R., Li, B., Phillips, G.L., Liang, X.H., Gerber, H.P., Hillan, K.J., and Ferrara, N. (2003). Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science *299*, 890–893.

Lee, C.G., Heijn, M., di Tomaso, E., Griffon-Etienne, G., Ancukiewicz, M., Koike, C., Park, K.R., Ferrara, N., Jain, R.K., Suit, H.D., and Boucher, Y. (2000). Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res. *60*, 5565–5570.

Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. Science *246*, 1306–1309.

Li, X., Padhan, N., Sjöström, E.O., Roche, F.P., Testini, C., Honkura, N., Sáinz-Jaspeado, M., Gordon, E., Bentley, K., Philippides, A., et al. (2016). VEGFR2 pY949 signalling regulates adherens junction integrity and metastatic spread. Nat. Commun. *7*, 11017.

Lima e Silva, R., Shen, J., Hackett, S.F., Kachi, S., Akiyama, H., Kiuchi, K., Yokoi, K., Hatara, M.C., Lauer, T., Aslam, S., et al. (2007). The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization. FASEB J. *21*, 3219–3230.

Long, D., Kanan, Y., Shen, J., Hackett, S.F., Liu, Y., Hafiz, Z., Khan, M., Lu, L., and Campochiaro, P.A. (2018). VEGF/VEGFR2 blockade does not cause retinal atrophy in AMD-relevant models. JCI Insight *3*.

Ma, W., and Wong, W.T. (2016). Aging Changes in Retinal Microglia and their Relevance to Age-related Retinal Disease. Adv. Exp. Med. Biol. *854*, 73–78.

Maclachlan, T.K., Lukason, M., Collins, M., Munger, R., Isenberger, E., Rogers, C., Malatos, S., Dufresne, E., Morris, J., Calcedo, R., et al. (2011). Preclinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration. Mol. Ther. *19*, 326–334.

Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell *161*, 1202–1214.

Maguire, M.G., Martin, D.F., Ying, G.S., Jaffe, G.J., Daniel, E., Grunwald, J.E., Toth, C.A., Ferris, F.L., 3rd, and Fine, S.L.; Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group (2016). Five-Year Outcomes with Anti-Vascular Endothelial Growth Factor Treatment of Neovascular Age-Related Macular Degeneration: The Comparison of Age-Related Macular Degeneration Treatments Trials. Ophthalmology *123*, 1751–1761.

Martin, D.F., Maguire, M.G., Ying, G.S., Grunwald, J.E., Fine, S.L., Jaffe, G.J., and Jaffe, G.J.; CATT Research Group (2011). Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N. Engl. J. Med. *364*, 1897–1908.

McDermott, D.F., Huseni, M.A., Atkins, M.B., Motzer, R.J., Rini, B.I., Escudier, B., Fong, L., Joseph, R.W., Pal, S.K., Reeves, J.A., et al. (2018). Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat. Med. *24*, 749–757.

Michaelson, I.C. (1948). The mode of development of the vascular system of the retina with some observations on its significance for certain retinal disorders. Trans. Ophthalmol. Soc. UK *68*, 137–180.

Miller, J.W. (1997). Vascular endothelial growth factor and ocular neovascularization. Am. J. Pathol. *151*, 13–23.

Miller, J.W., Le Couter, J., Strauss, E.C., and Ferrara, N. (2013). Vascular endothelial growth factor a in intraocular vascular disease. Ophthalmology *120*, 106–114.

Mintz-Hittner, H.A., Kennedy, K.A., and Chuang, A.Z.; BEAT-ROP Cooperative Group (2011). Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N. Engl. J. Med. *364*, 603–615.

Mitamura, T., Pradeep, S., McGuire, M., Wu, S.Y., Ma, S., Hatakeyama, H., Lyons, Y.A., Hisamatsu, T., Noh, K., Villar-Prados, A., et al. (2018). Induction of anti-VEGF therapy resistance by upregulated expression of microseminoprotein (MSMP). Oncogene *37*, 722–731.

Mitsuhashi, A., Goto, H., Saijo, A., Trung, V.T., Aono, Y., Ogino, H., Kuramoto, T., Tabata, S., Uehara, H., Izumi, K., et al. (2015). Fibrocyte-like cells mediate acquired resistance to anti-angiogenic therapy with bevacizumab. Nat. Commun. *6*, 8792.

Motz, G.T., and Coukos, G. (2013). Deciphering and reversing tumor immune suppression. Immunity *39*, 61–73.

Nagy, J.A., Chang, S.H., Dvorak, A.M., and Dvorak, H.F. (2009). Why are tumour blood vessels abnormal and why is it important to know? Br. J. Cancer *100*, 865–869.

Nakamura, R., Sene, A., Santeford, A., Gdoura, A., Kubota, S., Zapata, N., and Apte, R.S. (2015). IL10-driven STAT3 signalling in senescent macrophages promotes pathological eye angiogenesis. Nat. Commun. *6*, 7847.

Negri, L., and Ferrara, N. (2018). The Prokineticins: Neuromodulators and Mediators of Inflammation and Myeloid Cell-Dependent Angiogenesis. Physiol. Rev. *98*, 1055–1082.

Nguyen, Q.D., De Falco, S., Behar-Cohen, F., Lam, W.C., Li, X., Reichhart, N., Ricci, F., Pluim, J., and Li, W.W. (2018). Placental growth factor and its potential role in diabetic retinopathy and other ocular neovascular diseases. Acta Ophthalmol. *96*, e1–e9.

Ohm, J.E., and Carbone, D.P. (2001). VEGF as a mediator of tumor-associated immunodeficiency. Immunol. Res. *23*, 263–272.

Okabe, K., Kobayashi, S., Yamada, T., Kurihara, T., Tai-Nagara, I., Miyamoto, T., Mukouyama, Y.S., Sato, T.N., Suda, T., Ema, M., and Kubota, Y. (2014). Neurons limit angiogenesis by titrating VEGF in retina. Cell *159*, 584–596.

Oladipupo, S.S., Smith, C., Santeford, A., Park, C., Sene, A., Wiley, L.A., Osei-Owusu, P., Hsu, J., Zapata, N., Liu, F., et al. (2014). Endothelial cell FGF signaling is required for injury response but not for vascular homeostasis. Proc. Natl. Acad. Sci. USA *111*, 13379–13384.

Olsson, A.K., Dimberg, A., Kreuger, J., and Claesson-Welsh, L. (2006). VEGF receptor signalling - in control of vascular function. Nat. Rev. Mol. Cell Biol. *7*, 359–371.

Ott, P.A., Hodi, F.S., and Buchbinder, E.I. (2015). Inhibition of Immune Checkpoints and Vascular Endothelial Growth Factor as Combination Therapy for Metastatic Melanoma: An Overview of Rationale, Preclinical Evidence, and Initial Clinical Data. Front. Oncol. *5*, 202.

Pajusola, K., Aprelikova, O., Korhonen, J., Kaipainen, A., Pertovaara, L., Alitalo, R., and Alitalo, K. (1992). FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. Cancer Res. *52*, 5738–5743.

Park, J.E., Chen, H.H., Winer, J., Houck, K.A., and Ferrara, N. (1994). Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. J. Biol. Chem. *269*, 25646–25654.

Pastuskovas, C.V., Mundo, E.E., Williams, S.P., Nayak, T.K., Ho, J., Ulufatu, S., Clark, S., Ross, S., Cheng, E., Parsons-Reponte, K., et al. (2012). Effects of anti-VEGF on pharmacokinetics, biodistribution, and tumor penetration of trastuzumab in a preclinical breast cancer model. Mol. Cancer Ther. *11*, 752–762.

Penn, J.S., Tolman, B.L., and Henry, M.M. (1994). Oxygen-induced retinopathy in the rat: relationship of retinal nonperfusion to subsequent neovascularization. Invest. Ophthalmol. Vis. Sci. *35*, 3429–3435.

Poltorak, Z., Cohen, T., Sivan, R., Kandelis, Y., Spira, G., Vlodavsky, I., Keshet, E., and Neufeld, G. (1997). VEGF145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. J. Biol. Chem. *272*, 7151–7158.

Potente, M., Gerhardt, H., and Carmeliet, P. (2011). Basic and therapeutic aspects of angiogenesis. Cell *146*, 873–887.

Presta, L.G., Chen, H., O'Connor, S.J., Chisholm, V., Meng, Y.G., Krummen, L., Winkler, M., and Ferrara, N. (1997). Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res. *57*, 4593–4599.

Rajagopal, R., Bligard, G.W., Zhang, S., Yin, L., Lukasiewicz, P., and Semenkovich, C.F. (2016). Functional Deficits Precede Structural Lesions in Mice With High-Fat Diet-Induced Diabetic Retinopathy. Diabetes *65*, 1072–1084.

Rakoczy, E.P., Lai, C.M., Magno, A.L., Wikstrom, M.E., French, M.A., Pierce, C.M., Schwartz, S.D., Blumenkranz, M.S., Chalberg, T.W., Degli-Esposti, M.A., and Constable, I.J. (2015). Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. Lancet *386*, 2395–2403.

Regula, J.T., Lundh von Leithner, P., Foxton, R., Barathi, V.A., Cheung, C.M., Bo Tun, S.B., Wey, Y.S., Iwata, D., Dostalek, M., Moelleken, J., et al. (2016). Targeting key angiogenic pathways with a bispecific CrossMAb optimized for neovascular eye diseases. EMBO Mol. Med. *8*, 1265–1288.

Ruhrberg, C., Gerhardt, H., Golding, M., Watson, R., Ioannidou, S., Fujisawa, H., Betsholtz, C., and Shima, D.T. (2002). Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev. *16*, 2684–2698.

Saharinen, P., Eklund, L., and Alitalo, K. (2017). Therapeutic targeting of the angiopoietin-TIE pathway. Nat. Rev. Drug Discov. *16*, 635–661.

Saint-Geniez, M., Kurihara, T., Sekiyama, E., Maldonado, A.E., and D'Amore, P.A. (2009). An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. Proc. Natl. Acad. Sci. USA *106*, 18751–18756.

Sakurai, Y., Ohgimoto, K., Kataoka, Y., Yoshida, N., and Shibuya, M. (2005). Essential role of Flk-1 (VEGF receptor 2) tyrosine residue 1173 in vasculogenesis in mice. Proc. Natl. Acad. Sci. USA *102*, 1076–1081.

Schmittnaegel, M., Rigamonti, N., Kadioglu, E., Cassará, A., Wyser Rmili, C., Kiialainen, A., Kienast, Y., Mueller, H.J., Ooi, C.H., Laoui, D., and De Palma, M. (2017). Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. Sci. Transl. Med. *9*.

Scott, I.U., VanVeldhuisen, P.C., Ip, M.S., Blodi, B.A., Oden, N.L., Awh, C.C., Kunimoto, D.Y., Marcus, D.M., Wroblewski, J.J., and King, J.; SCORE2 Investigator Group (2017). Effect of Bevacizumab vs Aflibercept on Visual Acuity Among Patients With Macular Edema Due to Central Retinal Vein Occlusion: The SCORE2 Randomized Clinical Trial. JAMA *317*, 2072–2087.

Segarra, M., Ohnuki, H., Maric, D., Salvucci, O., Hou, X., Kumar, A., Li, X., and Tosato, G. (2012). Semaphorin 6A regulates angiogenesis by modulating VEGF signaling. Blood *120*, 4104–4115.

Semenza, G.L. (2000a). HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. *88*, 1474–1480.

Semenza, G.L. (2000b). HIF-1: using two hands to flip the angiogenic switch. Cancer Metastasis Rev. *19*, 59–65.

Sene, A., and Apte, R.S. (2014). Eyeballing cholesterol efflux and macrophage function in disease pathogenesis. Trends Endocrinol. Metab. *25*, 107–114.

Sene, A., Khan, A.A., Cox, D., Nakamura, R.E., Santeford, A., Kim, B.M., Sidhu, R., Onken, M.D., Harbour, J.W., Hagbi-Levi, S., et al. (2013). Impaired

cholesterol efflux in senescent macrophages promotes age-related macular degeneration. Cell Metab. *17*, 549–561.

Sene, A., Chin-Yee, D., and Apte, R.S. (2015). Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye. Trends Mol. Med. *21*, 43–51.

Senger, D.R., Galli, S.J., Dvorak, A.M., Perruzzi, C.A., Harvey, V.S., and Dvorak, H.F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science *219*, 983–985.

Senger, D.R., Connolly, D.T., Van de Water, L., Feder, J., and Dvorak, H.F. (1990). Purification and NH2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res. *50*, 1774–1778.

Sepah, Y.J., Sadiq, M.A., Boyer, D., Callanan, D., Gallemore, R., Bennett, M., Marcus, D., Halperin, L., Hassan, M., Campochiaro, P.A., et al.; READ-3 Study Group (2016). Twenty-four-Month Outcomes of the Ranibizumab for Edema of the Macula in Diabetes - Protocol 3 with High Dose (READ-3) Study. Ophthalmology *123*, 2581–2587.

Shalaby, F., Rossant, J., Yamaguchi, T.P., Gertsenstein, M., Wu, X.F., Breitman, M.L., and Schuh, A.C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature *376*, 62–66.

Shojaei, F., Wu, X., Malik, A.K., Zhong, C., Baldwin, M.E., Schanz, S., Fuh, G., Gerber, H.P., and Ferrara, N. (2007). Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. Nat. Biotechnol. *25*, 911–920.

Shojaei, F., Wu, X., Qu, X., Kowanetz, M., Yu, L., Tan, M., Meng, Y.G., and Ferrara, N. (2009). G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. Proc. Natl. Acad. Sci. USA *106*, 6742–6747.

Shrimali, R.K., Yu, Z., Theoret, M.R., Chinnasamy, D., Restifo, N.P., and Rosenberg, S.A. (2010). Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res. *70*, 6171–6180.

Smith, L.E. (2003). Pathogenesis of retinopathy of prematurity. Semin. Neonatol. *8*, 469–473.

Smith, L.E., Wesolowski, E., McLellan, A., Kostyk, S.K., D'Amato, R., Sullivan, R., and D'Amore, P.A. (1994). Oxygen-induced retinopathy in the mouse. Invest. Ophthalmol. Vis. Sci. *35*, 101–111.

Snuderl, M., Batista, A., Kirkpatrick, N.D., Ruiz de Almodovar, C., Riedemann, L., Walsh, E.C., Anolik, R., Huang, Y., Martin, J.D., Kamoun, W., et al. (2013). Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. Cell *152*, 1065–1076.

Socinski, M.A., Jotte, R.M., Cappuzzo, F., Orlandi, F., Stroyakovskiy, D., Nogami, N., Rodríguez-Abreu, D., Moro-Sibilot, D., Thomas, C.A., Barlesi, F., et al.; IMpower150 Study Group (2018). Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N. Engl. J. Med. *378*, 2288–2301.

Soker, S., Takashima, S., Miao, H.Q., Neufeld, G., and Klagsbrun, M. (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell *92*, 735–745.

Sonmez, K., Drenser, K.A., Capone, A., Jr., and Trese, M.T. (2008). Vitreous levels of stromal cell-derived factor 1 and vascular endothelial growth factor in patients with retinopathy of prematurity. Ophthalmology *115*, 1065–1070.

Spooner, K., Hong, T., Bahrami, B., and Chang, A. (2018). A meta-analysis of patients with treatment-resistant macular oedema secondary to retinal vein occlusions following switching to aflibercept. Acta Ophthalmol.

Stepien, K.E., Rosenfeld, P.J., Puliafito, C.A., Feuer, W., Shi, W., Al-Attar, L., Dubovy, S.R., Murray, T.G., Davis, J.L., Lee, W.H., et al. (2009). Comparison of intravitreal bevacizumab followed by ranibizumab for the treatment of neovascular age-related macular degeneration. Retina *29*, 1067–1073.

Sweeney, C.J., Miller, K.D., Sissons, S.E., Nozaki, S., Heilman, D.K., Shen, J., and Sledge, G.W., Jr. (2001). The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res. *61*, 3369–3372.

Terman, B.I., Dougher Vermazen, M., Carrion, M.E., Dimitrov, D., Armellino, D.C., Gospodarowicz, D., and Böhlen, P. (1992). Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem. Biophys. Res. Commun. *187*, 1579–1586.

Tong, R.T., Boucher, Y., Kozin, S.V., Winkler, F., Hicklin, D.J., and Jain, R.K. (2004). 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*, 3731–3736.

Van der Veldt, A.A., Lubberink, M., Bahce, I., Walraven, M., de Boer, M.P., Greuter, H.N., Hendrikse, N.H., Eriksson, J., Windhorst, A.D., Postmus, P.E., et al. (2012). Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. Cancer Cell *21*, 82–91.

Wacker, A., and Gerhardt, H. (2011). Endothelial development taking shape. Curr. Opin. Cell Biol. *23*, 676–685.

Wagle, N., Emery, C., Berger, M.F., Davis, M.J., Sawyer, A., Pochanard, P., Kehoe, S.M., Johannessen, C.M., Macconaill, L.E., Hahn, W.C., et al. (2011). Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. J. Clin. Oncol. *29*, 3085–3096.

Wakelee, H., Zvirbule, Z., De Braud, F., Kingsley, C.D., Mekhail, T., Lowe, T., Schütte, W., Lena, H., Lawler, W., Braiteh, F., et al. (2017). Efficacy and Safety of Onartuzumab in Combination With First-Line Bevacizumab- or Pemetrexed-Based Chemotherapy Regimens in Advanced Non-Squamous Non-Small-Cell Lung Cancer. Clin. Lung Cancer *18*, 50–59.

Wallin, J.J., Bendell, J.C., Funke, R., Sznol, M., Korski, K., Jones, S., Hernandez, G., Mier, J., He, X., Hodi, F.S., et al. (2016). Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat. Commun. *7*, 12624.

Warren, R.S., Yuan, H., Matli, M.R., Gillett, N.A., and Ferrara, N. (1995). Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J. Clin. Invest. *95*, 1789–1797.

Wechsler-Reya, R.J., and Barres, B.A. (1997). Retinal development: communication helps you see the light. Curr. Biol. *7*, R433–R436.

Weekes, C.D., Beeram, M., Tolcher, A.W., Papadopoulos, K.P., Gore, L., Hegde, P., Xin, Y., Yu, R., Shih, L.M., Xiang, H., et al. (2014). A phase I study of the human monoclonal anti-NRP1 antibody MNRP1685A in patients with advanced solid tumors. Invest. New Drugs *32*, 653–660.

Weekes, C.D., Rosen, L.S., Capasso, A., Wong, K.M., Ye, W., Anderson, M., McCall, B., Fredrickson, J., Wakshull, E., Eppler, S., et al. (2018). Phase I study of the anti-α5β1 monoclonal antibody MINT1526A with or without bevacizumab in patients with advanced solid tumors. Cancer Chemother. Pharmacol. *82*, 339–351.

Wells, J.A., Glassman, A.R., Ayala, A.R., Jampol, L.M., Aiello, L.P., Antoszyk, A.N., Arnold-Bush, B., Baker, C.W., Bressler, N.M., Browning, D.J., et al.; Diabetic Retinopathy Clinical Research Network (2015). Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N. Engl. J. Med. *372*, 1193–1203.

Willett, C.G., Boucher, Y., di Tomaso, E., Duda, D.G., Munn, L.L., Tong, R.T., Chung, D.C., Sahani, D.V., Kalva, S.P., Kozin, S.V., et al. (2004). Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat. Med. *10*, 145–147.

Winkler, F., Kozin, S.V., Tong, R.T., Chae, S.S., Booth, M.F., Garkavtsev, I., Xu, L., Hicklin, D.J., Fukumura, D., di Tomaso, E., et al. (2004). Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell *6*, 553–563.

Wise, G.N. (1956). Retinal neovascularization. Trans. Am. Ophthalmol. Soc. *54*, 729–826.

Xin, H., Zhong, C., Nudleman, E., and Ferrara, N. (2016). Evidence for Pro-angiogenic Functions of VEGF-Ax. Cell *167*, 275–284.

Yang, S., Toy, K., Ingle, G., Zlot, C., Williams, P.M., Fuh, G., Li, B., de Vos, A., and Gerritsen, M.E. (2002). Vascular endothelial growth factor-induced genes

in human umbilical vein endothelial cells: relative roles of KDR and Flt-1 receptors. Arterioscler. Thromb. Vasc. Biol. *22*, 1797–1803.

Yao, J., Wu, X., Zhuang, G., Kasman, I.M., Vogt, T., Phan, V., Shibuya, M., Ferrara, N., and Bais, C. (2011). Expression of a functional VEGFR-1 in tumor cells is a major determinant of anti-PlGF antibodies efficacy. Proc. Natl. Acad. Sci. USA *108*, 11590–11595.

Yaspan, B.L., Williams, D.F., Holz, F.G., Regillo, C.D., Li, Z., Dressen, A., van Lookeren Campagne, M., Le, K.N., Graham, R.R., Beres, T., et al.; MAHALO Study Investigators (2017). Targeting factor D of the alternative complement pathway reduces geographic atrophy progression secondary to age-related macular degeneration. Sci. Transl. Med. *9*.

Yonekawa, Y., Andreoli, C., Miller, J.B., Loewenstein, J.I., Sobrin, L., Eliott, D., Vavvas, D.G., Miller, J.W., and Kim, I.K. (2013). Conversion to aflibercept for chronic refractory or recurrent neovascular age-related macular degeneration. Am. J. Ophthalmol *156*, 29–35.

Zirlik, K., and Duyster, J. (2018). Anti-Angiogenics: Current Situation and Future Perspectives. Oncol. Res. Treat. *41*, 166–171.

Zissen, M.H., Kunz, P., Subbarayan, M., Chin, F.T., Conti, P.S., Fisher, G.A., and Quon, A. (2011). 18F-5-fluorouracil dynamic positron emission tomography/computed tomography shows decreased tracer activity after bevacizumab in colorectal metastases. Nucl. Med. Commun. *32*, 343–347.