Pharmacology & Therapeutics 164 (2016) 204–225

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Patel

Antagonist antibodies to vascular endothelial growth factor receptor 2 (VEGFR-2) as anti-angiogenic agents

Beverly L. Falcon [a], Sudhakar Chintharlapalli [a], Mark T. Uhlik [b], Bronislaw Pytowski [c,*]

[a] Eli Lilly and Company, Indianapolis, IN, USA
[b] Biothera, Eagan, MN, USA
[c] Eli Lilly and Company, New York, NY, USA

ARTICLE INFO

Available online 8 June 2016

Keywords:
Tumor vasculature
Angiogenesis
Endothelial cell
VEGFR-2
Ramucirumab
DC101

ABSTRACT

Interaction of numerous signaling pathways in endothelial and mesangial cells results in exquisite control of the process of physiological angiogenesis, with a central role played by vascular endothelial growth factor receptor 2 (VEGFR-2) and its cognate ligands. However, deregulated angiogenesis participates in numerous pathological processes. Excessive activation of VEGFR-2 has been found to mediate tissue-damaging vascular changes as well as the induction of blood vessel expansion to support the growth of solid tumors. Consequently, therapeutic intervention aimed at inhibiting the VEGFR-2 pathway has become a mainstay of treatment in cancer and retinal diseases. In this review, we introduce the concepts of physiological and pathological angiogenesis, the crucial role played by the VEGFR-2 pathway in these processes, and the various inhibitors of its activity that have entered the clinical practice. We primarily focus on the development of ramucirumab, the antagonist monoclonal antibody (mAb) that inhibits VEGFR-2 and has recently been approved for use in patients with gastric, colorectal, and lung cancers. We examine in-depth the pre-clinical studies using DC101, the mAb to mouse VEGFR-2, which provided a conceptual foundation for the role of VEGFR-2 in physiological and pathological angiogenesis. Finally, we discuss further clinical development of ramucirumab and the future of targeting the VEGF pathway for the treatment of cancer.

Contents

Abbreviations: Ang1, angiopoietin 1; Ang2, angiopoietin 2; bFGF, basic fibroblast growth factor; CEP, circulating endothelial progenitor cells; DCE-CT, dynamic contrast-enhanced computerized tomography; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; EC, endothelial cell; EGFR, epidermal growth factor receptor; FGFR-1, fibroblast growth factor receptor 1; GEM, genetically-engineered murine tumor model; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; IR, ionizing radiation; mAb, monoclonal antibody; MTD, maximum tolerated dose; NO, nitric oxide; NOS, nitric oxide synthase; OIR, oxygen-induced retinopathy; PDGF-C, platelet-derived growth factor C; PDGFRβ, platelet-derived growth factor receptor beta; PD-1, programmed death 1; PlGF, placental growth factor; PNET, pancreatic islet neuroendocrine tumors; RT, radiation therapy; RTK, receptor tyrosine kinases; sema 3A, semaphorin 3A; Treg, regulatory T cells; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; VDA, vascular disrupting agent; VEGF, vascular endothelial growth factor A (VEGF-A); VEGF-B, vascular endothelial growth factor B; VEGF-C, vascular endothelial growth factor C; VEGF-D, vascular endothelial growth factor D; VEGFR-1, vascular endothelial growth factor receptor 1; VEGFR-2, vascular endothelial growth factor receptor 2; VEGFR-3, vascular endothelial growth factor receptor 3; VPF, vascular permeability factor (VPF).

* Corresponding author at: Eli Lilly and Company, 450 East 29th Street, New York, NY, USA.
E-mail address: bronek.pytowski@lilly.com (B. Pytowski).

http://dx.doi.org/10.1016/j.pharmthera.2016.06.001

1. Introduction

Angiogenesis inhibitors have been approved for the treatment of a wide range of cancer types and for some ocular diseases. Many patients have been treated with these agents for local and metastatic solid tumors worldwide. Anti-angiogenic agents in clinical use can be broadly divided into two categories: 1. tyrosine kinase inhibitors (TKIs) which are chemical agents that inhibit the kinase activity of receptor tyrosine kinases (RTKs) and 2. protein biological agents. The latter category includes the monoclonal antibody (mAb) bevacizumab that blocks the function of vascular endothelial growth factor A (VEGF-A; VEGF hereafter) and ziv-aflibercept, a recombinant fusion protein that as a decoy VEGF receptor binds VEGF, VEGF-B and placental growth factor (PlGF). Recently, a novel biologic agent, ramucirumab, has been approved for use in a number of cancer indications. Ramucirumab is a human IgG1 mAb that selectively inhibits the vascular endothelial growth factor receptor 2 (VEGFR-2) and blocks the signaling pathways in endothelial cells (ECs) that mediate angiogenesis. Our aim is to describe in detail the development of ramucirumab and the extensive pre-clinical data that provided the rationale for targeting VEGFR-2 with this mAb. While maintaining this focus, we attempt to balance our analysis by including discussion of clinical and pre-clinical data generated with other agents that target the VEGF/VEGFR-2 pathway.

1.1. Introduction to angiogenesis

Angiogenesis is the biological process by which new blood vessels develop from pre-existing ones. The formation of new capillaries through angiogenesis allows for the expansion of the blood vessel network into newly created and avascular tissues. Angiogenesis is distinct from vasculogenesis, which is the de novo formation of a vascular plexus from endothelial progenitor cells (angioblasts). While both processes expand the blood vasculature, they do so through very different mechanisms. Vasculogenesis occurs primarily during early embryogenesis via a pre-programmed developmental pattern, while angiogenesis is triggered by localized biochemical cues in growing tissues. Together, these two processes accomplish the multiplicity of exquisitely orchestrated steps that create the vascular network. These steps include de novo differentiation of ECs, specification of arteriovenous fate of these cells, assembly of ECs into cords and the development of a vascular lumen. Subsequently, EC activation, tip cell formation and stalk cell proliferation lead to angiogenic sprouting, branching, capillary anastomoses and association with mural cells that establish functional and perfused vasculature. As may be expected, the spatial and temporal control of these events is under control of a myriad of molecular pathways [reviewed in (Potente et al., 2011)]. It is important, in the context of this review, to keep in mind that the VEGF/VEGFR-2 pathway is a vital but by no means sole driver of the angiogenic process.

1.1.1. Angiogenesis in normal development

Ordered expansion of the vascular network is essential throughout normal growth and development [reviewed in (Domigan & Iruela-Arispe, 2012; Tung et al., 2012)]. Angiogenesis is driven primarily as a response to the formation of oxygen gradients in tissues [reviewed in (Semenza, 2007)]. Cells residing in regions beyond the oxygen diffusion limits (100–200 μm) become hypoxic and initiate the secretion of pro-angiogenic growth factors and cytokines to recruit new blood vessels via angiogenesis [reviewed in (Torres Filho, Leunig et al., 1994; Semenza, 2007)]. During pregnancy, an initial primitive vascular plexus is established to provide nutrients to the developing embryo [reviewed in (Chen & Zheng, 2014)]. These earliest events of placental vascularization are marked by vasculogenesis, whereas by the third trimester of pregnancy placental angiogenesis drives an expansion of the primitive vascular network to create a more sophisticated and functional blood vessel network to meet the needs of the growing fetus. Within the developing embryo, angiogenesis is the major mode of production of new blood vessels within the brain and kidney and also works in concert with vasculogenesis in the developing lungs [reviewed in (Baldwin, 1996)]. Postnatally, angiogenesis has been shown to be required in the expanding blood vessel networks in expanding tissues during growth, particularly in the epiphysis (growth plate) [reviewed in (Hall et al., 2006)] and in the endometrium of post-pubertal females during menstruation [reviewed in (Jabbour et al., 2006)].

1.1.2. Angiogenesis in wound healing

Quiescent vasculature is a hallmark of homeostasis in normal adult tissues with the exception of the female reproductive organs. However, the process of angiogenesis may be robustly activated under a variety of tissue stresses and in diverse disease states. Acute tissue injury initiates a wound healing program marked by discrete phases of hemostasis, humoral inflammation, cellular inflammation, angiogenesis, and generation of mature connective tissue stroma [reviewed in (Dvorak, 2015)]. Angiogenesis during wound healing is driven primarily through the action of VEGF secreted from hypoxic cells in the inflammatory microenvironment. VEGF stimulates the sprouting of new vessels as well as induction of hyperpermeability, allowing for the increased passage of proteins (in particular fibrin and other clotting factors) and inflammatory cells (polymorphonuclear granulocytes, lymphocytes, monocytes, and fibroblasts). The complex actions of these factors and cell types result in both the reconstitution of the vasculature and "sealing" of the wound by fibrin deposition and stromal generation. Over time, the newly created vessels and desmoplastic stroma in the healing wound site gradually resolve back into a highly ordered and stable quiescent tissue, re-establishing a homeostatic balance.

1.1.3. Pathological angiogenesis

Many pathological conditions are characterized by aberrant angiogenesis. Insufficient or reduced angiogenesis has been demonstrated in diseases such as amyotrophic lateral sclerosis (ALS), diabetic ulcers, Crohn's disease, lupus, preeclampsia and coronary artery disease. Conversely, chronic activation or over-stimulation of angiogenesis is a hallmark in diabetic complications, arthritis, psoriasis, inflammatory bowel disease, endometriosis, and cancer [reviewed in (Carmeliet, 2005)].

Tumor blood vessels are torturous, hyperpermeable, and highly heterogeneous both morphologically and with regards to efficiency of tissue perfusion [reviewed in (Huang, Goel et al., 2013)]. Tumor angiogenesis was thrust into the limelight in the early 1970s when Judah Folkman proposed a hypothesis that angiogenesis is essential for tumor growth and that tumors secrete "tumor angiogenic factors" (TAFs) that induce the development of new blood vessels [reviewed in (Folkman, 1971, 1975)]. In retrospect, the relationship of tumor cells and vasculature is considerably more complicated. Solid tumors originate in proximity to pre-existing tissue vasculature. Many primary tumors initially grow along such tissue blood vessels, a phenomenon called vessel co-option. This phenomenon may be particularly important for the growth of metastatic lesions that frequently occur in highly vascularized tissues such as the lung and liver [reviewed in (Donnem et al., 2013)]. The Folkman hypothesis becomes valid when a tumor reaches a size where its further growth becomes dependent on recruitment of new blood vessels, an event referred-to as the "angiogenic switch" [reviewed in (Shaked, McAllister et al., 2014; Semenza, 2013)].

Growth of a tumor beyond the limits of efficient oxygen diffusion through the interstitial fluid, results in regions of hypoxia, nutrient depletion and metabolic imbalance. These conditions result in the production of various factors (TAFs) by both tumor cells and the associated stromal components. In the early 1980's the first of these TAFs were identified from tumors. Shing and Klagsbrun (Shing et al., 1984) purified a factor from a chondrosarcoma which later became known as basic fibroblast growth factor (bFGF) while Senger and Dvorak (Senger et al., 1983) purified a factor they called vascular permeability factor (VPF) from an ovarian tumor. A few years later the Ferrara

laboratory isolated a pro-angiogenic protein from bovine pituitary follicular cells that they termed VEGF (Ferrara & Henzel, 1989), which was found to be the same factor as VPF. Shortly thereafter, the Ferrara laboratory cloned the gene for VEGF (Leung et al., 1989). In a pivotal finding, the laboratory of Eli Keshet demonstrated that VEGF gene expression was stimulated by hypoxia (Shweiki et al., 1992). Mechanistically, this finding was explained by the discovery that the VEGF gene is under direct control of hypoxia inducible factor 1 (HIF) (Liu et al., 1995; Forsythe et al., 1996). Together, these findings ushered in a new era of understanding in the interplay between tumor growth, metabolism and angiogenesis [reviewed in (Hanahan & Weinberg, 2000)]. It should be noted that tumor hypoxia has been hypothesized to be a consequence of rapidly growing tumors outgrowing their vascular supply and the inherent inefficiency of the abnormal tumor vasculature, which we explore further in subsequent sections.

VEGF is regarded as the key pro-angiogenic factor that drives tumor angiogenesis [reviewed in (Ferrara, 2002)]. However, unlike the situation that exists during development and wound healing, VEGF expression is highly deregulated in primary tumors and in metastatic lesions. In tumors, VEGF is expressed at high levels and by a multiplicity of cell types including cancer cells, tumor stroma and invading myeloid cells leading to EC hyperproliferation and loss of the guidance mechanisms of angiogenic sprouting. Consequently, under conditions of VEGF over-expression, sprouting angiogenesis may only partially drive the development of a tumor vascular bed. In a series of pivotal experiments, the laboratory of Harold Dvorak used a reductionist model in which a range of blood vessel subtypes that morphologically resemble those of human tumors are generated in rodent tissues by the administration of adenovirus engineered to express murine $VEGF_{164}$ (Pettersson et al., 2000). The initial angiogenic response involves generation of enlarged vessels ("mother" vessels) (MVs) with poor pericyte coverage that seem to originate by hypertrophy of pre-existing vessels rather than by sprouting. With time, "mother" vessels transform to other vessel types such as glomeruloid malformations (GMP), vascular malformations (VM), feeding arteries (FA) and draining veins (DV). It is of major interest that the late-forming vessel types such as VMs, FAs and DVs express lower levels of VEGFR-2 than the early MVs and GMPs and exhibit significant resistance to anti-VEGF therapy (Sitohy et al., 2011).

1.2. Vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 family

While the past three decades have yielded an incredible wealth of information about the numerous mediators of angiogenesis, the activation of VEGFR-2 by VEGF is overwhelmingly regarded as the most critical driver of tumor angiogenesis [reviewed in (Hicklin & Ellis, 2005)]. VEGF has been shown to be expressed at high levels in many different types of carcinomas. Dozens of reviews have covered the role of VEGF/VEGFR signaling and its importance in tumor angiogenesis. As such, it remains the most studied pro-angiogenic pathway and target of anti-angiogenic therapeutic intervention. Here we will provide only a cursory review of the most salient features of the VEGF/VEGFR pathways.

1.2.1. Introduction to the vascular endothelial growth factor A/vascular endothelial growth factor receptor family

The VEGF family consists of 5 ligands (PlGF, VEGF-A, VEGF-B, VEGF-C, and VEGF-D) that bind to a family of 3 receptors (VEGF receptor-1 [VEGFR-1], VEGFR-2 and VEGFR-3) [reviewed in (Jeltsch et al., 2013; Shibuya, 2013a, 2013b)]. A considerable amount of cross-talk exists between ligand and receptor binding, with the most relevant and well-studied interactions to be VEGFR-1 binding to PlGF, VEGF-B and VEGF-A, VEGFR-2 binding to VEGF-A, VEGF-C and VEGF-D and VEGFR-3 binding to VEGF-C and VEGF-D (Fig. 1). VEGFR-1 is widely expressed on a variety of cell types, including tumor cells, ECs and monocytes. Signals mediated through VEGFR-1 are involved in angiogenesis (via PlGF and VEGF), immune cell recruitment (via VEGF), and fatty acid uptake (via VEGF-B). VEGFR-3 is highly expressed on lymphatic endothelium and binding of VEGF-C and VEGF-D to this RTK mediates the process of lymphangiogenesis. VEGFR-2 expression is primarily limited to ECs and signaling of VEGF through VEGFR-2 is the major driver of angiogenesis, although there is some data to suggest that VEGF-C and VEGF-D are also involved in VEGFR-2 mediated angiogenesis [reviewed in (Chen et al., 2012)] and (Jauhiainen et al., 2011). Although detailed discussion is beyond the scope of this review, expression of VEGFR-2 has also been reported on bone-marrow derived circulating cells and on subsets of myeloid and lymphoid leukocytes.

1.2.2. Vascular endothelial growth factor receptor 2 activation in angiogenesis

Shortly after the discovery of VPF/VEGF-A (Senger et al., 1983; Leung et al., 1989) and its primary receptor VEGFR-2 (originally identified as FLK-1, KDR in humans; (Matthews et al., 1991; Millauer et al., 1993), indispensable roles for this pathway in developmental and pathological angiogenesis were elucidated. Genetic inactivation (knockout) of either VEGF (Carmeliet et al., 1996; Ferrara et al., 1996) or VEGFR-2 (Shalaby et al., 1995) cause early embryonic lethality (E8.5-E9.5) as a result of defective hematopoietic and EC development and lack of establishment of blood islands. The vital importance of VEGF is highlighted by the fact that loss of even a single allele disrupts embryonic vascular development leading to lethality (Carmeliet et al., 1996; Ferrara et al., 1996). Knockout or knockdown of VEGF in tumor cells prevents or significantly delays their ability to establish and/or grow in preclinical models.

Binding of VEGF to VEGFR-2 initiates canonical signaling pathways similar to other RTKs [reviewed in (Koch & Claesson-Welsh, 2012; Shibuya, 2013a, 2013b; Domigan et al., 2015)]. The autophosphorylation of the VEGFR-2 kinase domain (Y1504 and Y1509) is one of the earliest events upon VEGF binding and is critical for activation of the kinase and subsequent phosphorylation events on the VEGFR-2 receptor. Numerous small molecule inhibitors have been developed to inhibit VEGFR-2 kinase activity and many of these have been developed as effective drugs in the clinic to treat various cancers. However, a vast majority of these inhibitors have potent activities against many other RTKs because of the structural similarities of the kinase domains within this family.

Several other tyrosine residues on VEGFR-2 outside of the kinase domain are phosphorylated in response to VEGF. The most important of these include Y951, Y1175 and Y1214, which have critical roles in mediating endothelial permeability (Y951), endothelial proliferation (Y1175), and endothelial migration (Y951, Y1175, and Y1214). The phosphorylation of these residues allows for other signaling mediators such as TSAD, SHB, SHC, PLCγ, GRB2 and NCK to bind and recruit additional factors. The establishment of these intracellular complexes on VEGFR-2 culminates in the activation of canonical pathways such as PKC, RAS/RAF/ERK/MAPK, and PI3K [reviewed in (Koch & Claesson-Welsh, 2012)].

While VEGF-C and VEGF-D have been demonstrated to bind and signal through VEGFR-2, their knockout phenotypes suggest they have more important roles in lymphangiogenesis. VEGF-C null mice die embryonically at E15.5–E17.5 as a result of impaired lymphatic vessel development and edema (Karkkainen et al., 2004). VEGF-D null mice are healthy and fertile with only minor effects noted on lymphatic development (Baldwin et al., 2005). Knockout of either VEGF-C and VEGF-D or compound deletion of both genes did not result in any significant effects on blood vessel development (Haiko et al., 2008), suggesting that VEGF is sufficient to promote angiogenesis through VEGFR-2 during development. However, it is not clear whether in pathological states VEGF-C alone can sufficiently activate VEGFR-2 to maintain angiogenesis under conditions where VEGF is potently neutralized. Experimental resolution of this question would be instrumental in

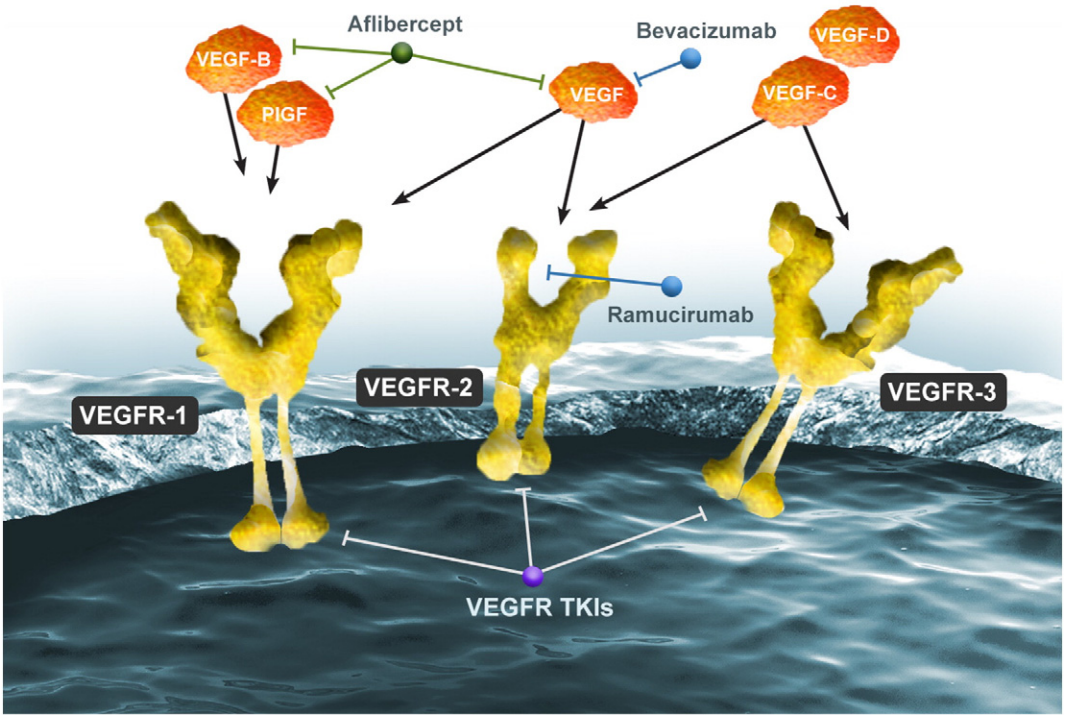

Fig. 1. The VEGF family and its inhibitors. There is considerable crosstalk within the VEGF family which consists of 5 ligands (PlGF, VEGF-A, VEGF-B, VEGF-C, and VEGF-D) that can bind to 3 different receptors (VEGFR-1, VEGFR-2 and VEGFR-3). VEGF binds to VEGFR-2 or VEGFR-1, PlGF and VEGF-B to VEGFR-1, and VEGF-C and VEGF-D to VEGFR-2 or VEGFR3. Many of the TKIs non-selectively inhibit the VEGF receptors. Large molecule biologic inhibitors are more selective and inhibit either VEGF pathway ligands (bevacizumab/B20/G6 or aflibercept) or receptors (ramucirumab/DC101). Bevacizumab (or mouse B20 or G6) inhibits all of the VEGF isoforms, while aflibercept targets the VEGF isoforms plus PlGF and VEGF-B. Ramucirumab (or mouse DC101) selectively inhibits the binding of VEGF, VEGF-C, and VEGF-D to VEGFR-2.

clearly delineating whether the effects of inhibiting VEGF can be differentiated from the effects of inhibiting VEGFR-2.

2. Introduction to therapeutic intervention in cancer

Historically, development of clinical agents that inhibit VEGF/VEGFR-2 signaling pathway began with generation of neutralizing antibodies to VEGF. Subsequently, TKIs, small molecular entities that inhibit intrinsic tyrosine kinase activity of RTKs were introduced. These agents were followed by novel biologic moieties such as VEGF-Trap and, most recently by an antagonist antibody to VEGFR-2. These agents are discussed in greater detail below.

2.1. Receptor tyrosine kinase inhibitors

A detailed discussion of TKIs is beyond the scope of this article and has been extensively reviewed elsewhere (Gotink & Verheul, 2010). Briefly, anti-angiogenic multi-targeted TKIs are small molecule inhibitors with potent inhibitory activity at the catalytic binding site on the VEGFR-2 intracellular domain. These include competitive inhibitors such as sunitinib, allosteric inhibitors such as sorafenib and covalent inhibitors such as vandetanib. Most of the first generation anti-angiogenic TKIs such as sunitinib, sorafenib and pazopanib have adverse effects unrelated to efficient VEGF blockade as they inhibit a wide range of kinase targets such as PDGFRs, c-kit, Flt3, RET, CSF1R, B-Raf in addition to the VEGFRs. The second generation anti-angiogenic TKIs such as axitinib, tivozanib, cediranib have improved potency and selectivity for VEGFRs [reviewed in (Bhargava & Robinson, 2011)]. Several of these TKIs have been approved by the FDA in solid tumors including metastatic renal cell carcinoma (Motzer et al., 2009), gastrointestinal stromal tumors (Demetri et al., 2006), pancreatic neuroendocrine tumors (PNET) (Raymond et al., 2011), unresectable hepatocellular carcinoma (Llovet, Ricci et al., 2008), advanced soft tissue sarcoma (Ranieri et al., 2014) and advanced medullary thyroid cancer (Duda, 2012).

2.2. Bevacizumab

Bevacizumab (Avastin) is a humanized anti-VEGF mAb which binds to and neutralizes all human VEGF isoforms and proteolytic fragments. It does not neutralize other members of the VEGF family [reviewed in (Ferrara et al., 2004)]. Currently bevacizumab has been approved as a monotherapy for recurrent glioblastoma (Vredenburgh et al., 2007), in combination with chemotherapy for metastatic colorectal cancer (Hurwitz et al., 2004), metastatic non-squamous non-small cell lung cancer (Sandler et al., 2006), platinum resistant ovarian cancer (Stockler et al., 2014) and metastatic cervical cancer (Penson et al., 2015) and in combination with IFNα for metastatic renal cell carcinoma (Escudier et al., 2010).

2.3. Aflibercept

Aflibercept, also known as VEGF-trap (aflibercept hereafter), is a recombinant decoy receptor fusion protein created by fusion of domain 2 of vascular endothelial growth factor receptor-1 (VEGFR-1) and domain 3 of VEGFR-2 with the Fc portion of human IgG1. It binds or "traps" the different isoforms of VEGF as well as VEGF-B, and PlGF [reviewed in (Gaya & Tse, 2012; Papadopoulos et al., 2012; Ciombor & Berlin, 2014)]. Aflibercept is reported to have a higher affinity for VEGF than VEGFR-2 or bevacizumab (Papadopoulos et al., 2012). In addition, unlike bevacizumab, aflibercept is able to target PlGF which has been reported to be increased in response to other anti-VEGF therapies and may play a role in the development of resistance to these drugs (Rini et al., 2008; Willett et al., 2009). Aflibercept has been approved

in combination with chemotherapy for 2nd line treatment of metastatic colorectal cancer (Van Cutsem et al., 2012).

2.4. Ramucirumab

Ramucirumab (IMC-1121B) is a fully-human IgG1 mAb that binds to the ligand-binding site of VEGFR-2 and prevents the activation of this RTK. Its development is discussed in detail below. Following an extensive clinical testing program (Table 1), ramucirumab received approval for use as a monotherapy and in combination with chemotherapy in metastatic gastric or gastroesophageal (GE) junction adenocarcinoma, in combination with chemotherapy for metastatic non-small cell lung cancer and for metastatic colorectal cancer [reviewed in (Calvetti, Pilotto et al., 2015)].

2.4.1. The development of ramucirumab

The development of therapeutic mAbs that antagonize human VEGFR-2 has been previously reviewed (Clarke & Hurwitz, 2013). Initially, a phage display-derived mouse/human chimeric IgG1 mAb cP1C11(IMC-1C11) was produced [reviewed in (Hunt, 2001)]. IMC-1C11 was extensively characterized using both cell-free and in vitro cell-based models (Zhu et al., 1998; Tille et al., 2003; Persaud et al., 2004). The in vivo results with IMC-1C11 are discussed in subsequent sections. This mAb entered a dose-escalating phase I clinical trial in 2000 in patients with liver metastatic colorectal cancer (Posey et al., 2003) but its further clinical development was not pursued. Subsequently, a fully-human mAb to human VEGFR-2, was produced by affinity maturation of a parent antagonist mAb to human VEGFR-2 called 2C6 (Lu et al., 2003). 2C6 was identified for high affinity binding to human VEGFR-2 utilizing a naive phage display library of human Fab fragments (Lu et al., 2002). Affinity maturation using chain shuffling in association with a tailored selection process identified a clone designated 1121. 1121 Fab and IgG forms showed 36- and 4-fold higher affinity for VEGFR-2 relative to the parental molecule 2C6, respectively and significantly enhanced the ability of the mAb to inhibit the interaction of VEGF with human VEGFR-2 (Lu et al., 2003; Zhu et al., 2003). 1121 (IMC-1121B; ramucirumab hereafter), bound to immobilized human VEGFR-2 (KDR) with an EC_{50} of 0.16 nM (B. Pytowski, Eli Lilly, unpublished data) and blocked VEGF/VEGFR-2 interaction with an IC_{50} of 0.8 nM (Lu et al., 2003). As determined by surface plasmon resonance on a BIAcore instrument, ramucirumab showed an overall affinity of 11–50 pM (Lu et al., 2003) (B. Pytowski, Eli Lilly, unpublished data). In in vitro cell-based functional assays, ramucirumab inhibited VEGF-stimulated VEGFR-2 activation and proliferation of human ECs as well as VEGF stimulated VEGFR-2 phosphorylation in both human umbilical vein ECs (HUVEC) and porcine aortic ECs transfected with human VEGFR-2 (PAE-KDR cells) (Lu et al., 2003; Zhu et al., 2003). In addition, ramucirumab inhibited VEGF-induced migration of human leukemia cells (Zhu et al., 2003). Co-crystallization of ramucirumab with the Ig domain 3 of human VEGFR-2 allowed the definition of the epitope of this mAb at high resolution and provided structural explanation for its antagonism of VEGF binding (Franklin et al., 2011). In vivo testing of IMC-1C11 and ramucirumab was difficult because these mAbs do not recognize murine VEGFR-2. However, both antibodies prolong the survival of immunodeficient mice inoculated with human leukemia cells that express VEGFR-2 suggesting that the growth of these cells is at least in part driven by autocrine or paracrine production of VEGF (Dias et al., 2000, 2001; Zhu et al., 2003).

VEGFR-2 is also activated by vascular endothelial growth factors C and D (VEGFs C and D) when these factors are proteolytically processed into their mature forms [reviewed in (Lohela et al., 2009)]. Structural studies predicted that conserved residues in VEGF-C and VEGF-D mediate binding to VEGFR-2 (Leppanen et al., 2010, 2011) in immunoglobulin-like (Ig) domains 2 and 3 also known to mediate the binding of VEGF (Ruch et al., 2007). In accordance with these findings, ramucirumab inhibited the binding of VEGF, VEGF-C and VEGF-D to soluble extracellular domain of human VEGFR-2 in a dose-depended manner and respective IC_{50} values reflected the difference in affinities of various ligands for VEGFR-2 (VEGF > VEGF-C > VEGF-D) (B. Pytowski, Eli Lilly, unpublished data). Ramucirumab prevented VEGF-C from binding to human VEGFR-2, and inhibited VEGF-C-induced activation of VEGFR-2 in both vascular and lymphatic ECs (Goldman et al., 2007; Tvorogov et al., 2010). Ramucirumab has also been shown to potently inhibit sprouting and proliferation of ECs following stimulation with VEGF-C (Miao et al., 2006; Tvorogov et al., 2010), and block VEGF-C-induced heterodimerization of VEGFR-2 and VEGFR-3 (Nilsson et al., 2010). The ability of ramucirumab to block all three VEGFR-2 ligands invites speculation whether it might offer therapeutic advantages over agents that neutralize VEGF. However, to date, no pre-clinical or clinical evidence has emerged to support this claim. A related unaddressed question is whether a combination of anti-VEGF and anti-VEGFR-2 agents may offer a benefit exceeding that of either agent alone.

Table 1
Completed Phase III Ramucirumab trials.

Trial	Indication	Design	Primary objective	Result	Ref.
REVEL	NSCLC[a] Second-line (N = 1253)	Docetaxel (75 mg/m^2) (60 mg/m^2 Korea/Taiwan) ± ramucirumab (10 mg/kg) q 3 weeks	Overall survival	Met	Garon, Ciuleanu et al., 2014
REGARD	G-OJ[b] Second-line (N = 355) 2:1	Ramucirumab (8 mg/kg) q 2 weeks vs. Placebo	Overall survival	Met	Fuchs, Tomasek et al., 2014
RAINBOW	G-OJ[b] Second-line (N = 665)	Paclitaxel (80 mg/m^2) ± ramucirumab (8 mg/kg) q 2 weeks	Overall survival	Met	Wilke, Muro et al., 2014
REACH	HCC[c] Second-line, post-sorafenib (N = 544)	Ramucirumab (8 mg/kg) q 2 weeks vs. placebo	Overall survival	Not met	Zhu et al., 2015
RAISE	mCRC[d] Second-line, FOLFOX/Bev Res (N = 1050)	FOLFIRI ± ramucirumab (8 mg/kg) q 2 weeks	Overall survival	Met	Tabernero, Yoshino et al., 2015
ROSE	mBC[e] First-line (N = 1144) 2:1 Ram:Dox	Docetaxel (75 mg/m^2) ± ramucirumab (10 mg/kg) q 3 weeks	Progression-free Survival	Not met	Mackey, Ramos-Vazquez et al., 2015

[a] Non-small-cell lung cancer.
[b] Gastric or gastro-oesophageal junction adenocarcinoma.
[c] Hepatocellular carcinoma.
[d] Metastatic colorectal carcinoma.
[e] Metastatic breast carcinoma.

3. Preclinical targeting of the vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 axis

Murine models of cancer, whether syngeneic or xenogeneic, depend on angiogenic expansion of the murine vasculature which, in turn, is partly supported by murine stroma. As described below, the need to accurately model in mice clinical targeting of either VEGF or VEGFR-2 with biologic agents presented researchers with significant technical challenges that were, to a significant degree, overcome through the use of surrogate, "proof-of-concept" antibodies.

3.1. The development of biologic reagents for pre-clinical targeting of vascular endothelial growth factor A and vascular endothelial growth factor receptor 2

Recent detailed reviews have outlined the preclinical studies of antibodies targeting VEGF (Crawford & Ferrara, 2009; Bagri et al., 2010) and aflibercept (Gaya & Tse, 2012; Ciombor & Berlin, 2014). Although bevacizumab and other mAbs to human VEGF decrease tumor growth in human tumor xenograft models, these agents can only inhibit the tumor derived VEGF (Crawford & Ferrara, 2009) but cannot target VEGF produced by mouse stroma, which has been shown to play an important part in the angiogenesis process (Gerber et al., 2000; Liang et al., 2006; Yu et al., 2008). Thus, to faithfully model in mice the therapeutic activity of VEGF blockade in patients, anti-VEGF mAbs must neutralize both human and mouse VEGF. To this end, two cross-species reactive function-blocking antibodies targeting VEGF (B20.4-1 and G6-31; B20 and G6 hereafter) have been developed (Liang et al., 2006). These antibodies neutralize VEGF with no detectable activity towards other VEGF-family members such as PlGF, VEGF-B, VEGF-C, or VEGF-D (Liang et al., 2006). In contrast, aflibercept binds to and inhibits both human and mouse VEGF and PlGF.

Even more dramatically than in the case of bevacizumab, the complete absence of cross-reactivity of IMC-1C11 and ramucirumab with murine VEGFR-2 meant that these mAbs could not be tested at all for anti-angiogenic activity in mice. Instead, the validity of targeting VEGFR-2 in cancer and other pathological conditions has been established almost exclusively with a rat anti-mouse VEGFR-2 mAb, DC101. Although DC101 is frequently described as having been made to provide a surrogate ("proof-of-concept") mAb for ramucirumab, its production and initial characterization predate ramucirumab by several years (Rockwell et al., 1995). While complete in vitro characterization of DC101 has not been published, binding experiments have shown that DC101, but not ramucirumab, binds to mouse VEGFR-2 (Flk-1) with an EC_{50} of about 0.28 nM. The kinetics of DC101 binding to mouse VEGFR-2 (Flk-1) were determined by surface plasmon resonance on a BIAcore instrument. The binding affinity (Kd) was established to be 0.11 nM, between 5 and 10-fold lower than the corresponding affinity of ramucirumab for human VEGFR-2 (Lu et al., 2003) (B. Pytowski, Eli Lilly, unpublished data). Specificity of DC101 for VEGFR-2 has also been demonstrated. DC101 did not bind to mouse VEGFR-1 (Luttun et al., 2002) (B. Pytowski, Eli Lilly, unpublished data). Similar to ramucirumab, DC101 inhibited the binding of VEGF and VEGF-C to mouse VEGFR-2, but was unable to inhibit binding of VEGF-C to mouse VEGFR-3 (Pytowski et al., 2005). DC101 was not tested as an antagonist of VEGF-D binding to VEGFR-2 since mouse VEGF-D binds only to VEGFR-3.

3.2. Effect of vascular endothelial growth factor receptor 2 inhibition on physiological angiogenesis and vascular function

In vivo target engagement by DC101 was demonstrated when systemic administration of DC101 to either normal or tumor-bearing mice was found to significantly raise plasma concentration of VEGF, in accord with the concept that ligand-induced receptor internalization represents a key mechanism of ligand clearance. (Bocci, Man et al., 2004). Of interest, in vivo treatment of either naïve or tumor-bearing mice with DC101 also leads to elevated plasma levels of PlGF through an as yet unknown mechanism raising the possibility that this factor might be a useful pharmacodynamic marker of ramucirumab exposure in patients (Fischer et al., 2007; Bais et al., 2010).

The availability of selective reagents that block the VEGF/VEGFR-2 pathway has been utilized to study the role of physiological angiogenesis in development. One of the first events in embryogenesis is vasculoneogenesis in which ECs differentiate from precursor cells, termed angioblasts. Experimentally, these events can be recapitulated in cultures of embryoid bodies derived from pluripotent murine embryonal stem cells (ESM). Using this approach, it was demonstrated that signaling through endothelial fibroblast growth factor receptor 1 (FGFR-1) was insufficient for capillary plexus formation from embryoid bodies if VEGFR-2 activation was prevented with either DC101 or antibodies to VEGF (Magnusson et al., 2004).

The use of DC101 in post-natal mice also facilitated the elucidation of the importance of VEGFR-2 signaling in organogenesis. DC101 potently inhibited physiological angiogenic sprouting in the retinas of neonatal mice (Tammela et al., 2008; Benedito, Rocha et al., 2012) and mice treated with DC101 in the perinatal period demonstrated impaired alveolization of the lungs (McGrath-Morrow, Cho et al., 2005). In a related finding, the extensive remodeling of the primitive vascular plexus of embryonic mouse tracheas that is initiated after birth was completely prevented by administration of DC101 (Ni, Lashnits et al., 2010) and the development of kidneys was disrupted by administration of DC101 leading to formation of cysts, abnormal glomeruli and consequent proteinuria (McGrath-Morrow, Cho et al., 2006). VEGFR-2 signaling has also been demonstrated to play an important role in adipogenesis and in the sensitivity of fat to insulin. Administration of DC101 reduced angiogenesis and adipose tissue growth and inhibited preadipocyte differentiation by disrupting paracrine interaction between ECs and preadipocytes (Fukumura, Ushiyama et al., 2003). Similarly, DC101 and anti-VEGF antibodies led to changes of adipose tissue vascularization, adipocyte sizes, and insulin sensitivity although the effect varied depending on the age of the mice (Honek, Seki et al., 2014). Furthermore, in experiments on the role of angiogenesis on early pregnancy in the mouse, DC101 has been shown to block the growth of uterine decidua and the development of corpora lutea (Pauli et al., 2005; Douglas et al., 2009). Similarly, IMC-1C11 and aflibercept inhibited ovary follicle development, a process dependent on angiogenesis, during the menstrual cycle in monkeys (Zimmermann et al., 2001; Taylor et al., 2007). These studies demonstrated the crucial role of VEGFR-2 in mediating post-natal angiogenesis that is crucial for normal organ development and the maintenance of pregnancy.

The VEGF-VEGFR-2 autocrine loop has also been invoked in the differentiation of murine ESC, hematopoiesis, and the generation of red blood cells. Either genetic knock-down of VEGFR-2 or its blockade with the kinase inhibitor (sunitinib) or DC101 in vitro allowed the maintenance of a pluripotent state of ESCs even in the absence of leukemia inhibitory factor (LIF) (Chen et al., 2014b). Hematopoiesis requires VEGFR-2 signaling to support the development of marrow capillaries and sinusoidal lining and stimulate the expansion of VEGFR-2^+ circulating endothelial progenitor cells (CEP). Both events were stimulated by injecting either VEGF or angiopoietin-1 (Ang1) into mice but only VEGF-induced hematopoiesis was inhibited by concurrent administration of DC101 (Hattori et al., 2001). In a related study, potent neutralization of VEGF/VEGFR-2 signaling with either aflibercept or DC101 induced production of red blood cells through elevated production of erythropoietin by the liver. Since hepatocytes do not express VEGFR-2, this phenomenon was ascribed to paracrine signaling between hepatocytes and hepatic ECs (Tam et al., 2006).

Quiescent endothelium is believed to be only minimally dependent on VEGFR-2 signaling. In fact, conditional depletion of VEGFR-2 in ECs of adult mice does not affect the viability of the mice (Ding et al., 2010). Chronic administration of DC101 or anti-VEGF antibodies led to

capillary regression in the intestinal villi, liver and the uterus. Both modes of inhibition resulted in marked reduction in endothelial fenestrations in several endocrine organs (Yang et al., 2013a). Similarly, aflibercept and adenoviral expression of soluble VEGFR-1 or VEGFR-2, which act as decoy receptors, decreased the vasculature in multiple organs. The vasculature of these normal organs were less sensitive to VEGF inhibition than tumor vessels, tended to be fenestrated, and had relatively high expression of VEGFR-2 and VEGFR-3 (Kamba et al., 2006). Together, these results indicate that the effects of VEGF pathway inhibition on normal organs are a class-specific consequence of inhibiting VEGFR-2 function [reviewed in (Cao, 2014)] and that dependence on VEGF for vascular stability is variable from one vascular bed to another. The changes imparted by VEGF/VEGFR-2 blockade on normal endothelium are likely to mediate the adverse events associated with the use of anti-angiogenic therapeutics in humans [reviewed in (Kamba & McDonald, 2007)].

Formation of new vessels is a crucial component of re-establishment of tissue homeostasis following injury. Organ regeneration offers one of the best examples of this relationship. In a mouse model, liver regeneration and hepatic cell proliferation were reduced to a modest but statistically-significant extent by systemic administration of DC101, concomitant with reduction in the activation of liver endothelium (Van et al., 2008). In contrast, while systemic administration of VEGF accelerated compensatory right lung growth following left lung pneumonectomy, co-administration of DC101 or the anti-VEGFR-1 mAb MF1 had no inhibitory effect suggesting VEGF acted through a yet undefined mechanism (Sakurai et al., 2007).

Interestingly, VEGFR-2 signaling is also crucial for the regeneration of the bone marrow. Bone marrow sinusoidal endothelial cells (SECs) express both VEGFR-2 and VEGFR-3. However, in a SEC injury model induced by lethal irradiation, engraftment of transplanted bone marrow was severely impaired by systemic blockade with DC101 but not by mAb mediated inhibition of VEGFR-3 demonstrating the central role of VEGFR-2 in the regeneration of sinusoidal endothelium (Hooper et al., 2009). It is of interest to note in the context of discussing tissue regeneration that while the formation of the tumor vascular bed is thought to primarily result from angiogenesis, considerable evidence suggests that, in addition, bone marrow-derived VEGFR-2$^{(+)}$ CEPs can be found in the circulation of mice. CEPs are able to incorporate into distal sites of developing blood vessels and differentiate into mature ECs, a process termed adult vasculoneogenesis. This has been demonstrated in models of tissue revascularization following experimentally induced limb ischemia. In these models, VEGF has been shown to be produced by various leukocytes that invade the ischemic tissue and the VEGF-induced tissue vascularization can be blocked by inhibition of VEGFR-2 (Heissig et al., 2005; Ohki et al., 2005).

In addition to its important role in angiogenesis and vascular homeostasis, the VEGF pathway has been shown to regulate vascular permeability and blood pressure. VEGF was originally discovered as a VPF (Senger et al., 1986) and this activity is now known to be mediated through the activation of VEGFR-2. Mice deficient in β3-integrin show enhanced VEGF-mediated permeability. In this model, administration of DC101 abolished the vascular leakage and linked enhanced permeability to elevated expression of VEGFR-2 (Robinson et al., 2004). As discussed later, loss of endothelial integrity due to excess VEGF is also a hallmark of many pathological processes. Furthermore, administration of DC101 to normal mice revealed that VEGFR-2 signaling is involved in the maintenance of normal blood pressure by regulating expression of nitric oxide synthases (NOS) in the kidney (Facemire et al., 2009).

4. Effect of vascular endothelial growth factor receptor 2 inhibition in models of acute and chronic tissue injury

Blockade of the VEGF/VEGFR-2 pathway in vivo with highly specific biologics was instrumental in illuminating the crucial role of this receptor in controlling angiogenesis in tissue response to acute or chronic injury. As we discuss below, depending on the pathological setting, angiogenesis can either contribute to tissue damage or to tissue recovery following injury. We begin with models of ocular pathology because of their central importance in elucidating the mechanisms of normal and pathological angiogenesis, and then extend the discussion to other models of tissue injury.

4.1. Mouse models of ocular pathologies

Oxygen-induced retinopathy (OIR) is one of the most commonly used models of pathological angiogenesis. Intravitreal administration of DC101 or bevacizumab resulted in similar reduction in retinal blood vessel proliferation (Hollanders et al., 2015a). Similar observations were obtained using a canine model of OIR with IMC-1C11 or aflibercept (McLeod et al., 2002; Lutty et al., 2011). Another model of vascular ocular pathology is laser-induced choroidal neovascularization (CNV). Blood vessel density and vascular leakage were reduced by intravitreal injections of DC101 in the mouse (Huang et al., 2011; Hollanders et al., 2015b) and by tanibirumab in rat CNV models (Kim et al., 2014). Similar studies with aflibercept or anti-VEGF antibodies illustrate the central role for the VEGF pathway in ocular pathologies (Lutty et al., 2011; Hollanders et al., 2015a, 2015b). Such pre-clinical data spurred the development of biologic therapeutics that target VEGF that have revolutionized the treatment of macular degeneration and other ocular diseases [reviewed in (Zhang et al., 2015)]. A clinical program for ramucirumab in ocular indications has not been initiated.

4.2. Mouse models of tissue injury

Inflammation is the cardinal sign of tissue injury and pro-inflammatory mediators frequently upregulate VEGF production and angiogenesis which, in turn, can potentiate the process by providing a pathway for extravasation of leukocytes and plasma proteins. For example, treatment with DC101 in a model of contact sensitivity reduced both dermal angiogenesis and inflammatory infiltrate (Watanabe et al., 2004). Angiogenesis is clearly required for tissue healing following organ transplantation but can contribute to chronic rejection of allografts. Alloimmune response appears to involve VEGFR-1 expressed by infiltrating myeloid cells and VEGFR-2 expressed on blood endothelium. In a model of cardiac transplantation, combined antibody blockade of VEGF receptors was optimal for reducing inflammation in the allografts with the VEGFR-1 mAb MF1 inhibition reducing the inflammatory infiltrate and DC101 reducing blood vessel density (Raisky et al., 2007). In another study, only the combined inhibition of VEGFR-1 and VEGFR-2 prolonged allograft survival (Sho et al., 2005).

Inflammation is also critically dependent on the function of lymphatic vessels which are major conduits for immune cell trafficking. Inflammatory processes are typically accompanied by the development of new lymphatics known as lymphangiogenesis. Lymphangiogenesis is primarily mediated by VEGF receptor 3 (VEGFR-3) and its cognate ligands VEGFs C and D. However, a role of VEGFR-2 in this process has also been suggested [reviewed in (Tammela & Alitalo, 2010)] and the ability to specifically block this receptor in vivo with DC101 was critical in providing experimental proof for this hypothesis. In a transgenic model of VEGF over-expression in the skin, lymphangiogenesis was strongly induced at sites of skin wound healing and could be inhibited by treatment with DC101 (Hong et al., 2004). Similarly, in a model of skin regeneration in normal mice, only co-neutralization of VEGFR-2 with DC101 and VEGFR-3-specific mAb mF4-31C1 completely prevented formation of functional lymphatic capillaries (Goldman et al., 2007). Furthermore, in a model of lung inflammation induced by persistent infection of mouse trachea with *Mycoplasma*, only combined inhibition of VEGFR-2 and VEGFR-3 prevented the pathological expansion of lung lymphatics (Baluk et al., 2014). Chronic inflammation of the cornea can induce pathological angiogenesis and lymphangiogenesis in this

normally avascular tissue. In a model of corneal suture-induced inflammation, both VEGFR-3 and VEGFR-2 were involved in lymphangiogenesis and significant inhibition was only achieved by a combined mAb blockade (Yuen et al., 2011). These studies demonstrate that VEGFR-2 plays a significant role in lymphangiogenesis during both tissue regeneration and chronic inflammation. Of interest, in both the lung and corneal models of chronic inflammation, lymphangiogenesis was only inhibited in early stages of the pathological processes indicating that established lymphatic vessels become independent of VEGFR-2 and VEGFR-3 signaling.

5. Inhibition of vascular endothelial growth factor receptor 2 in mouse models of tumor angiogenesis

Pre-clinical development of anti-angiogenic drugs provided a rich armamentarium of inhibitors whose use allowed in depth investigation of mechanisms driving tumor angiogenesis (see Section 1). Here, we focus our attention on the contributions made to this body of knowledge through the use of antibodies targeting VEGFR-2, while providing select examples where the neutralization of VEGF with either mAbs or aflibercept was used in similar experimental settings. An important illustration of the Folkman principle that tumors only grow to a few microns without additional support from the vasculature was provided by a study of lung tumor development. As expected, DC101 had no effect on the number of induced lung tumors but significantly reduced the total tumor burden (Karoor, Le et al., 2010). Numerous studies have shown that systemic administration of DC101 potently inhibits the growth of orthotopic and subcutaneous xenograft models (Prewett et al., 1999; Kunkel et al., 2001; Bruns et al., 2002; Yu et al., 2002; Raisky et al., 2007) and models of spontaneous cancer (Izumi et al., 2003; Fenton et al., 2005). We explore the mechanism of this inhibition in greater detail later but, broadly stated, DC101 treatment potently reduces tumor vascularity with vessels in the tumor parenchyma inhibited more readily than those found in the tumor stroma.

Similarly, inhibiting the VEGF pathways with anti-VEGF mAbs or aflibercept also led to rapid and sustainable reductions in tumor vascular density and profound effects on tumor growth in subcutaneous xenografts, orthotopic tumor models, and genetically-engineered murine tumor models (GEM). B20 was tested in over 30 different models with the magnitude of the response varying depending on the tumor model. However, responsiveness was not dependent on tumor histology, growth rate, or VEGF levels (Bagri et al., 2010). Administration of G6, a more potent inhibitor of mouse and human VEGF, effectively decreased (~90%) the growth of multiple tumor models irrespective of how stromalized the xenografts were (Liang et al., 2006). G6 decreased blood volume within 24–48 h in the HM-7 colorectal xenograft model (O'Connor et al., 2009). In syngeneic tumor models, G6 treatment showed varying levels of tumor responsiveness that was dependent on the recruitment of CD11b + Gr1 + myeloid cells (Shojaei et al., 2007). Long-term treatment of multiple GEM and xenograft tumor models showed improved survival with B20 treatment (Bagri et al., 2010) and long-term administration of G6 led to a substantial increase in survival of APC +/min and Men1 mice (Korsisaari et al., 2007, 2008). Similarly, treatment of a diverse array of tumor models in mice with aflibercept resulted in potent inhibition of tumor angiogenesis and, consequently, tumor growth. For example, aflibercept reduced the growth of breast cancer xenografts in a dose dependent manner that was correlated with decreased density of tumor vessels (Le et al., 2008). Aflibercept also decreased vascular density and tumor volume in models of melanoma, rhabdomyosarcoma, and glioma (Holash et al., 2002) as well as in subcutaneous and orthotopic pancreatic cancer models (Fukasawa & Korc, 2004). In an orthotopic murine model of renal cancer, potency of tumor growth inhibition by aflibercept depended on how early the treatment began, a phenomenon commonly seen in murine cancer models and which we address in greater detail below (Verheul et al., 2007). Aflibercept increased the survival of mice bearing intracranial glioma xenografts either when given during the initial onset or during advanced phases of tumor development. Longer treatments (6 weeks vs. 3 weeks) showed even longer survival, however, a resistant phenotype was observed in these long-term surviving mice (Gomez-Manzano et al., 2008).

5.1. Mechanistic aspects of vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 inhibition on tumor blood vessels

The effects of VEGFR-2 inhibition on the genesis of the tumor vasculature are based on the interruption of the various signaling pathways that are activated in ECs following binding of VEGF [reviewed in (Olsson et al., 2006); see also Section 1]. Activated VEGFR-2 mediates several distinct biological responses of ECs including proliferation, survival, and adhesion and migration. In turn, each of these responses is deregulated during the growth of the tumor vasculature.

The important role of VEGFR-2 in EC proliferation was demonstrated in a reductionist model of inducible HIF-1 in the skin of normal mice (Oladipupo et al., 2011). Pathological neovascularization is largely mediated by induction of the HIF family (primarily HIF-1 and HIF-2) which in turn directly upregulates the expression of VEGF. HIF-1 induction in the skin of these mice indeed led to an increase of VEGF and activation of VEGFR-2. Initial stages of angiogenesis were characterized by marked activation of VEGFR-2 and EC proliferation resulting in generation of new capillaries and increase in blood flow. The induction of the new capillary bed was sensitive to DC101. However, after 14 days of HIF-1 expression, EC expansion stopped despite continued activation of VEGFR-2. This more mature vasculature was in turn insensitive to treatment with DC101. Reduction of VEGFR-2 activation with DC101 has also been demonstrated by in situ detection of receptor phosphorylation in whole tumor sections of bladder cancer xenografts (Davis et al., 2004). Furthermore, reduction in the proliferation of tumor ECs has been directly demonstrated in experimental skin papillomas (Beck et al., 2011) and in skin carcinoma heterotransplants (Miller et al., 2005).

While the VEGF/VEGFR-2 axis plays an important role in EC survival; apoptosis of ECs and tumor blood vessel regression has only been demonstrated in a small number of studies (Bruns et al., 2002; Sweeney et al., 2002; Kiessling et al., 2004; Miller et al., 2005; Mancuso et al., 2006). In one model, treatment with DC101 induced apoptosis of tumor vessel endothelium of colorectal cancer metastases to the liver. EC apoptosis preceded initiation of apoptosis in tumor cells (Bruns et al., 2002). Similarly, in a syngeneic model of peritoneal colon carcinomatosis, DC101 prolonged the survival of the mice. Histological assessment showed reduced tumor vascularity and EC apoptosis (Shaheen et al., 2001b). However, in a model of soft tissue sarcoma, DC101 enhanced EC apoptosis induced by low-dose treatment with doxorubicin but did not promote apoptosis as a monotherapy (Zhang et al., 2002).

As already mentioned, while angiogenesis is thought to be the main mechanism of the formation of new blood vessels in adults, vasculoneogenesis has also been observed under pathological conditions. This phenomenon is mediated by the incorporation of CEPs into the endothelium of new microvessels in sites of tissue injury. The same phenomenon also operates during the development of the tumor vasculature. In a pivotal study, the number of CEPs in circulation were shown to correlate with the genetic background of the mice, and, in turn, mice with the highest levels of CEPs appeared to mount the strongest angiogenic responses in various in vivo models of angiogenesis in syngeneic as well as xenograft tumor models. While CEP levels were elevated in tumor-bearing mice, presumably due to increased systemic VEGF, these cells were reduced by DC101 treatment to nearly the levels found in normal control animals (Shaked et al., 2005). The same group showed that certain forms of chemotherapy, for example, paclitaxel, induce CEP mobilization and subsequent homing to tumor blood vessels while others such as gemcitabine, did not. This mobilization

appeared to be mediated by the chemokine SDF-1 and could be blocked by administration of DC101 (Shaked et al., 2008). More recently, blockade of either VEGFR-1 with mAb MF1 or VEGFR-2 with DC101 significantly reduced growth of human esophageal cancer xenografts in immunodeficient mice. In parallel, DC101 led to a reduction of the number of VEGFR-2$^{(+)}$ CEP in the bone marrow of the mice (Xu et al., 2015).

5.2. Effects of inhibiting vascular endothelial growth factor A/ vascular endothelial growth factor receptor 2 signaling on the function of the tumor vasculature

There are multiple mechanisms that can affect the function of tumor vessels. We will focus on the effects of anti-angiogenic therapy on vessel wall integrity and the ability of blood vessels to perfuse solid tumors and metastatic lesions. VEGF acting through VEGFR-2 is a major mediator of blood vessel permeability. In some solid cancers, this increase in permeability can lead to an accumulation of fluid in abdominal spaces termed malignant ascites. Ascites occurs most frequently in patients with ovarian and gastrointestinal cancers and this condition has been re-capitulated in mouse tumor models. One expectation therefore, would be that inhibition of VEGFR-2 activation in these models would reduce ascites formation. Indeed, systemic administration of DC101 resulted in reduced extravasation of Evans Blue dye in mice that received intravenous infusions of either recombinant VEGF or malignant effusions from patients with various cancers. Both the degree of permeability and the efficacy of DC101 correlated with the level of VEGF in the malignant effusions (Zebrowski et al., 1999). In a model of experimental malignant ascites formation, mice inoculated intraperitoneally with sarcoma tumor cells developed peritoneal ascites. When the malignant ascites was drained, its recurrence was prevented by the administration of either DC101 or soluble human VEGFR-1 used as a trap (Stoelcker et al., 2000). Similarly, accumulation of ascites induced by intraperitoneal injection of a human hepatocellular carcinoma cell line was significantly reduced to a similar extent by administration of either DC101 or neutralizing antibodies to VEGF (Yoshiji et al., 2001). Others have looked at the extent of vascular leakage by injection of tracers, antibodies, or staining of fibrinogen (Nakahara et al., 2006; Verheul et al., 2007). Inhibition of the VEGF pathway with aflibercept showed decreased vascular leakage in an orthotopic syngeneic renal cell cancer model and reduced ascites in an ovarian carcinoma model (Byrne et al., 2003; Verheul et al., 2007). Using a combined method of multispectral segmentation and dynamic contrast enhanced MRI in a colorectal xenograft model, investigators showed that anti-VEGF treatment decreased vascular permeability at 24 h in viable tissue regions (Berry et al., 2008). Vascular leakage was also observed when fibrosarcoma cells over-expressing VEGF-C were implanted in skin-window chambers of immune-deficient mice. This increase could be reversed with administration of DC101, demonstrating that the permeability-promoting action of VEGF-C was through activation of VEGFR-2 and not its primary receptor, VEGFR-3 (Kadambi et al., 2001).

In contrast to the effect of VEGF/VEGFR-2 targeted therapy on vascular leakage, there exists a surprising degree of controversy regarding the effect of anti-angiogenic agents on tumor vessel perfusion. One view is that inhibition of tumor angiogenesis or regression of pre-existing tumor vasculature induces intra-tumoral hypoxia and reduces tumor growth by affecting blood perfusion. As a consequence, one might expect lowered levels of oxygen and an accumulation of metabolic waste products. Indeed, the related processes of tumor blood perfusion, induction of tumor hypoxia, and effects on tumor growth have been extensively studied. Reduction in blood flow of pre-existing xenograft tumors after DC101 administration was measured by micro-ultrasound in several xenograft models of carcinoma cells with reduction in blood flow correlating with loss of vascularity observed by immunohistochemistry (IHC) and preceding the decrease in tumor volume (Krix et al., 2003; Cheung et al., 2007; Jugold et al., 2008). Preclinical studies using ex vivo microcomputed tomography and in vivo ultrasound imaging also showed decreased perfusion and reduced tumor blood volume within 24–48 h of anti-VEGF treatment (O'Connor et al., 2009). In a model of pancreatic islet neuroendocrine tumors (PNET), aflibercept decreased endothelial fenestrations, vascular density, and decreased tumor vessel patency and blood flow measured by lectin perfusion (Inai et al., 2004). The extent of tumor growth inhibition by aflibercept was also shown to correlate to relative perfusion changes measured by dynamic contrast enhanced ultrasound in multiple murine models of cancer (Eichten et al., 2013). In one study, DC101 reduced tumor microvascular density and blood flow as measured by high-frequency micro-ultrasound. This has been reported to increase the hypoxic tumor fraction as measured with pimonidazole and to induce the expression of HIF-1 (Franco et al., 2006). Responses of the tumor vasculature to DC101 or aflibercept have also been observed using dynamic contrast-enhanced computerized tomography (DCE-CT) and by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) that can concurrently quantify a number of vascular physiological parameters such as perfusion, fractional plasma volume and permeability each of which contribute to tumor hypoxia (Kiessling et al., 2004; Cheung et al., 2007; Stantz et al., 2011; Hoff et al., 2012). Reduction in perfusion was observed with these techniques, and was correlated with decreased tumor vascularity measure by IHC. The work of Kiessling et al. also showed that the decreased vascularity and perfusion preceded the reduction in tumor volume.

An alternative and seemingly, counter-intuitive hypothesis for which considerable experimental evidence has been provided by the laboratory of Rakesh Jain proposes that anti-angiogenic therapy induces "vascular normalization" that can improve tumor perfusion by removing the dysfunctional vasculature induced by excess production of pro-angiogenic growth factors [reviewed in (Goel et al., 2011)]. The normalization concept has been demonstrated in a number of preclinical models with targeting the VEGF ligand or receptor (Byrne et al., 2003; Winkler et al., 2004). When androgen-dependent mouse mammary carcinoma cells implanted into dorsal skin chambers of immunodeficient mice were treated with DC101, the initial response was characterized by vessel regression and tumor hypoxia. However, the initial phase was followed by renewed tumor growth and increased tumor oxygenation that could be followed non-invasively in the skin chamber. (Hansen-Algenstaedt et al., 2000). When intravital microscopy was used to examine the effect of DC101 administration on functional parameters of the vasculature in several types of xenografts, blockade of VEGFR-2 led to the expected pruning of immature vessels. However, the treatment improved the function of the remaining tumor vasculature as measured by the decrease in interstitial fluid pressure (IFP), decreased vessel permeability and histological evidence of maturation such as increased pericyte coverage and production of collagen IV. (Tong et al., 2004). An increase of normal morphology of the tumor vasculature after a brief treatment with DC101 has also been demonstrated in a skin carcinoma model using ultrastructural analysis. The blood vessels in DC101-treated tumors exhibited a continuous basement membrane, normal pericyte coverage and regular intracellular junctions (Miller et al., 2005).

The understanding of the mechanistic basis by which anti-angiogenic therapy modulates tumor vessel perfusion is of paramount importance for elucidating the efficacy of this therapeutic modality when combined with either chemotherapy or radiation. We return to this subject in Sections 5.4 and 5.5 that deal with these topics.

Finally, while angiogenesis is clearly critical for growth of solid tumors, its role in the progression of liquid tumors has been controversial. As mentioned earlier, bone marrow endothelium provides a supportive microenvironment for the development of hematopoietic stem cells. In one pivotal study, researchers in the laboratory of Shahin Raffi used the specificity of anti-VEGFR-2 mAbs IMC-1C11 and DC101 to examine the roles of either autocrine or paracrine VEGF stimulation in a series of models using VEGFR-2(+) human leukemia cells

implanted into immunodeficient mice. The human receptor-specific mAb IMC-1C11 interrupted the autocrine loop by preventing human VEGFR-2 activation by tumor-derived human VEGF and prolonged the survival of the engrafted mice. Treatment with DC101, which can only inhibit murine VEGFR-2, also prolonged the survival of the mice indicating that the growth of the leukemic cells was partly supported by signals produced by murine endothelium. Interestingly, only the treatment with both antibodies led to long-term remission of the disease (Dias et al., 2001).

5.3. Resistance of tumor vasculature to mAb-mediated inhibition of vascular endothelial growth factor receptor 2

Generally disappointing clinical results of anti-angiogenic agents in the treatment of solid tumors stimulated a concerted research effort aimed at understanding the mechanisms mediating both intrinsic and acquired resistance of tumor blood vessels to VEGF/VEGFR-2 targeted therapies. Here we discuss how efficacy of anti-VEGF/VEGFR-2 therapy is dependent on the state of tumor vessel maturation, degree of tumor stroma, and the location of the tumor in the primary versus metastatic sites. We then go on to address how tumor resistance to these modes of treatment may be mediated by the emergence of alternative, pro-angiogenic pathways. Finally, we briefly discuss the role of myeloid cell infiltration of tumors in the context of resistance to anti-angiogenic therapy and end with the somewhat distantly related subject of vascular niche that may affect therapy by providing a microenvironment supportive of survival of cancer stem cells (CSCs).

It is a common observation that there exists an inverse relationship between efficacy of anti-angiogenic therapy and the timing of initial treatment in preclinical models of angiogenesis. This has been interpreted to demonstrate gradual blood vessel maturation concurrent with the acquisition of independence from VEGF signaling. As mentioned earlier, in a transgenic inducible model of HIF-1 expression in the skin of normal mice, DC101 became ineffective in the more mature vasculature. Turning off the HIF-1 transgene after 14 days also led to maturation of the skin vasculature that was insensitive to DC101 (Oladipupo et al., 2011). This phenomenon extends to other forms of pathological angiogenesis since in corneal neovascularization models anti-VEGF treatment is more effective when given early (Chen et al., 2014a). Similarly, DC101 as a monotherapy showed some efficacy in GEMs, however the efficacy was lost in late-stage tumors (Casanovas et al., 2005; Hassan et al., 2011; Wicki et al., 2012). In addition, in either syngeneic transplant or spontaneous murine tumor models, a delay in DC101 administration relative to tumor growth initiation adversely affected the efficacy of treatment (Fenton et al., 2004a).

Another element that appears to negatively impact the efficacy of DC101 is the relative abundance of tumor stroma and localization of tumor blood vessels within the tumor parenchyma versus stromal regions. In a series of well characterized tumor xenograft models, tumor containing vessels in the parenchyma were sensitive to DC101, whereas the tumors with vessels within stromal tracts were refractory to treatment (Smith et al., 2013). Extensive fibroblast-rich desmoplastic tumor stroma which characterizes pancreatic ductal adenocarcinoma (PDAC) is formed in part due to the action of sonic hedgehog (Shh) secreted by neoplastic cells. Shh-deficient tumors have a reduced stromal component, are more aggressive and proliferative and exhibit increased vascularity. Interestingly, treatment of Shh-deficient but not parental PDAC tumors with DC101 results in reduced vascularity and prolonged the survival of tumor-bearing mice demonstrating that tumor stroma may reduce the efficacy of some anti-angiogenic treatments in this disease (Rhim et al., 2014).

While most murine tumor models reflect primary disease, in a vast majority of cancer patients the most common target of therapy is a tumor that has metastasized to distant organs (see Section 5.7). Benefits of anti-angiogenic therapy in the metastatic setting of preclinical models have been somewhat modest. Triple-negative human breast cancer cells in a visceral metastatic setting were completely resistant to treatment with DC101 (Francia et al., 2008). In another study, both DC101 and the TKI, sunitinib, were efficacious in treating established orthotopic primary tumors. However, sunitinib was ineffective using the same cells in a model of postsurgical advanced metastatic disease. While DC101 was effective in this metastatic setting, the relative potency was much lower than what was observed in the primary tumors (Guerin et al., 2013). The mechanisms that convey the resistance of metastatic tumors to anti-angiogenic therapy are unclear but may involve more cooption of the existing vasculature than what is seen in primary tumors. However, somewhat different results were obtained in models of metastatic lesions resulting from orthotopic implantation of prostate cancer tumor cell lines. In both cases, growth of the primary tumor was sensitive to treatment with DC101 (Sweeney et al., 2002; Burton et al., 2008). In these models, DC101 also potently reduced growth of the lung metastases (Burton et al., 2008) and also inhibited tumor growth and bone destruction when tumors were directly implanted into the articular surface of the bone (Sweeney et al., 2002). It is unclear why blockade of VEGFR-2 shows differential ability to inhibit growth of metastasized tumors in these various models.

Many mechanisms of acquired and innate resistance to inhibitors of the VEGF/VEGFR-2 pathway have been proposed based on non-clinical investigation. These pathways involve the action of alternative pro-angiogenic factors such as PlGF, VEGF-C and -D, basic FGF and platelet derived growth factor C (PDGF-C). VEGFR-1 (Flt1) is a high-affinity receptor for VEGF, PlGF and VEGF-B. The role of VEGFR-1 in pathological angiogenesis, however, has been controversial because VEGFR-1 has low tyrosine kinase activity and an alternative slicing event produces a soluble form of the receptor that acts as a trap for VEGF and is crucial for proper angiogenic patterning during development (Kappas et al., 2008). Furthermore, mice that express kinase-dead variant of VEGFR-1 are fully viable (Hiratsuka et al., 1998). For these reasons, VEGFR-1 has been generally considered a negative modulator rather than mediator of angiogenesis. An additional complication arises from the expression of VEGFR-1 by a diverse array of circulating progenitor cells as well as circulating and tissue myeloid cells that can affect angiogenesis indirectly by secreting pro-angiogenic factors (Fischer et al., 2007). Nevertheless, blockade of VEGFR-1 with the anti-mouse VEGFR-1 mAb MF1 showed benefit in angiogenic disorders such as retinal ischemia, arthritis and atherosclerosis. MF1 also significantly reduced the growth of human epidermoid xenograft tumors and rat gliomas; although to a lesser extent than DC101 (Luttun et al., 2002). More recently, the same laboratory demonstrated that anti-PlGF antibodies can enhance the effect of DC101 (Fischer et al., 2007). Another example of the pro-angiogenic action of VEGFR-1 was provided in a study where subcutaneous growth of melanoma cells was only inhibited by a simultaneous blockade of VEGFR-1 with MF1 and VEGFR-2 with DC101 (Gille et al., 2007). PlGF transfected murine fibrosarcoma cells formed more normalized tumor vasculature than parental tumors and treatment with MF1 did not significantly reduce tumor growth rates. In contrast, treatments of parental tumors with DC101 or the TKI sunitinib, resulted in significant normalization of the tumor vasculature and decreased tumor growth rates (Hedlund et al., 2009). In support of the role of VEGFR-1 as a negative regulator of VEGFR-2/VEGF pro-angiogenic signaling, reduced production of PlGF using shRNA in a human choriocarcinoma significantly accelerated tumor growth. Tumors with down-modulated PlGF production exhibited resistance to anti-angiogenic drugs including DC101. In contrast, gain-of-function of PlGF in tumors showed increased sensitivity to anti-VEGF antibodies. Furthermore MF1 and DC101 antibodies had opposing effects on tumor angiogenesis whereby VEGFR-2 blockade was inhibitory and VEGFR-1 blockade resulted in enhanced tumor angiogenesis (Hedlund et al., 2013). Surprisingly, when a VEGF-null fibrosarcoma cell line generated from VEGF deficient mice was transfected with PlGF, the growth of the tumors was accelerated but was still sensitive to inhibition by DC101 or anti-VEGF antibodies

(Yang et al., 2013b). One explanation for VEGF-dependence of PlGF-stimulated angiogenesis would be that VEGF is produced by VEGFR-1 + myeloid cells that infiltrate tumors that produce high levels of PlGF.

Another member of the VEGF receptor family whose activity has been invoked as a possible resistance mechanism for anti-VEGF/VEGFR-2 therapies is the vascular endothelial growth factor receptor 3 (VEGFR-3). VEGFR-3 is expressed in embryonic endothelium but in adults is primarily a mediator of lymphangiogenesis. Expression of VEGFR-3 has been documented in tumor but not normal blood vessels and a neutralizing mAb to mouse VEGFR-3 (mF4-31C1) has shown inhibitory activity in a number of tumor xenograft models (Laakkonen et al., 2007). Furthermore, the combination mF4-31C1 and DC101 led to a significant decrease in blood vessel density in human LNM35 lung cancer xenografts and B16 syngeneic tumors as compared to administration of either antibody alone (Tammela et al., 2008).

Members of the fibroblast growth factor family are known activators of ECs, driving EC proliferation, migration, and capillary tube formation in vitro and tumor angiogenesis in vivo. When DC101 treatment of pancreatic xenograft tumors was combined with SSR128129E (SSR), a small molecule allosteric inhibitor of FGF receptor (FGFR) signaling, both tumor weight and volume were more potently inhibited by the combined therapy than by either monotherapy. However, it is unclear whether the combination effect was due to co-targeting of the ECs since FGFs also stimulate multiple cell types in tumor stroma and thus the anticancer potential of SSR is likely due to a combined effect on many cell types (Bono et al., 2013). In a mouse model of PNET, DC101 initially impaired angiogenesis in pancreatic islet dysplasias, decreased vessel density and reduced tumor burden. However, the effect was transient and followed by renewed tumor progression coincident with re-induction of angiogenesis. The resumption of tumor growth was paralleled by elevated production of alternative pro-angiogenic factors including FGF1, FGF2, FGF7, FGF8, Ephrin-A1, and Angiopoietin-2 (Ang2) suggesting emergence of VEGF/VEGFR-2 independent blood vasculature. In fact, treatment of the refractory tumor vessels following VEGFR-2 inhibition with a FGF-trap decreased tumor vessels and tumor growth (Casanovas et al., 2005). Interestingly, bFGF may also drive VEGF-dependent angiogenesis. Overexpression of bFGF in a model of hepatocellular carcinoma led to increased VEGF production and reduced anti-tumor activity of DC101 (Yoshiji et al., 2002).

The Ang2–Tie2 pathway has also been implicated in the resistance to therapy targeting the VEGF pathway. GEM models of both PNET and mammary adenocarcinomas become refractory to DC101 treatment. Additional testing showed that DC101 increased Ang2 within the PNET model but not the mammary model. Targeting both Ang2 and VEGFR-2 was able to suppress the revascularization and progression of most tumors in the PNET model (Rigamonti et al., 2014).

Another event that may lead to a tumor vasculature resistant to anti-VEGFR-2 therapy is an increase in pericyte coverage of blood vessels, a cardinal sign of vessel maturation. Pericytes are specialized mesenchymal cells that share the basement membrane of ECs and help in establishing quiescence and stabilization of mature blood vessels. Pericyte-EC signaling involves multiple pathways. Initial attraction of mesenchymal cells to growing blood vessels during angiogenesis and their differentiation into pericytes is thought to be mediated in part by the activation of the platelet-derived growth factor receptor *beta* (PDGFRβ) by its cognate ligands, PDGFs BB and CC (Benjamin et al., 1998). PDGFRβ knockout mice die perinatally due to incomplete coverage of blood vessels by pericytes and consequent hemorrhage (Soriano, 1994). PDGFRβ signaling is abnormal in tumor vasculature. Thus, co-targeting of PDGFRβ and VEGFR-2 appears to be a possible path to more potent anti-angiogenic treatment of solid tumors. Overexpression of the PDGFRβ ligand, PDGF-C in a human glioblastoma cell line led to tumors with smaller vessel diameters and lower vascular permeability when the cell line was implanted in a cranial window in nude mice. In contrast to parental tumors, PDGF-C-over-expressing tumors were insensitive to DC101, reinforcing the notion that VEGFR-2 targeted therapies act optimally on newly formed, immature blood vessels (di Tomaso et al., 2009). In addition, studies combining inhibition of VEGF with bevacizumab with an aptamer targeting PDGF-B showed greater effects on tumor growth of orthotopic ovarian models of cancer (Lu et al., 2010) indicating that loss of pericyte coverage on tumor vessels leads to better efficacy of anti-VEGF therapy. Indeed, treatment of mice bearing pancreatic tumor xenografts with a mAb specific for mouse PDGFRβ gave a modest anti-tumor effect but enhanced the anti-angiogenic effect of DC101 leading to superior control of tumor growth (Shen et al., 2007). Similarly, combination of DC101 with another mAb capable of blocking both mouse and human PDGFRβ (IMC-2C5) resulted in significantly enhanced antitumor activity in several xenograft tumor models compared with either treatment alone. In addition, IMC-2C5 attenuated the expression of VEGF and bFGF in tumor stroma elevated by VEGFR-2 inhibition (Shen et al., 2009). However, targeting PDGFRβ needs to be approached with caution since pericyte depletion by various methods, including PDGFRβ promoter-driven selective cell killing, has been shown in some models to promote metastatic tumor dissemination (Cooke et al., 2012).

A major factor that appears to limit the efficacy of anti-VEGF/VEGFR-2 therapy in cancer is the infiltration of the tumor by cells that mediate innate immunity. Several subtypes of such myeloid cells promote both initial tumor growth and resistance to anti-VEGF/VEGFR-2 therapy by secreting a large array of pro-angiogenic and pro-inflammatory factors [reviewed in (Rivera & Bergers, 2015b)]. This adverse effect is potentiated by the fact that subsets of myeloid cells (MDSCs) can also promote an immunosuppressive tumor microenvironment. A recent publication demonstrated the link between tumor-induced inflammation and sensitivity of tumor vessels to anti-VEGFR-2 agents. In breast cancer models, anti-angiogenic therapies were shown to induce expression of cyclooxygenase-2 (Cox-2), leading to elevated levels of its product prostaglandin E2 (PGE2). Cox-2 inhibition normalized PGE2 levels in tumors and enhanced the activity of DC101 and the TKI, sunitinib (Ben-Batalla et al., 2015). Myeloid cell infiltration can be modeled in reductionist, non-tumor models in vivo. Matrigel plugs impregnated with a combination of hepatocyte growth factor (HGF) and bFGF were rapidly infiltrated with M2-like macrophages which are known to promote angiogenesis by secretion of angiogenic growth factors such as VEGF, FGF-2 and TGFβ and various matrix metalloproteinases. The HGF/FGF-2 plugs became well vascularized and this angiogenic response could be inhibited with either DC101 or with macrophage depletion with clodronate; implicating the M2 macrophages as a major source of host-derived VEGF (Barbay et al., 2015). In transgenic models of PNET, treatment with either the TKI sorafenib or with DC101 led to tumor stasis followed by resumption of tumor growth. Sensitivity to VEGF inhibition was associated with myeloid-cell derived angiostatic and immunostimulatory chemokines such as CXCL14 and CXCL4. Further studies revealed that PI3K signaling in myeloid cells leads to the immunosuppressive and proangiogenic phenotype in relapsed tumors (Rivera et al., 2015). While increased Gr1 + CD11b + cells were observed in tumors refractory to anti-VEGF agents [reviewed in (Ferrara, 2010)], targeting of these cells was not sufficient to sensitize tumors to anti-angiogenic agents due to compensatory up regulation of tumor-associated macrophages (Rivera et al., 2015).

Finally, it is worth mentioning that tumor blood vessels have been proposed to create a specialized microenvironment, termed the vascular niche, in which the proximity to the ECs supports the long-term survival and self-renewal of CSC also known as tumor stem cells (TSCs; hereafter) which are capable of generating the entire heterogeneous population seen in tumors (Krishnamurthy et al., 2010). In turn, survival of the TSC pool may represent a mechanism of long-term resistance to anti-angiogenic therapy. This hypothesis has been investigated in a number of murine models of TSC-derived cancers using in vivo VEGFR-2 blockade with DC101. In a rat glioma xenograft model, DC101 or chemotherapy alone inhibited tumor growth and the combination treatment was more potent than either therapy alone. However,

neither treatment alone had an effect on the TSC fraction of the tumors as measured by the number of tumor sphere-forming units, indicating that the TSC cells represent a form of resistance to anti-angiogenic therapy. The TSC fraction was only reduced in the combination group (Folkins et al., 2007). However, an opposite result was obtained in the mouse model of skin tumors in which DC101 not only caused tumor regression associated with a significant decrease in EC proliferation and tumor microvascular density but also reduced the proportion of CD34(+) TSCs (Beck et al., 2011). Together, these studies indicate that the role of targeting the VEGF pathway in TSC biology remains controversial.

5.4. Inhibition of vascular endothelial growth factor receptor 2 in tumor vasculature in relation to efficacy of concurrent chemotherapy

With the exception of the approval of ramucirumab in gastric cancer, all other approved indications for selective VEGF pathway inhibitors have been in combination with chemotherapeutics. Numerous in vivo pre-clinical studies have shown that antibody-mediated blockade of VEGFR-2 potentiates the therapeutic effect of concurrent chemotherapy. DC101 was effective when combined with paclitaxel in xenograft models of bladder cancer (Inoue et al., 2000), doxorubicin in models of soft-tissue sarcoma (Zhang et al., 2002, 2006), gemcitabine in models of pancreatic cancer (Bruns et al., 2002) and vinblastine in models of neuroblastoma (Klement et al., 2000). Similar combination effects of anti-VEGF or aflibercept with a number of cytotoxic agents have been seen in multiple tumor models (Hu et al., 2005; Chiron et al., 2007; Bagri et al., 2010; Singh et al., 2012).

As mentioned previously, vascular normalization has been invoked to explain why in multiple randomized phase III trials, the anti-VEGF antibody bevacizumab prolonged survival only when combined with chemotherapy [reviewed in (Jain, 2005)]. This hypothesis suggests that improved perfusion increases access of the chemotherapeutic to the tumor cells when combined with bevacizumab. In a colorectal model, pretreatment with bevacizumab or a VEGFR-2 TKI (pazopanib) lowered the interstitial fluid pressure (IFP), increased penetration of chemotherapy delivered intraperitoneally, and delayed tumor growth (Gremonprez et al., 2015). In several tumor models, treatment with DC101 led to the reduction of IFP and induction of a hydrostatic pressure gradient across the tumor endothelium. These events correlated with elevated extravasation of fluorescently-labeled BSA into the tumor parenchyma which was used as a surrogate assay for chemotherapeutic drug delivery (Tong et al., 2004). In an interesting clinical study, glioblastoma patients treated with the pan-VEGF TKI cediranib were assessed for tumor blood perfusion. In this study, improvement of perfusion was correlated with increased survival, raising the possibility that cediranib acted both to induce vascular normalization and to directly affect tumor growth by inhibiting RTKs expressed by tumor cells (Sorensen et al., 2012). It should be noted that the normalization hypothesis remains controversial and a number of studies have shown the opposite effect; i.e. reduction in the delivery of chemotherapeutic drugs to the tumor. For example, positron emission tomography of non-small cell carcinoma patients showed rapid reduction of uptake of radioactive docetaxel upon administration of bevacizumab (Van der Veldt et al., 2012). Taken together, the studies highlight our limited understanding of how anti-angiogenic drugs act in concert with chemotherapy in cancer patients.

Chemotherapeutic agents exert their action primarily by cytotoxic damage to tumor cells. Most of these agents are administered at close to the maximum tolerated dose (MTD) at which life-threatening toxicity becomes unacceptable. Due to this narrow therapeutic window, chemotherapy is usually administered with prolonged breaks between successive cycles. Generally, this leads to profound response followed by regrowth after cessation of treatment. Anti-VEGF therapy may either delay or prevent this relapse during these chemotherapy drug holidays (Bagri et al., 2010), but it is not known whether extended VEGF depletion in this context can ultimately lead to the demise of the tumor. An alternative chemotherapy dosing approach referred to as "metronomic" chemotherapy has been developed, which consists of administration of chemotherapeutic agents at levels well below MTD but either continuously or at frequent intervals. Interestingly, "metronomic" chemotherapy appears to act in part through damage to tumor endothelium and this effect can be potentiated by co-administration of targeted anti-angiogenic agents [reviewed in (Kerbel & Kamen, 2004)]. When an established orthotopic breast tumor xenograft in SCID mice was treated with DC101 and either low dose cyclophosphamide (CTX) given continuously in drinking water or MTD CTX given every other day; both regimens proved significantly superior to any single monotherapy. However, mice treated with DC101 and CTX at MTD showed rapid weight loss and died significantly earlier than the mice in the DC101 and low dose CTX group (Man et al., 2002). These findings were further substantiated using a combination of DC101 and low-dose chemotherapy in models of breast cancer exhibiting multi-drug resistance due to over-expression of P-glycoprotein (Klement et al., 2002). The relative advantage of DC101 combined with metronomic chemotherapy was also observed in models of melanoma where the treatment was initiated after removal of the primary tumor and the disease had metastasized to the lungs (Cruz-Munoz et al., 2009) and in a model of advanced hepatocellular carcinoma where the tumor cells were orthotopically implanted into the liver (Tang et al., 2010).

Finally, another hypothesis for the effectiveness of combining anti-angiogenic agents with chemotherapy suggests that such combinations may reduce the fraction of tumor cells that exhibit a TSC phenotype. As discussed earlier, only the combination therapy of DC101 and cyclophosphamide reduced the number TSCs in a glioblastoma tumor model (Folkins et al., 2007).

5.5. Inhibition of vascular endothelial growth factor receptor 2 in tumor vasculature in relation to efficacy of concurrent ionizing radiation therapy (RT)

Despite an extensive clinical testing program for bevacizumab and to a lesser extent, aflibercept, to date, no anti-angiogenic therapy has been approved for use with concurrent RT. However, considerable pre-clinical and clinical evidence suggests that anti-angiogenic therapy can act as a radiosensitizer in a variety of tumor types. Concurrent anti-angiogenic treatment with RT can either potentiate the magnitude of tumor response to RT or extend the time of tumor stasis [reviewed in (Verheij et al., 2010)]. It is generally believed that the extent of radiation-induced DNA damage is critically dependent on tumor oxygen levels as a key mechanism of RT is the generation of reactive oxygen intermediates. As mentioned above, anti-angiogenic treatment induces tumor hypoxia so the use of anti-angiogenic agents and RT seems counterintuitive. Nevertheless, a number of non-clinical studies have demonstrated beneficial combinatorial effect of systemic DC101 and RT in mouse tumor models. In xenograft models of glioblastoma and small cell lung carcinoma in athymic mice, DC101 reduced the dose of RT required to control local growth of 50% of the tumors (Kozin et al., 2001). The combination of DC101 and RT also significantly reduced tumor growth in two models of squamous cell carcinoma (Li et al., 2005). In another xenograft model of human lung cancer, local RT led to the reduction of tumor growth rate but resulted in eventual relapse. Treatment with DC101 resulted in a reduction of the recurrent tumor, compared to primary or "radiation-naïve" tumors (Kozin et al., 2007). Furthermore, in a syngeneic model of murine mammary carcinoma, the potency of the combination of DC101 treatment with RT was greater than either monotherapy despite the fact that RT or DC101 induced tumor hypoxia (Fenton et al., 2004b).

One explanation for the efficacy of DC101 combined with RT is that the anti-angiogenic effect of VEGFR-2 blockade provides a "window" of tumor vessel normalization that reduces hypoxia and enhances the effect of RT. Consistent with this idea, DC101 improved the effectiveness

of ionizing radiation (IR) by decreasing hypoxia, which is known to adversely affect the efficacy of IR. When γ-radiation or DC101 was used in an orthotopic model of human glioma, radiation but not DC101 significantly delayed the tumor growth. However, when RT was given several days after DC101 treatment began, the tumor growth delay significantly exceeded the expected additive effect (Winkler et al., 2004). The observed synergy was suggested to result from an increase in the number of mature tumor vessels due to DC101-mediated up-regulation of expression of Ang1 and consequent recruitment of pericytes.

Additional support for the normalization model comes from a study in which blood perfusion of subcutaneous pancreatic tumor xenografts was measured using DCE-CT. Either low or high dose of DC101 given one week prior to RT delayed tumor growth compared to RT alone. Physiological maps of tumors obtained by DCE-CT suggested a significant decrease in the heterogeneity of the tumors suggesting more uniform and potentially more efficient blood flow (Cao et al., 2014). Similarly, anti-VEGF therapy with bevacizumab given five days before RT decreased tumor growth compared to RT or anti-VEGF alone, which was associated with vascular normalization and increased pO_2 levels (Myers et al., 2010). In general, while vascular normalization can improve the efficacy of chemotherapeutics and RT, its effectiveness appears limited by acute dose-dependence, and difficulty in creating a persistent "normalized" phenotype without further pruning the tumor vessels leading to hypoxia. Current efforts are focusing on the ability to sustain vessel normalization [reviewed in (Rivera & Bergers, 2015a)].

An alternative view was offered in a study of the induction of apoptosis of ECs by RT. Irradiation of ECs in culture induced apoptosis mediated by a wave of ceramide production due to activation of acid sphingomyelinase (asmase). The apoptosis of ECs after in vitro RT was rescued by exogenous VEGF but potentiated by DC101. In vivo studies using a fibrosarcoma model showed that neither anti-VEGF therapy nor DC101 induced tumor cell apoptosis alone but both treatments potentiated the pro-apoptotic effect of RT on tumor ECs. The radiosensitizing effect was observed only when RT was given within a short time period (1 h) after the anti-angiogenic treatment and was lost by 24 h. However, the potentiation of RT-induced EC apoptosis by either anti-VEGF or DC101 was abrogated in tumors grown in asmase$^{-/-}$ mice strongly suggesting that the mechanism of synergy of anti-angiogenic treatment and RT is due to potentiation of damage to tumor endothelium (Truman et al., 2010).

The combination of VEGFR-2 blockade with DC101 and RT in some models led to significant adverse events. Nearly half of mice bearing small cell lung carcinoma tumors and treated with DC101 and RT developed peritoneal ascites (Kozin et al., 2001). This observation is difficult to reconcile with the known ability of DC101 to reduce pathological vascular leakage. Furthermore, DC101 treatment was studied in irradiated and non-irradiated intracerebral GBM-bearing mice. Surprisingly, systemic administration of DC101 led to increased mortality of the mice when given alone or in combination with RT as compared to RT monotherapy. This toxicity was mainly characterized by edematous changes in the pancreas and the intestine. The adverse effect of DC101 on mortality was difficult to explain as histological examination of the tumors showed a significant combination effect of the two treatments in terms of reduced tumor area, reduced tumor proliferation, and increased apoptosis (Verhoeff et al., 2009). These concerns are not unique to anti-VEGFR-2 inhibition as anti-VEGF therapy combined with RT also leads to toxicities in normal tissues (Mangoni et al., 2012).

5.6. Therapeutic combinations of anti-vascular endothelial growth factor receptor 2 treatment and other therapies

In other experimental approaches, VEGFR-targeted mAbs have been combined with agents that either disrupt or alter the function of the vascular endothelium or with biologics that target oncogenic pathways. VEGF regulates blood pressure and vascular permeability by up-regulating levels of nitric oxide (NO), primarily via endothelial NOS in ECs. In turn, there have been reports that NO can up-regulate VEGF by enhancing the action of HIF-1. When systemic DC101 treatment was combined with an oral NOS inhibitor, N-nitro-L-arginine (NNLA), the combination reduced growth of pancreatic tumor xenografts better than either agent alone. This combination was also more potent than either agent in reducing tumor blood vessel permeability. On the other hand, blockade of NO synthesis but not treatment with DC101 reduced mean vessel diameter in tumors (Camp et al., 2006). When sub-dermally implanted tumors were treated with either OXi-4503, a second-generation derivative of the vascular disrupting agent (VDA) combretastin-A4 phosphate (CA4-P) or DC101, OXi-4503 produced central tumor hypoxia and necrosis while DC101 resulted only in hypoxia. In both types of monotherapies, the tumors retained a well-perfused viable rim of tumor cells. The combination of the two agents significantly decreased perfusion and increased hypoxia and necrosis, which was associated with suppressed tumor growth. One mechanistic explanation for this combination effect was that VDA treatment induces production of CEP cells that enhance angiogenesis in the tumor viable rim. This raise in CEP was not observed in mice treated with DC101 before the administration of the VDA (Shaked et al., 2006). Finally, combination treatment of anti-VEGF (B20) with an antibody targeting a tumor vessel secreted protein (EGFL7), caused increased progression-free and overall survival benefits associated with decreased tumor volume and tumor vessels in GEMM models (Johnson et al., 2013).

Combining targeted agents that affect the vasculature with agents that block the initiation of oncogenic signaling hold promise because of the relatively low toxicity profiles of these molecules as opposed to chemotherapy and multi-targeted TKIs. MAbs directed against the epidermal growth factor receptor (EGFR) are used for the treatment of wild-type RAS metastatic colorectal cancer (cetuximab, and panitumumab), metastatic non-small cell lung cancer, and head and neck cancer (cetuximab); while the mAb trastuzumab is used in breast cancer patients whose tumors express the epidermal growth factor receptor 2 (HER2). Clinical results of combining anti-EGFR antibodies with bevacizumab, however, have been uniformly disappointing [reviewed in (Di Maio et al., 2014)]. These failures highlight the difficulty in translating the results of pre-clinical models to the clinic despite a significant body of literature showing the efficacy of such combinations in xenograft tumor models. Synergistic effects of combining cetuximab with DC101 were shown in a subcutaneous xenograft model of colorectal cancer (Tonra et al., 2006). Similar results were obtained in a xenograft model of colon cancer peritoneal carcinomatosis (Shaheen et al., 2001a). In both models, an additive effect on tumor cell apoptosis was observed. EGFR overexpression is also found in a subset of gastric cancers and has been reported in a majority of pancreatic tumors. In this respect, combination of DC101 and cetuximab proved beneficial in xenograft models of gastric and pancreatic cancers (Jung et al., 2002; Tonra et al., 2006). In addition, anti-EGFR mAbs are currently in development or in clinical trials for the treatment of glioblastoma multiforme (GBM). GBM is of particular interest with respect to anti-angiogenic therapy because genetic ablation of VEGF or treatment with DC101 has been shown to increase local tumor invasion leading to per-tumoral metastatic sites referred to as "satellites" (Paez-Ribes et al., 2009). In two mouse models of GBM, the combination of DC101 and cetuximab was more potent than either monotherapy (Diao et al., 2010; Yi et al., 2011). In both of these studies, DC101 increased the number of microsatellites but this pro-metastatic effect was reduced by co-administration of cetuximab. In another study, the combination was no better than DC101 alone (Lamszus et al., 2005). Comparatively less information exists on pre-clinical use of combined anti-VEGFR-2 antibodies and trastuzumab in breast cancer models. In one study, the combination of DC101 and trastuzumab led to significant improvement in the survival of mice bearing HER2+ tumors in the brain (Kodack et al., 2012). Interestingly, when HER2+ breast cancer

cells were implanted orthotopically, resistance to trastuzumab emerged after about one month of therapy. These resistant tumors exhibited sensitivity to the anti-VEGF antibody bevacizumab probably because the resistant tumors produced elevated levels of VEGF (du Manoir et al., 2006). In addition, in a HER2 overexpressing breast tumor xenograft model low dose aflibercept and low dose trastuzumab were shown to have additive effects on tumor growth, vascular density, and cell proliferation (Le et al., 2008).

5.7. Inhibition of vascular endothelial growth factor receptor 2 in tumor vasculature and tumor metastasis

The contribution of angiogenesis to the metastatic dissemination of primary tumors is widely accepted and the mechanisms by which tumor cells enter blood vessels has been extensively studied [reviewed in (Reymond et al., 2013)]. Although many anti-angiogenic treatments, for example aflibercept (Verheul et al., 2007) and DC101 (Sweeney et al., 2002) inhibit distant organ metastasis in murine tumor models, the mechanism of this inhibition is likely to be complex. Anti-angiogenic agents inhibit the size of the primary tumors and reduce tumor vascularity thus simultaneously decreasing the number of tumor cells that can metastasize and the vascular surface available for extravasation. As also mentioned previously, anti-angiogenic treatment normalizes the tumor vasculature and the resulting "normal" vessels may be harder for tumor cells to penetrate than the chaotic and disorganized tumor blood vessels. The readouts of distant organ metastasis can also complicate study interpretation as anti-angiogenic treatment may reduce the growth of lesions in distant organs making these micrometastases difficult to detect. This, in turn, gives a falsely low measure of the metastatic rate. For these reasons, we will focus our discussion on the effects of anti-angiogenic therapy on the invasion of the tumor cells into the regional normal tissue, a subject of considerable recent controversy.

One of the earliest studies of local tumor invasion also represents the first in vivo use of DC101. Transplantation of benign and malignant (*ras*-transformed) keratinocytes under the skin of mice resulted in distinct patterns of angiogenesis and local tumor invasion. Malignant cells induced a more aggressive invasion into the local stroma accompanied by aggressive and directional growth of capillaries. This vascular expansion was driven by elevated tumor-derived VEGF and increased expression of VEGFR-2 on the tumor endothelium. Treatment of established malignant tumors with DC101 reduced tumor vascularity to the level seen in transplants of non-malignant keratinocytes and dramatically reduced tumor invasion into the surrounding stroma (Skobe et al., 1997). Using similar skin heterotransplant models, the same laboratory further demonstrated that treatment with DC101 normalizes the tumor vessels and stroma in part by down-modulating expression of stromal matrix metalloproteinases (Miller et al., 2005; Vosseler et al., 2005).

In contrast to these earlier findings, recently much attention has been generated by non-clinical studies showing that anti-angiogenic treatment can increase local invasiveness and, in a few cases, distant metastasis. Several publications document increased invasiveness induced by inhibition of the VEGF pathway in PNETs. In the first study, both tumor cell-specific deletion of the VEGF gene or systemic treatment with DC101 resulted in increased local, lymph node, and distant metastasis and the effect persisted after the cessation of DC101 treatment. Furthermore, DC101 induced hypoxia in both the primary islet tumors as well as in the liver metastases. (Paez-Ribes et al., 2009). Similar findings in this model were obtained in another study but, interestingly, the pro-invasive effect of DC101 was reversed by concurrent treatment with semaphorin 3A (sema 3A) (Maione et al., 2012). Sunitinib or inhibition of VEGF with an antibody has also shown increased hypoxia associated with increased tumor cell migration and invasiveness in PNET tumors. This increase in invasiveness was reduced by co-treatment of these VEGF pathway inhibitors with inhibition of cMET or sema 3A, which reduced tumor expression of HIF-1 and phosphorylation of cMET (Maione et al., 2012; Sennino et al., 2012). Together, these studies indicate that anti-angiogenic treatment elevates tumor hypoxia, which induces MET activity and increases tumor cell migration and invasiveness. However, a recent study in this PNET model contradicts the findings described above. In this study, DC101 or a mAb to Ang 2 inhibited the growth of the tumors and the combination showed superior anti-angiogenic effect and tumor control (Rigamonti et al., 2014). Despite seeing potent anti-angiogenic blockade and strong induction of hypoxia, these authors did not observe any increase in tumor invasiveness.

It is important to state that at this junction the increase in local tumor invasion has only been demonstrated clinically in patients with high grade glioblastoma (de Groot et al., 2010) despite thousands of patients representing diverse solid tumor types having been treated world-wide with biologic and TKI anti-angiogenics. The clinical findings with glioblastoma have been recapitulated in mouse models of intracerebral glioblastoma xenografts in which either bevacizumab (de Groot et al., 2010) or DC101 showed pro-metastatic effects as evidenced by an increase in local satellite tumors (Lamszus et al., 2005). In the later study, DC101-induced invasion was ameliorated by concurrent treatment with the EGFR antagonist antibody cetuximab. It is important to note that, due to its species specificity, DC101 can only mediate its pro-invasive activity by blocking VEGFR-2 on the vascular endothelium of tumor xenografts, as an important role for VEGFR-2 on tumor cells has recently emerged. In orthotopically implanted murine glioblastoma cells that were found to express VEGFR-2 in complex with the RTK cMET, a receptor for HGF, either genetic knock-out of VEGF expression or pharmacological inhibition of VEGF with B20 increased local invasion. This pro-metastatic effect was ascribed to the induction of cell migration by HGF-activated cMET that was no longer being inhibited by VEGFR-2 signaling (Lu et al., 2012). Taken in toto, preclinical and clinical findings suggest that metastasis induction by anti-angiogenic treatment is limited. Where this phenomenon does occurs, however, it is still not clear whether the induction of tumor hypoxia after anti-VEGF/VEGFR-2 therapy selects for tumor sub-populations with increased aggressiveness, increased ability to use alternative angiogenic pathways, or both.

We conclude this section with a brief mention of the role played by VEGFR-2 in metastasis of tumor to regional lymph nodes as revealed by the use of antagonist mAbs. The negative prognostic value of tumor dissemination to regional lymph nodes has been recognized for a long time although the clinical significance of tumor spread through lymphatic vessels has been controversial [reviewed in (Alitalo & Detmar, 2012)]. As already discussed, VEGFR-2 is expressed on lymphatic vessels although, unlike the case of angiogenesis where VEGFR-2 signaling is paramount, lymphangiogenesis is primarily mediated by activation of VEGFR-3 [reviewed in (Tammela & Alitalo, 2010)]. In an orthotopic model of breast cancer tumor spread to lymph nodes and lungs, both DC101 and the anti-VEGFR-3 mAb mF4-31C1 reduced tumor lymphangiogenesis. While DC101 was much more potent in reducing the size of the primary tumor, inhibition of VEGFR-3 activation more potently suppressed regional and distant metastases. Finally, the combination of the two treatments was more potent than either treatment alone. As in the case of dissemination through blood vessels, an earlier (prevention) therapy, given soon after implantation of the tumor cells, was more effective than later (intervention) therapy (Roberts et al., 2006). This study supports the concept that VEGFR-2 plays a role in tumor lymphangiogenesis although clearly not as pivotal as that played by VEGFR-3. In contrast, in an orthotopic model of prostate cancer, mF4-31C1 potently reduced tumor lymphangiogenesis and metastasis to regional lymph nodes and distal vital organs without influencing tumor growth. DC101, however, inhibited growth of the primary tumor and reduced distant metastasis but had little effect on lymphangiogenesis and tumor spread to lymph nodes (Burton et al., 2008). Also, in an ear model of tumor spread to lymph nodes, the anti-

VEGFR-3 mAb potently blocked tumor dissemination, while DC101 primarily reduced the growth of the metastatic lesions (Hoshida et al., 2006). These studies indicate that at this time the exact role played by VEGFR-2 in tumor metastasis through the lymphatic vasculature remains to be delineated.

5.8. Blockade of vascular endothelial growth factor receptor 2 signaling in models of tumor immunity

The relationship between tumor angiogenesis and anti-tumor immune response is highly complex, involving cells that mediate both innate and acquired immune response. Inhibitors of VEGF/VEGFR-2 alter tumor blood vessels and may facilitate extravasation and function of effector T cells [reviewed in (Lanitis et al., 2015)]. At the same time, these agents induce tumor hypoxia which is known to polarize myeloid cells to an immunosuppressive phenotype and pro-angiogenic phenotype [reviewed in (Rivera & Bergers, 2015b). Despite these uncertainties, the recent successes of cancer immunotherapies have raised the interest of combining these novel modalities of cancer treatment with inhibitors of angiogenesis. Cancer immunotherapy can depend on several immune mechanisms such as antibody-dependent cell mediated cytotoxicity (ADCC), enhancement of immunity through tumor vaccines, or blockade of immune checkpoint molecules. At least in murine models, each of these forms of immunotherapy can be successfully combined with anti-angiogenic therapy mediated by VEGFR-2 blockade with DC101. One of the first studies involved co-targeting VEGFR-2 with DC101 with direct induction of anti-melanoma ADCC using the mAb TA99 which recognizes TYRP-1/gp75 (tyrosinase-related protein-1). The growth of subcutaneous melanoma tumors was significantly suppressed by DC101 and by TA99 treatment alone. The combined treatment resulted in a significant enhancement of tumor growth suppression. In addition, significant reduction of lung metastases resulting from tail vein injection of the tumor cells was observed with the single agent treatments and this response was enhanced with combined therapy (Patel et al., 2008). In another study with melanoma cells, adoptive cell transfer (ACT), synergized with either DC101 or anti-VEGF mAb B20 in reducing the growth of the tumors. However, only treatment with B20 increased the numbers of autologous tumor-infiltrating lymphocytes (TILs) and improved survival (Shrimali, Yu et al., 2010). The mechanism for this differential action of the two modes of anti-angiogenic therapy in this study is unclear. However, it should be pointed out that the effect of anti-angiogenic therapy on the infiltration of immune cells into tumors may be context dependent. In a model of spontaneous colon tumors that develop in adenomatous polyposis coli conditional knockout mice, continuous administration of DC101 impeded the infiltration of CD4 + and CD8 + cells into the tumor region (Yang, Choi et al., 2015). In contrast, low doses of DC101 were shown to increase T-cell tumor infiltration in a breast cancer model (Huang et al., 2012).

Concurrent administration of DC101 also appeared to be efficacious in enhancing tumor vaccine treatment in two models of breast cancer. Effect of HER-2/*neu* (*neu*) — targeted vaccination in FBV mice was potentiated by treatment with DC101 with increased tumor-specific CD8 + T cells and tumor regression. In contrast, in *Neu*-N mice which are derived from the parental FVB strain but exhibit peripheral immune tolerance, *neu*-specific vaccination was ineffective and addition of DC101 reduced tumor growth without inducing tumor regression or antigen-specific T-cell activation (Manning et al., 2007). In a second study which used whole cancer cell vaccine therapy in mice bearing orthotopic breast cancer tumors, the effect of vaccination was potentiated by subsequent treatment with DC101. However, one provocative finding was that the effect was observed only with low (10 mg/kg) and not high (40 mg/kg) dose DC101 although the high dose resulted in superior anti-angiogenic response and reduction in tumor growth. This result was interpreted to mean that lower doses of DC101 have a superior vascular normalizing effect that reverses the immune-suppressive tumor environment and facilitates tumor penetration by effector T cells (Huang et al., 2012).

It should be pointed out that agents targeting the VEGF/VEGFR-2 axis can also excert their effects directly on immune cells. One group followed up on the clinical observation that bevacizumab reduces the number of regulatory T cells (Treg) in peripheral blood of patients with metastatic colon cancer (mCRC). These authors found that Treg from mice bearing colorectal carcinoma tumors expressed VEGFR-2 and directly proliferated in response to VEGF. In these mice, anti-VEGF mAb B20 and sunitinib treatments reduced Treg. This treatment seemed to affect Treg percentages but did not induce any changes in their function. Similar effects were seen with DC101 (Terme et al., 2013).

The clinical successes with cancer immunotherapy have been primarily obtained with mAbs directed at CTLA-4 and the programmed death 1 (PD-1) receptor and its ligands PD-L1 and PD-L2. A recent review highlights the preclinical rationale of combining angiogenesis inhibition and immune checkpoint inhibition for cancer treatments (Ott et al., 2015). To date, only a single study combined in vivo VEGFR-2 blockade with an antibody to PD-1. Using a colon adenocarcinoma model, these investigators found that simultaneous blockade of PD-1 and VEGFR-2 produced a synergistic anti-tumor effect (Yasuda et al., 2013). Although DC101 but not anti-PD-1 disrupted tumor vessels, the infiltration of T cells into tumors treated with anti-VEGFR-2 mAb or combination was unaffected. Despite the limited amount of experimental data available to date, there is ample reason for optimism that targeting the tumor vasculature may positively affect T cell function either by altering the endothelial barrier to facilitate T cell entry or by reducing the immune inhibitory activity directly promoted by the ECs [reviewed in (Lanitis et al., 2015)].

6. Clinical targeting of the vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 pathway in cancer – the road forward

Several biologic anti-angiogenic agents targeting the VEGF/VEGFR pathway that are currently approved provide significant clinical benefits. Some patients do achieve complete responses but in the majority of patients, average increases in PFS or OS is only weeks to months. Clinical efforts are underway to expand and extend the scope of clinical utility of anti-angiogenic agents by exploring novel indications, earlier interventions, and combinations with other forms of therapy.

6.1. Bevacizumab and aflibercept — clinical development

While extensive review of the current clinical development of bevacizumab and aflibercept is beyond the scope of this review, a brief overview of some current trials is provided below and within the supplemental table. The majority of the ongoing clinical trials are focused on expanding the benefit of anti-VEGF based therapies in approved indications such as glioblastoma, mRCC, mCRC, gastroesophageal cancers either by combining them with agents that inhibit alternate pro-angiogenic pathways such Ang/Tie2 (trebananib), BMP9/ALK1 (TRC105), or by combining with cancer immunotherapy agents such as ipilumumab (anti-CTLA4 antibody), nivolumab/pembrolizumab (anti-PD1 antibody), atezolizumab (anti-PD-L1 antibody) and rindopepimut (a 14-mer peptide vaccine that spans the length of EGF receptor variant III) [reviewed in (Swartz et al., 2014)]. Some of the ongoing clinical trials are focused on new indications such as melanoma, which would broaden the patient population that could potentially benefit from anti-angiogenic therapy by combining them with recently approved targeted agents such as vemurafenib (BRAF inhibitor), cobimetinib (MEK inhibitor) or immunotherapy agents such as ipilumumab.

6.2. Additional vascular endothelial growth factor A pathway inhibitors in the clinic

Additional inhibitors of the VEGF pathway have also been described. These include another inhibitor targeting VEGFR2 and a bispecific antibody targeting VEGF and Ang2. Tanibirumab (TTAC-0001) is a novel antagonist antibody to VEGFR-2 that has been developed from a fully human naïve single chain variable fragment (ScFv) phage library and has recently completed a phase I clinical trial in solid tumors. Although primary literature on the characterization and clinical trial outcome of Tanibirumab is not available, this mAb is reported to cross-react with mouse, rat, human, and monkey VEGFR-2. It also appears to be effective in various tumor models in rodents, suggesting anti-angiogenic activity [reviewed in (Lee, 2011)].

A bispecific antibody blocking VEGF-A based on bevacizumab on one arm and Ang-2 based on an Ang2 selective human IgG1 on the other (R05520985; Ang-2-VEGF-A CrossMab) has been developed (Kienast et al., 2013). It is currently being tested in a phase I trial in metastatic solid tumors and in a phase II trial in combination with FOLFOX in untreated metastatic colorectal cancer.

6.3. Ramucirumab — clinical development

Several clinical trials of ramucirumab are currently underway (Table 2). Of particular interest is RAINFALL, a randomized, double-blind, placebo-controlled phase III study of cisplatin plus a fluoropyrimidine with or without ramucirumab as first-line therapy in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. The clinical development plan for ramucirumab also includes a number of early and late phase studies exploring subsets of patients, additional tumor indications, and combinations with agents targeting tumor vessels (cMet), tumor cells (EGFR, CDK4/6, cMet), or the immune system (TGFβR1, PD-1, PD-L1). While the results from the REACH trial in second line HCC did not achieve its primary endpoint in overall survival, there was an improvement in overall survival in a population of patients with high alpha-fetoprotein (Zhu et al., 2015). The REACH-2 study will examine whether ramucirumab can improve survival in patients with high alpha-fetoprotein in second line HCC patients following sorafenib treatment. Ramucirumab is also being explored in a phase III trial (RANGE) in combination with docetaxel in urothelial carcinomas which would represent a new histology for this class of inhibitors. Finally, ramucirumab trials explore combinations with agents targeting EGFR (erlotinib) in NSCLC, CDK4/6 (abemaciclib) in NSCLC, cMet (Emibetuzumab) in advanced cancers, a multikinase inhibitor (merestinib) in advanced cancers, TGFβR1 (galunisertib) in HCC, PD-1 (pembrolizumab) in multiple cancers, or PD-L1 (durvalumab) in gastrointestinal or thoracic cancers.

6.4. Key challenges

Many patients progress after initial disease stabilization due to acquired resistance to anti-angiogenic drugs. Some of the challenges for intrinsic or acquired resistance to anti-angiogenic agents include multiple other pro-angiogenic factors besides VEGF (Kopetz et al., 2010), resistance due to myeloid cell infiltration [reviewed in (Bergers & Hanahan, 2008)], inflammatory host response, and angiogenesis independent growth through vasculogenesis, vessel co-option or vascular mimicry [reviewed in (Loges et al., 2010)]. Tumors produce multiple

Table 2
Ongoing Ramucirumab clinical trials.

Trial	Name	Phase	Indication	Patient segment(s)	Combination[a]
Ramucirumab plus anti-PD-1 (pembrolizumab)	NA	I	G-OJ[b], NSCLC[c], or TCCU[d]	Locally advanced and unresectable or metastatic	Pembrolizumab
Ramucirumab plus anti-PD-L1 (MEDI4736)	NA	I	Gastrointestinal or thoracic malignancies including G-OJ[b], NSCLC[c], or HCC[e]	Locally advanced and unresectable or metastatic	MEDI4736
Abemaciclib (LY2835219) in combination with other anti-cancer drugs[k]	NA	I	NSCLC[b]	Stage IV	Abemaciclib
Multikinase inhibitor of MET, MST1R and others (LY2801653) in advanced cancer[l]	NA	I	Advanced cancer including CRC[f], HNSCC[g], Uveal melanoma with liver metastasis, CC[h], NSCLC[c]	Advanced cancer	LY2801653
Ramucirumab or necitumumab (anti-EGFR) with investigational third-generation EGFR inhibitor AZD9291	NA	I	NSCLC[c]	Advanced EGFR T790M positive	AZD9291
Antibody to cMET (LY2875358) in combination with ramucirumab in participants with advanced cancer	NA	1/II	Advanced Cancer G-OJ[b], HCC[e],RCC[i], NSCLC[c]	Advanced cancer	LY2875358
TGF-bR1 inhibitor (LY2157299) in participants With HCC[e]	NA	II	HCC[e]	Progressed on or ineligible for sorafenib with no previous systemic treatment	LY2157299
Ramucirumab versus placebo in patients with HCC[e] and elevated baseline alpha-fetoprotein	REACH-2	III	HCC[e]	Second line following first-line therapy with sorafenib	
Ramucirumab in combination with erlotinib in participants with EGFR mutation-positive metastatic NSCLC[c]	RELAY	III	NSCLC[c]	Untreated patients with EGFR mutation positive metastatic NSCLC[c]	Erlotinib
Ramucirumab plus docetaxel in participants with urothelial cancer	RANGE	III	UC[j]	Locally advanced or Unresectable or Metastatic UC[j] who progressed on or after platimun-based therapy	Docetaxel
Cisplatin plus a fluoropyrimidine with or without ramucirumab as first-line therapy in patients with metastatic gastric or gastroesophageal junction adenocarcinoma	RAIN-FALL	III	G-OJ[b]	Metastatic gastric or G-OJ[b] adenocarcinoma with no prior systemic chemotherapy except for (neo)adjuvant	Cisplatin plus a fluoropyrimidine

[a] Ramucirumab combined with indicated therapeutic.
[b] Gastric or gastro-oesophageal junction adenocarcinoma.
[c] Non-small-cell lung cancer.
[d] Transitional cell carcinoma of the urothelium.
[e] Hepatocellular carcinoma.
[f] Metastatic colorectal carcinoma.
[g] Head and neck squamous cell carcinoma.
[h] Cholangiocarcinoma.
[i] Renal Cell Carcinoma.
[j] Urothelial carcinoma.
[k] Abemaciclib also combined with Pemetrexed, Gemcitabine or PI3K/mTOR dual Inhibitor (LY3023414).
[l] LY2801653 also combined with Cetuximab, Cisplatin or Gemcitabine.

angiogenic molecules and different stages of tumor development may depend on different angiogenic factors and therefore blocking a single pro-angiogenic molecule might have very little impact or no impact on tumor growth and overall survival. Combination strategies and approaches such as intermittent dosing schedules, sub-MTDs to delay hypoxia onset thereby improving oxygenation and delivery of co-administered drugs is the future of anti-angiogenic therapy. Novel combination approaches include simultaneously targeting multiple pro-angiogenic factors that impact tumor growth at different stages and influence different stages of blood vessel maturation.

In addition to novel combinations of anti-angiogenic agents, the use of anti-angiogenic therapy in conjunction with selective agents targeting the tumor signaling pathways is also being explored. Aside from ramucirumab in second line gastric cancer, biologic anti-angiogenic agents have only been approved in combination with chemotherapeutics. Combinations of anti-angiogenic agents with agents targeting tumor cells may improve response with non-overlapping toxicities, unlike the current combinations.

Patient responses to anti-angiogenic therapies are variable within and across indications. Most of the reported outcomes are averages but in reality these vary quite extensively from complete responses to no responses. Thus, it is very important to find predictive biomarkers to identify patients that benefit from anti-angiogenic therapies. Several biomarkers including circulating biomarkers (soluble proangiogenic ligands), tissue biomarkers (receptors), physiological response biomarkers (hypertension, blood flow), genetic biomarkers (SNPs), and imaging biomarkers (DCE-MRI) have been explored with variable results [reviewed in (Murukesh et al., 2010)]. To date no predictive or surrogate response biomarker has been identified which could identify responders or non-responders to anti-angiogenic therapies [reviewed in (Hatch et al., 2015)].

The future of anti-angiogenic therapies depends upon identifying predictive biomarkers to monitor response and upon creative, novel combination approaches that can overcome innate resistance, avoid induction of acquired resistance, and provide a meaningful survival benefit to the patients.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pharmthera.2016.06.001.

Conflict of interest statement

As of the writing of the manuscript, all the authors were employees and shareholders of Eli Lilly and Company. This review discusses in part the development and clinical use of ramucirumab, a monoclonal antibody marketed by Eli Lilly as a cancer therapeutic under the trade name of Cyramza®.

Acknowledgments

The authors wish to thank Dr. Laura Benjamin for her critical reading of the manuscript and Dr. Jessica Martin Rege for providing the ramucirumab clinical trial information.

References

Alitalo, A., & Detmar, M. (2012). Interaction of tumor cells and lymphatic vessels in cancer progression. *Oncogene 31*(42), 4499–4508.

Bagri, A., Berry, L., Gunter, B., Singh, M., Kasman, I., Damico, L. A., ... Plowman, G. D. (2010). Effects of anti-VEGF treatment duration on tumor growth, tumor regrowth, and treatment efficacy. *Clin Cancer Res 16*(15), 3887–3900.

Bais, C., Wu, X., Yao, J., Yang, S., Crawford, Y., McCutcheon, K., ... Ferrara, N. (2010). PlGF blockade does not inhibit angiogenesis during primary tumor growth. *Cell 141*(1), 166–177.

Baldwin, H. S. (1996). Early embryonic vascular development. *Cardiovasc Res 31* (Spec No: E34-45).

Baldwin, M. E., Halford, M. M., Roufail, S., Williams, R. A., Hibbs, M. L., Grail, D., ... Achen, M. G. (2005). Vascular endothelial growth factor D is dispensable for development of the lymphatic system. *Mol Cell Biol 25*(6), 2441–2449.

Baluk, P., Adams, A., Phillips, K., Feng, J., Hong, Y. K., Brown, M. B., & McDonald, D. M. (2014). Preferential lymphatic growth in bronchus-associated lymphoid tissue in sustained lung inflammation. *Am J Pathol 184*(5), 1577–1592.

Barbay, V., Houssari, M., Mekki, M., Banquet, S., Edwards-Levy, F., Henry, J. P., ... Brakenhielm, E. (2015). Role of M2-like macrophage recruitment during angiogenic growth factor therapy. *Angiogenesis 18*(2), 191–200.

Beck, B., Driessens, G., Goossens, S., Youssef, K. K., Kuchnio, A., Caauwe, A., ... Blanpain, C. (2011). A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. *Nature 478*(7369), 399–403.

Ben-Batalla, I., Cubas-Cordova, M., Udonta, F., Wroblewski, M., Waizenegger, J. S., Janning, M., ... Loges, S. (2015). Cyclooxygenase-2 blockade can improve efficacy of VEGF-targeting drugs. *Oncotarget 6*(8), 6341–6358.

Benedito, R., Rocha, S. F., Woeste, M., Zamykal, M., Radtke, F., Casanovas, O., ... Adams, R. H. (2012). Notch-dependent VEGFR3 upregulation allows angiogenesis without VEGF-VEGFR2 signaling. *Nature 484*(7392), 110–114.

Benjamin, L. E., Hemo, I., & Keshet, E. (1998). A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development 125*(9), 1591–1598.

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

Berry, L. R., Barck, K. H., Go, M. A., Ross, J., Wu, X., Williams, S. P., ... Carano, R. A. (2008). Quantification of viable tumor microvascular characteristics by multispectral analysis. *Magn Reson Med 60*(1), 64–72.

Bhargava, P., & Robinson, M. O. (2011). Development of second-generation VEGFR tyrosine kinase inhibitors: current status. *Curr Oncol Rep 13*(2), 103–111.

Bocci, G., Man, S., Green, S. K., Francia, G., Ebos, J. M., du Manoir, J. M., ... Kerbel, R. S. (2004). Increased plasma vascular endothelial growth factor (VEGF) as a surrogate marker for optimal therapeutic dosing of VEGF receptor-2 monoclonal antibodies. *Cancer Res 64*(18), 6616–6625.

Bono, F., De Smet, F., Herbert, C., De Bock, K., Georgiadou, M., Fons, P., ... Carmeliet, P. (2013). Inhibition of tumor angiogenesis and growth by a small-molecule multi-FGF receptor blocker with allosteric properties. *Cancer Cell 23*(4), 477–488.

Bruns, C. J., Shrader, M., Harbison, M. T., Portera, C., Solorzano, C. C., Jauch, K. W., ... Ellis, L. M. (2002). Effect of the vascular endothelial growth factor receptor-2 antibody DC101 plus gemcitabine on growth, metastasis and angiogenesis of human pancreatic cancer growing orthotopically in nude mice. *Int J Cancer 102*(2), 101–108.

Burton, J. B., Priceman, S. J., Sung, J. L., Brakenhielm, E., An, D. S., Pytowski, B., ... Wu, L. (2008). Suppression of prostate cancer nodal and systemic metastasis by blockade of the lymphangiogenic axis. *Cancer Res 68*(19), 7828–7837.

Byrne, A. T., Ross, L., Holash, J., Nakanishi, M., Hu, L., Hofmann, J. I., ... Jaffe, R. B. (2003). Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. *Clin Cancer Res 9*(15), 5721–5728.

Calvetti, L., Pilotto, S., Carbognin, L., Ferrara, R., Caccess, M., Tortora, G., & Bria, E. (2015). The coming of ramucirumab in the landscape of anti-angiogenic drugs: potential clinical and translational perspectives. *Expert Opin Biol Ther 15*(9), 1359–1370.

Camp, E. R., Yang, A., Liu, W., Fan, F., Somcio, R., Hicklin, D. J., & Ellis, L. M. (2006). Roles of nitric oxide synthase inhibition and vascular endothelial growth factor receptor-2 inhibition on vascular morphology and function in an in vivo model of pancreatic cancer. *Clin Cancer Res 12*(8), 2628–2633.

Cao, Y. (2014). VEGF-targeted cancer therapeutics-paradoxical effects in endocrine organs. *Nat Rev Endocrinol 10*(9), 530–539.

Cao, N., Cao, M., Chin-Sinex, H., Mendonca, M., Ko, S. C., & Stantz, K. M. (2014). Monitoring the effects of anti-angiogenesis on the radiation sensitivity of pancreatic cancer xenografts using dynamic contrast-enhanced computed tomography. *Int J Radiat Oncol Biol Phys 88*(2), 412–418.

Carmeliet, P. (2005). Angiogenesis in life, disease and medicine. *Nature 438*(7070), 932–936.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., ... Nagy, A. (1996). Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature 380*(6573), 435–439.

Casanovas, O., Hicklin, D. J., Bergers, G., & Hanahan, D. (2005). Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell 8*(4), 299–309.

Chen, D. B., & Zheng, J. (2014). Regulation of placental angiogenesis. *Microcirculation 21*(1), 15–25.

Chen, W. L., Chen, Y. M., Chu, H. S., Lin, C. T., Chow, L. P., Chen, C. T., & Hu, F. R. (2014a). Mechanisms controlling the effects of bevacizumab (avastin) on the inhibition of early but not late formed corneal neovascularization. *PLoS One 9*(4), e94205.

Chen, G., Xu, X., Zhang, L., Fu, Y., Wang, M., Gu, H., & Xie, X. (2014b). Blocking autocrine VEGF signaling by sunitinib, an anti-cancer drug, promotes embryonic stem cell self-renewal and somatic cell reprogramming. *Cell Res 24*(9), 1121–1136.

Chen, J. C., Chang, Y. W., Hong, C. C., Yu, Y. H., & Su, J. L. (2012). The role of the VEGF-C/VEGFRs axis in tumor progression and therapy. *Int J Mol Sci 14*(1), 88–107.

Cheung, A. M., Brown, A. S., Cucevic, V., Roy, M., Needles, A., Yang, V., ... Foster, F. S. (2007). Detecting vascular changes in tumour xenografts using micro-ultrasound and micro-ct following treatment with VEGFR-2 blocking antibodies. *Ultrasound Med Biol 33*(8), 1259–1268.

Chiron, M., Vrignaud, P., Lejeune, P., Demer, B., Leopold, W., & Bissery, M. (2007). Synergistic activity of aflibercept (VEGF Trap) in combination with 5-fluorouracil and irinotecan in preclinical tumor models. *Mol Cancer Ther*San Francisco, CA: AACR-NCI-EORTC.

Ciombor, K. K., & Berlin, J. (2014). Aflibercept–a decoy VEGF receptor. *Curr Oncol Rep 16*(2), 368.

Clarke, J. M., & Hurwitz, H. I. (2013). Targeted inhibition of VEGF receptor 2: An update on ramucirumab. *Expert Opin Biol Ther 13*(8), 1187–1196.

Cooke, V. G., LeBleu, V. S., Keskin, D., Khan, Z., O'Connell, J. T., Teng, Y., ... Kalluri, R. (2012). Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. *Cancer Cell 21*(1), 66–81.

Crawford, Y., & Ferrara, N. (2009). VEGF inhibition: insights from preclinical and clinical studies. *Cell Tissue Res 335*(1), 261–269.

Cruz-Munoz, W., Man, S., & Kerbel, R. S. (2009). Effective treatment of advanced human melanoma metastasis in immunodeficient mice using combination metronomic chemotherapy regimens. *Clin Cancer Res 15*(15), 4867–4874.

Davis, D. W., Inoue, K., Dinney, C. P., Hicklin, D. J., Abbruzzese, J. L., & McConkey, D. J. (2004). Regional effects of an antivascular endothelial growth factor receptor monoclonal antibody on receptor phosphorylation and apoptosis in human 253J B-V bladder cancer xenografts. *Cancer Res 64*(13), 4601–4610.

de Groot, J. F., Fuller, G., Kumar, A. J., Piao, Y., Eterovic, K., Ji, Y., & Conrad, C. A. (2010). Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. *Neuro-Oncology 12*(3), 233–242.

Demetri, G. D., van Oosterom, A. T., Garrett, C. R., Blackstein, M. E., Shah, M. H., Verweij, J., ... Casali, P. G. (2006). Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: A randomised controlled trial. *Lancet 368*(9544), 1329–1338.

Di Maio, M., Morabito, A., Piccirillo, M. C., Daniele, G., Giordano, P., Costanzo, R., ... Perrone, F. (2014). Combining anti-epidermal growth factor receptor (EGFR) and anti-angiogenic strategies in advanced NSCLC: We should have known better. *Curr Pharm Des 20*(24), 3901–3913.

di Tomaso, E., London, N., Fuja, D., Logie, J., Tyrrell, J. A., Kamoun, W., ... Jain, R. K. (2009). PDGF-C induces maturation of blood vessels in a model of glioblastoma and attenuates the response to anti-VEGF treatment. *PLoS One 4*(4), e5123.

Diao, Y., Tian, X. H., Huang, Y. L., Chen, L. K., Lin, X. N., & Zhuang, Z. W. (2010). Enhanced cancer therapy with the combination of EGFR and VEGFR-2 targeting in an orthotopic glioblastoma model. *J Chemother 22*(6), 407–412.

Dias, S., Hattori, K., Heissig, B., Zhu, Z., Wu, Y., Witte, L., ... Rafii, S. (2001). Inhibition of both paracrine and autocrine VEGF/VEGFR-2 signaling pathways is essential to induce long-term remission of xenotransplanted human leukemias. *Proc Natl Acad Sci U S A 98*(19), 10857–10862.

Dias, S., Hattori, K., Zhu, Z., Heissig, B., Choy, M., Lane, W., ... Rafii, S. (2000). Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. *J Clin Invest 106*(4), 511–521.

Ding, B. S., Nolan, D. J., Butler, J. M., James, D., Babazadeh, A. O., Rosenwaks, Z., ... Rafii, S. (2010). Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature 468*(7321), 310–315.

Domigan, C. K., & Iruela-Arispe, M. L. (2012). Recent advances in vascular development. *Curr Opin Hematol 19*(3), 176–183.

Domigan, C. K., Ziyad, S., & Iruela-Arispe, M. L. (2015). Canonical and noncanonical vascular endothelial growth factor pathways: New developments in biology and signal transduction. *Arterioscler Thromb Vasc Biol 35*(1), 30–39.

Donnem, T., Hu, J., Ferguson, M., Adighibe, O., Snell, C., Harris, A. L., ... Pezzella, F. (2013). Vessel co-option in primary human tumors and metastases: An obstacle to effective anti-angiogenic treatment? *Cancer Med 2*(4), 427–436.

Douglas, N. C., Tang, H., Gomez, R., Pytowski, B., Hicklin, D. J., Sauer, C. M., ... Zimmermann, R. C. (2009). Vascular endothelial growth factor receptor 2 (VEGFR-2) functions to promote uterine decidual angiogenesis during early pregnancy in the mouse. *Endocrinology 150*(8), 3845–3854.

du Manoir, J. M., Francia, G., Man, S., Mossoba, M., Medin, J. A., Viloria-Petit, A., ... Kerbel, R. S. (2006). Strategies for delaying or treating in vivo acquired resistance to trastuzumab in human breast cancer xenografts. *Clin Cancer Res 12*(3 Pt 1), 904–916.

Duda, D. G. (2012). Molecular biomarkers of response to antiangiogenic therapy for cancer. *ISRN Cell Biol 2012*.

Dvorak, H. F. (2015). Tumors: Wounds that do not heal—Redux. *Cancer Immunol Res 3*(1), 1–11.

Eichten, A., Adler, A. P., Cooper, B., Griffith, J., Wei, Y., Yancopoulos, G. D., ... Thurston, G. (2013). Rapid decrease in tumor perfusion following VEGF blockade predicts long-term tumor growth inhibition in preclinical tumor models. *Angiogenesis 16*(2), 429–441.

Escudier, B., Bellmunt, J., Negrier, S., Bajetta, E., Melichar, B., Bracarda, S., ... Sneller, V. (2010). Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): Final analysis of overall survival. *J Clin Oncol 28*(13), 2144–2150.

Facemire, C. S., Nixon, A. B., Griffiths, R., Hurwitz, H., & Coffman, T. M. (2009). Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. *Hypertension 54*(3), 652–658.

Fenton, B. M., Paoni, S. F., & Ding, I. (2004a). Effect of VEGF receptor-2 antibody on vascular function and oxygenation in spontaneous and transplanted tumors. *Radiother Oncol 72*(2), 221–230.

Fenton, B. M., Paoni, S. F., & Ding, I. (2004b). Pathophysiological effects of vascular endothelial growth factor receptor-2-blocking antibody plus fractionated radiotherapy on murine mammary tumors. *Cancer Res 64*(16), 5712–5719.

Fenton, B. M., Paoni, S. F., Grimwood, B., & Ding, I. (2005). Varied response of spontaneous tumors to antiangiogenic agents. *Adv Exp Med Biol 566*, 59–65.

Ferrara, N. (2002). VEGF and the quest for tumour angiogenesis factors. *Nat Rev Cancer 2*(10), 795–803.

Ferrara, N. (2010). Role of myeloid cells in vascular endothelial growth factor-independent tumor angiogenesis. *Curr Opin Hematol 17*(3), 219–224.

Ferrara, N., & Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun 161*(2), 851–858.

Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K. S., ... Moore, M. W. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature 380*(6573), 439–442.

Ferrara, N., Hillan, K. J., Gerber, H. P., & Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov 3*(5), 391–400.

Fischer, C., Jonckx, B., Mazzone, M., Zacchigna, S., Loges, S., Pattarini, L., ... Carmeliet, P. (2007). Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell 131*(3), 463–475.

Folkins, C., Man, S., Xu, P., Shaked, Y., Hicklin, D. J., & Kerbel, R. S. (2007). Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. *Cancer Res 67*(8), 3560–3564.

Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *N Engl J Med 285*(21), 1182–1186.

Folkman, J. (1975). Tumor angiogenesis: A possible control point in tumor growth. *Ann Intern Med 82*(1), 96–100.

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., & Semenza, G. L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol 16*(9), 4604–4613.

Francia, G., Emmenegger, U., Lee, C. R., Shaked, Y., Folkins, C., Mossoba, M., ... Kerbel, R. S. (2008). Long-term progression and therapeutic response of visceral metastatic disease non-invasively monitored in mouse urine using beta-human choriogonadotropin secreting tumor cell lines. *Mol Cancer Ther 7*(10), 3452–3459.

Franco, M., Man, S., Chen, L., Emmenegger, U., Shaked, Y., Cheung, A. M., ... Kerbel, R. S. (2006). Targeted anti-vascular endothelial growth factor receptor-2 therapy leads to short-term and long-term impairment of vascular function and increase in tumor hypoxia. *Cancer Res 66*(7), 3639–3648.

Franklin, M. C., Navarro, E. C., Wang, Y., Patel, S., Singh, P., Zhang, Y., ... Kussie, P. (2011). The structural basis for the function of two anti-VEGF receptor 2 antibodies. *Structure 19*(8), 1097–1107.

Fuchs, C. S., Tomasek, J., Yong, C. J., Dumitru, F., Passalacqua, R., Goswami, C., ... RECARD Trial Investigators (2014). Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomized, multicenter, placebo-controlled, phase 3 trial. *Lancet 383*(9911), 31–39.

Fukasawa, M., & Korc, M. (2004). Vascular endothelial growth factor-trap suppresses tumorigenicity of multiple pancreatic cancer cell lines. *Clin Cancer Res 10*(10), 3327–3332.

Fukumura, D., Ushiyama, A., Duda, D. G., Xu, L., Tam, J., Krishna, V., ... Jain, R. K. (2003). Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. *Circ Res 93*(9), e88–e97.

Garon, E. B., Ciuleanu, T. E., Arrieta, O., Prabhash, K., Syrigos, K. N., Goksel, T., ... Perol, M. (2014). Pamucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicenter, double-blind, randomized phase 3 trial. *Lancet 384*(9944), 665–673.

Gaya, A., & Tse, V. (2012). A preclinical and clinical review of aflibercept for the management of cancer. *Cancer Treat Rev 38*(5), 484–493.

Gerber, H. P., Kowalski, J., Sherman, D., Eberhard, D. A., & Ferrara, N. (2000). Complete inhibition of rhabdomyosarcoma xenograft growth and neovascularization requires blockade of both tumor and host vascular endothelial growth factor. *Cancer Res 60*(22), 6253–6258.

Gille, J., Heidenreich, R., Pinter, A., Schmitz, J., Boehme, B., Hicklin, D. J., ... Breier, G. (2007). Simultaneous blockade of VEGFR-1 and VEGFR-2 activation is necessary to efficiently inhibit experimental melanoma growth and metastasis formation. *Int J Cancer 120*(9), 1899–1908.

Goel, S., Duda, D. G., Xu, L., Munn, L. L., Boucher, Y., Fukumura, D., & Jain, R. K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev 91*(3), 1071–1121.

Goldman, J., Rutkowski, J. M., Shields, J. D., Pasquier, M. C., Cui, Y., Schmokel, H. G., ... Swartz, M. A. (2007). Cooperative and redundant roles of VEGFR-2 and VEGFR-3 signaling in adult lymphangiogenesis 1. *FASEB J 21*(4), 1003–1012.

Gomez-Manzano, C., Holash, J., Fueyo, J., Xu, J., Conrad, C. A., Aldape, K. D., ... Yung, W. K. (2008). VEGF Trap induces antiglioma effect at different stages of disease. *Neuro-Oncology 10*(6), 940–945.

Gotink, K. J., & Verheul, H. M. (2010). Anti-angiogenic tyrosine kinase inhibitors: What is their mechanism of action? *Angiogenesis 13*(1), 1–14.

Gremonprez, F., Descamps, B., Izmer, A., Vanhove, C., Vanhaecke, F., Wever, O., & Ceelen, W. (2015). Pretreatment with VEGF(R)-inhibitors reduces interstitial fluid pressure, increases intraperitoneal chemotherapy drug penetration, and impedes tumor growth in a mouse colorectal carcinomatosis model. *Oncotarget 6*(30), 29889–29900.

Guerin, E., Man, S., Xu, P., & Kerbel, R. S. (2013). A model of postsurgical advanced metastatic breast cancer more accurately replicates the clinical efficacy of antiangiogenic drugs. *Cancer Res 73*(9), 2743–2748.

Haiko, P., Makinen, T., Keskitalo, S., Taipale, J., Karkkainen, M. J., Baldwin, M. E., ... Alitalo, K. (2008). Deletion of vascular endothelial growth factor C (VEGF-C) and VEGF-D is not equivalent to VEGF receptor 3 deletion in mouse embryos. *Mol Cell Biol 28*(15), 4843–4850.

Hall, A. P., Westwood, F. R., & Wadsworth, P. F. (2006). Review of the effects of anti-angiogenic compounds on the epiphyseal growth plate. *Toxicol Pathol 34*(2), 131–147.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell 100*(1), 57–70.

Hansen-Algenstaedt, N., Stoll, B. R., Padera, T. P., Dolmans, D. E., Hicklin, D. J., Fukumura, D., & Jain, R. K. (2000). Tumor oxygenation in hormone-dependent tumors during vascular

endothelial growth factor receptor-2 blockade, hormone ablation, and chemotherapy. *Cancer Res 60*(16), 4556–4560.
Hassan, S., Buchanan, M., Jahan, K., Aguilar-Mahecha, A., Gaboury, L., Muller, W. J., ... Basik, M. (2011). CXCR4 peptide antagonist inhibits primary breast tumor growth, metastasis and enhances the efficacy of anti-VEGF treatment or docetaxel in a transgenic mouse model. *Int J Cancer 129*(1), 225–232.
Hatch, A. J., Clarke, J. M., Nixon, A. B., & Hurwitz, H. I. (2015). Identifying blood-based protein biomarkers for antiangiogenic agents in the clinic: A decade of progress. *Cancer J 21*(4), 322–326.
Hattori, K., Dias, S., Heissig, B., Hackett, N. R., Lyden, D., Tateno, M., ... Rafii, S. (2001). Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med 193*(9), 1005–1014.
Hedlund, E. M., Hosaka, K., Zhong, Z., Cao, R., & Cao, Y. (2009). Malignant cell-derived PlGF promotes normalization and remodeling of the tumor vasculature 2. *Proc Natl Acad Sci U S A 106*(41), 17505–17510.
Hedlund, E. M., Yang, X., Zhang, Y., Yang, Y., Shibuya, M., Zhong, W., ... Cao, Y. (2013). Tumor cell-derived placental growth factor sensitizes antiangiogenic and antitumor effects of anti-VEGF drugs. *Proc Natl Acad Sci U S A 110*(2), 654–659.
Heissig, B., Rafii, S., Akiyama, H., Ohki, Y., Sato, Y., Rafael, T., ... Hattori, K. (2005). Low-dose irradiation promotes tissue revascularization through VEGF release from mast cells and MMP-9-mediated progenitor cell mobilization. *J Exp Med 202*(6), 739–750.
Hicklin, D. J., & Ellis, L. M. (2005). Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol 23*(5), 1011–1027.
Hiratsuka, S., Minowa, O., Kuno, J., Noda, T., & Shibuya, M. (1998). Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. *Proc Natl Acad Sci U S A 95*(16), 9349–9354.
Hoff, B. A., Bhojani, M. S., Rudge, J., Chenevert, T. L., Meyer, C. R., Galban, S., ... Galban, C. J. (2012). DCE and DW-MRI monitoring of vascular disruption following VEGF-Trap treatment of a rat glioma model. *NMR Biomed 25*(7), 935–942.
Holash, J., Davis, S., Papadopoulos, N., Croll, S. D., Ho, L., Russell, M., ... Rudge, J. S. (2002). VEGF-Trap: A VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci U S A 99*(17), 11393–11398.
Hollanders, K., Van Bergen, T., Kindt, N., Castermans, K., Leysen, D., Vandewalle, E., ... Stalmans, I. (2015a). The effect of AMA0428, a novel and potent ROCK inhibitor, in a model of neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci 56*(2), 1335–1348.
Hollanders, K., Van Bergen, T., Van de Velde, S., Sijnave, D., Vandewalle, E., Moons, L., & Stalmans, I. (2015b). Bevacizumab revisited: its use in different mouse models of ocular pathologies. *Curr Eye Res 40*(6), 611–621.
Honek, J., Seki, T., Iwamoto, H., Fischer, C., Li, J., Lim, S., ... Cao, Y. (2014). Modulation of age-related insulin sensitivity by VEGF-dependent vascular plasticity in adipose tissues. *Proc Natl Acad Sci U S A 111*(41), 14906–14911.
Hong, Y. K., Lange-Asschenfeldt, B., Velasco, P., Hirakawa, S., Kunstfeld, R., Brown, L. F., ... Detmar, M. (2004). VEGF-A promotes tissue repair-associated lymphatic vessel formation via VEGFR-2 and the alpha1beta1 and alpha2beta1 integrins. *FASEB J 18*(10), 1111–1113.
Hooper, A. T., Butler, J. M., Nolan, D. J., Kranz, A., Iida, K., Kobayashi, M., ... Rafii, S. (2009). Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell 4*(3), 263–274.
Hoshida, T., Isaka, N., Hagendoorn, J., di Tomaso, E., Chen, Y. L., Pytowski, B., ... Jain, R. K. (2006). Imaging steps of lymphatic metastasis reveals that vascular endothelial growth factor-C increases metastasis by increasing delivery of cancer cells to lymph nodes: therapeutic implications. *Cancer Res 66*(16), 8065–8075.
Hu, L., Hofmann, J., Holash, J., Yancopoulos, G. D., Sood, A. K., & Jaffe, R. B. (2005). Vascular endothelial growth factor trap combined with paclitaxel strikingly inhibits tumor and ascites, prolonging survival in a human ovarian cancer model. *Clin Cancer Res 11*(19 Pt 1), 6966–6971.
Huang, H., Shen, J., & Vinores, S. A. (2011). Blockade of VEGFR1 and 2 suppresses pathological angiogenesis and vascular leakage in the eye. *PLoS One 6*(6), e21411.
Huang, Y., Goel, S., Duda, D. G., Fukumura, D., & Jain, R. K. (2013). Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res 73*(10), 2943–2948.
Huang, Y., Yuan, J., Righi, E., Kamoun, W. S., Ancukiewicz, M., Nezivar, J., ... Poznansky, M. C. (2012). Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci U S A 109*(43), 17561–17566.
Hunt, S. (2001). Technology evaluation: IMC-1C11, ImClone systems. *Curr Opin Mol Ther 3*(4), 418–424.
Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., ... Kabbinavar, F. (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med 350*(23), 2335–2342.
Inai, T., Mancuso, M., Hashizume, H., Baffert, F., Haskell, A., Baluk, P., ... McDonald, D. M. (2004). Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. *Am J Pathol 165*(1), 35–52.
Inoue, K., Slaton, J. W., Davis, D. W., Hicklin, D. J., McConkey, D. J., Karashima, T., ... Dinney, C. P. (2000). Treatment of human metastatic transitional cell carcinoma of the bladder in a murine model with the anti-vascular endothelial growth factor receptor monoclonal antibody DC101 and paclitaxel. *Clin Cancer Res 6*(7), 2635–2643.
Izumi, Y., di, T. E., Hooper, A., Huang, P., Huber, J., Hicklin, D. J., ... Suit, H. D. (2003). Responses to antiangiogenesis treatment of spontaneous autochthonous tumors and their isografts. *Cancer Res 63*(4), 747–751.
Jabbour, H. N., Kelly, R. W., Fraser, H. M., & Critchley, H. O. (2006). Endocrine regulation of menstruation. *Endocr Rev 27*(1), 17–46.
Jain, R. K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science 307*(5706), 58–62.
Jauhiainen, S., Hakkinen, S. K., Toivanen, P. I., Heinonen, S. E., Jyrkkanen, H. K., Kansanen, E. , ... Yla-Herttuala, S. (2011). Vascular endothelial growth factor (VEGF)-D stimulates VEGF-A, stanniocalcin-1, and neuropilin-2 and has potent angiogenic effects. *Arterioscler Thromb Vasc Biol 31*(7), 1617–1624.
Jeltsch, M., Leppanen, V. M., Saharinen, P., & Alitalo, K. (2013). Receptor tyrosine kinase-mediated angiogenesis. *Cold Spring Harb Perspect Biol 5*(9).
Johnson, L., Huseni, M., Smyczek, T., Lima, A., Yeung, S., Cheng, J. H., ... Hegde, P. S. (2013). Anti-EGFL7 antibodies enhance stress-induced endothelial cell death and anti-VEGF efficacy. *J Clin Invest 123*(9), 3997–4009.
Jugold, M., Palmowski, M., Huppert, J., Woenne, E. C., Mueller, M. M., Semmler, W., & Kiessling, F. (2008). Volumetric high-frequency Doppler ultrasound enables the assessment of early antiangiogenic therapy effects on tumor xenografts in nude mice. *Eur Radiol 18*(4), 753–758.
Jung, Y. D., Mansfield, P. F., Akagi, M., Takeda, A., Liu, W., Bucana, C. D., ... Ellis, L. M. (2002). Effects of combination anti-vascular endothelial growth factor receptor and anti-epidermal growth factor receptor therapies on the growth of gastric cancer in a nude mouse model. *Eur J Cancer 38*(8), 1133–1140.
Kadambi, A., Carreira, C. M., Yun, C. O., Padera, T. P., Dolmans, D. E., Carmeliet, P., ... Jain, R. K. (2001). Vascular endothelial growth factor (VEGF)-C differentially affects tumor vascular function and leukocyte recruitment: Role of VEGF-receptor 2 and host VEGF-A. *Cancer Res 61*(6), 2404–2408.
Kamba, T., & McDonald, D. M. (2007). Mechanisms of adverse effects of anti-VEGF therapy for cancer. *Br J Cancer 96*(12), 1788–1795.
Kamba, T., Tam, B. Y., Hashizume, H., Haskell, A., Sennino, B., Mancuso, M. R., ... McDonald, D. M. (2006). VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol 290*(2), H560–H576.
Kappas, N. C., Zeng, G., Chappell, J. C., Kearney, J. B., Hazarika, S., Kallianos, K. G., ... Bautch, V. L. (2008). The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching. *J Cell Biol 181*(5), 847–858.
Karkkainen, M. J., Haiko, P., Sainio, K., Partanen, J., Taipale, J., Petrova, T. V., ... Alitalo, K. (2004). Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. *Nat Immunol 5*(1), 74–80.
Karoor, V., Le, M., Merrick, D., Dempsey, E. C., & Miller, Y. E. (2010). Vascular endothelial growth factor receptor 2-targeted chemoprevention of murine lung tumors. *Cancer Prev Res (Phila) 3*(9), 1141–1147.
Kerbel, R. S., & Kamen, B. A. (2004). The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer 4*(6), 423–436.
Kienast, Y., Klein, C., Scheuer, W., Raemsch, R., Lorenzon, E., Bernicke, D., ... Thomas, M. (2013). Ang-2-VEGF-A CrossMab, a novel bispecific human IgG1 antibody blocking VEGF-A and Ang-2 functions simultaneously, mediates potent antitumor, antiangiogenic, and antimetastatic efficacy. *Clin Cancer Res 19*(24), 6730–6740.
Kiessling, F., Farhan, N., Lichy, M. P., Vosseler, S., Heilmann, M., Krix, M., ... Delorme, S. (2004). Dynamic contrast-enhanced magnetic resonance imaging rapidly indicates vessel regression in human squamous cell carcinomas grown in nude mice caused by VEGF receptor 2 blockade with DC101. *Neoplasia 6*(3), 213–223.
Kim, J., Kim, T. E., Kim, J. A., Yun, J. H., Sohn, S., Shim, S. R., ... Kim, S. J. (2014). Intravitreal tanibirumab, a fully human monoclonal antibody against vascular endothelial growth factor receptor 2, partially suppresses and regresses laser-induced choroidal neovascularization in a rat model. *J Ocul Pharmacol Ther 30*(10), 847–853.
Klement, G., Baruchel, S., Rak, J., Man, S., Clark, K., Hicklin, D. J., ... 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*(8), R15–R24.
Klement, G., Huang, P., Mayer, B., Green, S. K., Man, S., Bohlen, P., ... Kerbel, R. S. (2002). Differences in therapeutic indexes of combination metronomic chemotherapy and an anti-VEGFR-2 antibody in multidrug-resistant human breast cancer xenografts. *Clin Cancer Res 8*(1), 221–232.
Koch, S., & Claesson-Welsh, L. (2012). Signal transduction by vascular endothelial growth factor receptors. *Cold Spring Harb Perspect Med 2*(7), a006502.
Kodack, D. P., Chung, E., Yamashita, H., Incio, J., Duyverman, A. M., Song, Y., ... Jain, R. K. (2012). Combined targeting of HER2 and VEGFR2 for effective treatment of HER2-amplified breast cancer brain metastases. *Proc Natl Acad Sci U S A 109*(45), E3119–E3127.
Kopetz, S., Hoff, P. M., Morris, J. S., Wolff, R. A., Eng, C., Glover, K. Y., ... Heymach, J. V. (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*(3), 453–459.
Korsisaari, N., Kasman, I. M., Forrest, W. F., Pal, N., Bai, W., Fuh, G., ... Ferrara, N. (2007). Inhibition of VEGF-A prevents the angiogenic switch and results in increased survival of Apc+/min mice. *Proc Natl Acad Sci U S A 104*(25), 10625–10630.
Korsisaari, N., Ross, J., Wu, X., Kowanetz, M., Pal, N., Hall, L., ... Ferrara, N. (2008). Blocking vascular endothelial growth factor-A inhibits the growth of pituitary adenomas and lowers serum prolactin level in a mouse model of multiple endocrine neoplasia type 1. *Clin Cancer Res 14*(1), 249–258.
Kozin, S. V., Boucher, Y., Hicklin, D. J., Bohlen, P., Jain, R. K., & Suit, H. D. (2001). Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. *Cancer Res 61*(1), 39–44.
Kozin, S. V., Winkler, F., Garkavtsev, I., Hicklin, D. J., Jain, R. K., & Boucher, Y. (2007). Human tumor xenografts recurring after radiotherapy are more sensitive to anti-vascular endothelial growth factor receptor-2 treatment than treatment-naive tumors. *Cancer Res 67*(11), 5076–5082.

Krishnamurthy, S., Dong, Z., Vodopyanov, D., Imai, A., Helman, J. I., Prince, M. E., ... Nor, J. E. (2010). Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells. *Cancer Res 70*(23), 9969–9978.

Krix, M., Kiessling, F., Vosseler, S., Farhan, N., Mueller, M. M., Bohlen, P., ... Delorme, S. (2003). Sensitive noninvasive monitoring of tumor perfusion during antiangiogenic therapy by intermittent bolus-contrast power Doppler sonography. *Cancer Res 63*(23), 8264–8270.

Kunkel, P., Ulbricht, U., Bohlen, P., Brockmann, M. A., Fillbrandt, R., Stavrou, D., ... Lamszus, K. (2001). Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor-2. *Cancer Res 61*(18), 6624–6628.

Laakkonen, P., Waltari, M., Holopainen, T., Takahashi, T., Pytowski, B., Steiner, P., ... Alitalo, K. (2007). Vascular endothelial growth factor receptor 3 is involved in tumor angiogenesis and growth. *Cancer Res 67*(2), 593–599.

Lamszus, K., Brockmann, M. A., Eckerich, C., Bohlen, P., May, C., Mangold, U., ... Westphal, M. (2005). Inhibition of glioblastoma angiogenesis and invasion by combined treatments directed against vascular endothelial growth factor receptor-2, epidermal growth factor receptor, and vascular endothelial-cadherin. *Clin Cancer Res 11*(13), 4934–4940.

Lanitis, E., Irving, M., & Coukos, G. (2015). Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol 33*, 55–63.

Le, X. F., Mao, W., Lu, C., Thornton, A., Heymach, J. V., Sood, A. K., & Bast, R. C., Jr. (2008). Specific blockade of VEGF and HER2 pathways results in greater growth inhibition of breast cancer xenografts that overexpress HER2. *Cell Cycle 7*(23), 3747–3758.

Lee, S. H. (2011). Tanibirumab (TTAC-0001): A fully human monoclonal antibody targets vascular endothelial growth factor receptor 2 (VEGFR-2). *Arch Pharm Res 34*(8), 1223–1226.

Leppanen, V. M., Jeltsch, M., Anisimov, A., Tvorogov, D., Aho, K., Kalkkinen, N., ... Alitalo, K. (2011). Structural determinants of vascular endothelial growth factor-D receptor binding and specificity. *Blood 117*(5), 1507–1515.

Leppanen, V. M., Prota, A. E., Jeltsch, M., Anisimov, A., Kalkkinen, N., Strandin, T., ... Alitalo, K. (2010). Structural determinants of growth factor binding and specificity by VEGF receptor 2. *Proc Natl Acad Sci U S A 107*(6), 2425–2430.

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V., & Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science 246*(4935), 1306–1309.

Li, J., Huang, S., Armstrong, E. A., Fowler, J. F., & Harari, P. M. (2005). Angiogenesis and radiation response modulation after vascular endothelial growth factor receptor-2 (VEGFR2) blockade. *Int J Radiat Oncol Biol Phys 62*(5), 1477–1485.

Liang, W. C., Wu, X., Peale, F. V., Lee, C. V., Meng, Y. G., Gutierrez, J., ... Fuh, G. (2006). Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF. *J Biol Chem 281*(2), 951–961.

Liu, Y., Cox, S. R., Morita, T., & Kourembanas, S. (1995). Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5′ enhancer. *Circ Res 77*(3), 638–643.

Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., ... S. I. S. Group (2008). Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med 359*(4), 378–390.

Loges, S., Schmidt, T., & Carmeliet, P. (2010). Mechanisms of resistance to anti-angiogenic therapy and development of third-generation anti-angiogenic drug candidates. *Genes Cancer 1*(1), 12–25.

Lohela, M., Bry, M., Tammela, T., & Alitalo, K. (2009). VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol 21*(2), 154–165.

Lu, K. V., Chang, J. P., Parachoniak, C. A., Pandika, M. M., Aghi, M. K., Meyronet, D., ... Bergers, G. (2012). VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. *Cancer Cell 22*(1), 21–35.

Lu, D., Jimenez, X., Zhang, H., Bohlen, P., Witte, L., & Zhu, Z. (2002). Selection of high affinity human neutralizing antibodies to VEGFR2 from a large antibody phage display library for antiangiogenesis therapy. *Int J Cancer 97*(3), 393–399.

Lu, C., Shahzad, M. M., Moreno-Smith, M., Lin, Y. G., Jennings, N. B., Allen, J. K., ... Sood, A. K. (2010). Targeting pericytes with a PDGF-B aptamer in human ovarian carcinoma models. *Cancer Biol Ther 9*(3), 176–182.

Lu, D., Shen, J., Vil, M. D., Zhang, H., Jimenez, X., Bohlen, P., ... Zhu, Z. (2003). Tailoring in vitro selection for a picomolar affinity human antibody directed against vascular endothelial growth factor receptor 2 for enhanced neutralizing activity. *J Biol Chem 278*(44), 43496–43507.

Luttun, A., Tjwa, M., Moons, L., Wu, Y., Angelillo-Scherrer, A., Liao, F., ... Carmeliet, P. (2002). Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med 8*(8), 831–840.

Lutty, G. A., McLeod, D. S., Bhutto, I., & Wiegand, S. J. (2011). Effect of VEGF trap on normal retinal vascular development and oxygen-induced retinopathy in the dog. *Invest Ophthalmol Vis Sci 52*(7), 4039–4047.

Mackey, J. R., Ramos-Vazquez, M., Lipatov, O., McCarthy, N., Krasnozhon, D., Semiglazov, V., ... Martin, M. (2015). Primary results of ROSE/TRIO-12, a randomized placebo-controlled phase III trial evaluating the addition of ramucirumab to first-line docetaxel chemotherapy in metastatic breast cancer. *J Clin Oncol 33*(2), 141–148.

Magnusson, P., Rolny, C., Jakobsson, L., Wikner, C., Wu, Y., Hicklin, D. J., & Claesson-Welsh, L. (2004). Deregulation of Flk-1/vascular endothelial growth factor receptor-2 in fibroblast growth factor receptor-1-deficient vascular stem cell development. *J Cell Sci 117*(Pt 8), 1513–1523.

Maione, F., Capano, S., Regano, D., Zentilin, L., Giacca, M., Casanovas, O., ... Giraudo, E. (2012). Semaphorin 3A overcomes cancer hypoxia and metastatic dissemination induced by antiangiogenic treatment in mice. *J Clin Invest 122*(5), 1832–1848.

Man, S., Bocci, G., Francia, G., Green, S. K., Jothy, S., Hanahan, D., ... Kerbel, R. S. (2002). Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res 62*(10), 2731–2735.

Mancuso, M. R., Davis, R., Norberg, S. M., O'Brien, S., Sennino, B., Nakahara, T., ... McDonald, D. M. (2006). Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J Clin Invest 116*(10), 2610–2621.

Mangoni, M., Vozenin, M. C., Biti, G., & Deutsch, E. (2012). Normal tissues toxicities triggered by combined anti-angiogenic and radiation therapies: Hurdles might be ahead. *Br J Cancer 107*(2), 308–314.

Manning, E. A., Ullman, J. G., Leatherman, J. M., Asquith, J. M., Hansen, T. R., Armstrong, T. D., ... Emens, L. A. (2007). A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. *Clin Cancer Res 13*(13), 3951–3959.

Matthews, W., Jordan, C. T., Gavin, M., Jenkins, N. A., Copeland, N. G., & Lemischka, I. R. (1991). A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. *Proc Natl Acad Sci U S A 88*(20), 9026–9030.

McGrath-Morrow, S. A., Cho, C., Cho, C., Zhen, L., Hicklin, D. J., & Tuder, R. M. (2005). Vascular endothelial growth factor receptor 2 blockade disrupts postnatal lung development. *Am J Respir Cell Mol Biol 32*(5), 420–427.

McGrath-Morrow, S., Cho, C., Molls, R., Burne-Taney, M., Haas, M., Hicklin, D. J., ... Rabb, H. (2006). VEGF receptor 2 blockade leads to renal cyst formation in mice. *Kidney Int 69*(10), 1741–1748.

McLeod, D. S., Taomoto, M., Cao, J., Zhu, Z., Witte, L., & Lutty, G. A. (2002). Localization of VEGF receptor-2 (KDR/Flk-1) and effects of blocking it in oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci 43*(2), 474–482.

Miao, H. Q., Hu, K., Jimenez, X., Navarro, E., Zhang, H., Lu, D., ... Zhu, Z. (2006). Potent neutralization of VEGF biological activities with a fully human antibody Fab fragment directed against VEGF receptor 2. *Biochem Biophys Res Commun 345*(1), 438–445.

Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N. P., Risau, W., & Ullrich, A. (1993). High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell 72*(6), 835–846.

Miller, D. W., Vosseler, S., Mirancea, N., Hicklin, D. J., Bohlen, P., Volcker, H. E., ... Fusenig, N. E. (2005). Rapid vessel regression, protease inhibition, and stromal normalization upon short-term vascular endothelial growth factor receptor 2 inhibition in skin carcinoma heterotransplants. *Am J Pathol 167*(5), 1389–1403.

Motzer, R. J., Hutson, T. E., Tomczak, P., Michaelson, M. D., Bukowski, R. M., Oudard, S., ... Figlin, R. A. (2009). Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol 27*(22), 3584–3590.

Murukesh, N., Dive, C., & Jayson, G. C. (2010). Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. *Br J Cancer 102*(1), 8–18.

Myers, A. L., Williams, R. F., Ng, C. Y., Hartwich, J. E., & Davidoff, A. M. (2010). Bevacizumab-induced tumor vessel remodeling in rhabdomyosarcoma xenografts increases the effectiveness of adjuvant ionizing radiation. *J Pediatr Surg 45*(6), 1080–1085.

Nakahara, T., Norberg, S. M., Shalinsky, D. R., Hu-Lowe, D. D., & McDonald, D. M. (2006). Effect of inhibition of vascular endothelial growth factor signaling on distribution of extravasated antibodies in tumors. *Cancer Res 66*(3), 1434–1445.

Ni, A., Lashnits, E., Yao, L. C., Baluk, P., & McDonald, D. M. (2010). Rapid remodeling of airway vascular architecture at birth. *Dev Dyn 239*(9), 2354–2366.

Nilsson, I., Bahram, F., Li, X., Gualandi, L., Koch, S., Jarvius, M., ... Claesson-Welsh, L. (2010). VEGF receptor 2/-3 heterodimers detected in situ by proximity ligation on angiogenic sprouts. *EMBO J 29*(8), 1377–1388.

O'Connor, J. P., Carano, R. A., Clamp, A. R., Ross, J., Ho, C. C., Jackson, A., ... Jayson, G. C. (2009). Quantifying antivascular effects of monoclonal antibodies to vascular endothelial growth factor: Insights from imaging. *Clin Cancer Res 15*(21), 6674–6682.

Ohki, Y., Heissig, B., Sato, Y., Akiyama, H., Zhu, Z., Hicklin, D. J., ... Ohsaka, A. (2005). Granulocyte colony-stimulating factor promotes neovascularization by releasing vascular endothelial growth factor from neutrophils. *FASEB J 19*(14), 2005–2007.

Oladipupo, S. S., Hu, S., Santeford, A. C., Yao, J., Kovalski, J. R., Shohet, R. V., ... Arbeit, J. M. (2011). Conditional HIF-1 induction produces multistage neovascularization with stage-specific sensitivity to VEGFR inhibitors and myeloid cell independence. *Blood 117*(15), 4142–4153.

Olsson, A. K., Dimberg, A., Kreuger, J., & Claesson-Welsh, L. (2006). VEGF receptor signalling — In control of vascular function. *Nat Rev Mol Cell Biol 7*(5), 359–371.

Ott, P. A., Hodi, F. S., & 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.

Paez-Ribes, M., Allen, E., Hudock, J., Takeda, T., Okuyama, H., Vinals, F., ... Casanovas, O. (2009). Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell 15*(3), 220–231.

Papadopoulos, N., Martin, J., Ruan, Q., Rafique, A., Rosconi, M. P., Shi, E., ... Wiegand, S. J. (2012). Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis 15*(2), 171–185.

Patel, D., Bassi, R., Hooper, A. T., Sun, H., Huber, J., Hicklin, D. J., & Kang, X. (2008). Enhanced suppression of melanoma tumor growth and metastasis by combined therapy with anti-VEGF receptor and anti-TYRP-1/gp75 monoclonal antibodies. *Anticancer Res 28*(5 A), 2679–2686.

Pauli, S. A., Tang, H., Wang, J., Bohlen, P., Posser, R., Hartman, T., ... Zimmermann, R. C. (2005). The vascular endothelial growth factor (VEGF)/VEGF receptor 2 pathway is critical for blood vessel survival in corpora lutea of pregnancy in the rodent. *Endocrinology 146*(3), 1301–1311.

Penson, R. T., Huang, H. Q., Wenzel, L. B., Monk, B. J., Stockman, S., Long, H. J., III, ... Tewari, K. S. (2015). Bevacizumab for advanced cervical cancer: Patient-reported outcomes of a randomised, phase 3 trial (NRG Oncology-Gynecologic Oncology Group protocol 240). *Lancet Oncol 16*(3), 301–311.

Persaud, K., Tille, J. C., Liu, M., Zhu, Z., Jimenez, X., Pereira, D. S., ... Pytowski, B. (2004). Involvement of the VEGF receptor 3 in tubular morphogenesis demonstrated with a human anti-human VEGFR-3 monoclonal antibody that antagonizes receptor activation by VEGF-C. *J Cell Sci 117*(Pt 13), 2745–2756.

Pettersson, A., Nagy, J. A., Brown, L. F., Sundberg, C., Morgan, E., Jungles, S., ... Dvorak, H. F. (2000). Heterogeneity of the angiogenic response induced in different normal adult tissues by vascular permeability factor/vascular endothelial growth factor. *Lab Investig 80*(1), 99–115.

Posey, J. A., Ng, T. C., Yang, B., Khazaeli, M. B., Carpenter, M. D., Fox, F., ... LoBuglio, A. F. (2003). A phase I study of anti-kinase insert domain-containing receptor antibody, IMC-1C11, in patients with liver metastases from colorectal carcinoma. *Clin Cancer Res 9*(4), 1323–1332.

Potente, M., Gerhardt, H., & Carmeliet, P. (2011). Basic and therapeutic aspects of angiogenesis. *Cell 146*(6), 873–887.

Prewett, M., Huber, J., Li, Y., Santiago, A., O'Connor, W., King, K., ... Hicklin, D. J. (1999). Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res 59*(20), 5209–5218.

Pytowski, B., Goldman, J., Persaud, K., Wu, Y., Witte, L., Hicklin, D. J., ... Swartz, M. A. (2005). Complete and specific inhibition of adult lymphatic regeneration by a novel VEGFR-3 neutralizing antibody. *J Natl Cancer Inst 97*(1), 14–21.

Raisky, O., Nykanen, A. I., Krebs, R., Hollmen, M., Keranen, M. A., Tikkanen, J. M., ... Lemstrom, K. B. (2007). VEGFR-1 and -2 regulate inflammation, myocardial angiogenesis, and arteriosclerosis in chronically rejecting cardiac allografts. *Arterioscler Thromb Vasc Biol 27*(4), 819–825.

Ranieri, G., Mammi, M., Donato Di Paola, E., Russo, E., Gallelli, L., Citraro, R., ... De Sarro, G. (2014). Pazopanib a tyrosine kinase inhibitor with strong anti-angiogenetic activity: a new treatment for metastatic soft tissue sarcoma. *Crit Rev Oncol Hematol 89*(2), 322–329.

Raymond, E., Dahan, L., Raoul, J. L., Bang, Y. J., Borbath, I., Lombard-Bohas, C., ... Ruszniewski, P. (2011). Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. *N Engl J Med 364*(6), 501–513.

Reymond, N., d'Agua, B. B., & Ridley, A. J. (2013). Crossing the endothelial barrier during metastasis. *Nat Rev Cancer 13*(12), 858–870.

Rhim, A. D., Oberstein, P. E., Thomas, D. H., Mirek, E. T., Palermo, C. F., Sastra, S. A., ... Stanger, B. Z. (2014). Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell 25*(6), 735–747.

Rigamonti, N., Kadioglu, E., Keklikoglou, I., Wyser Rmili, C., Leow, C. C., & De Palma, M. (2014). Role of angiopoietin-2 in adaptive tumor resistance to VEGF signaling blockade. *Cell Rep 8*(3), 696–706.

Rini, B. I., Michaelson, M. D., Rosenberg, J. E., Bukowski, R. M., Sosman, J. A., Stadler, W. M., ... George, D. J. (2008). Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol 26*(22), 3743–3748.

Rivera, L. B., & Bergers, G. (2015a). CANCER. Tumor angiogenesis, from foe to friend. *Science 349*(6249), 694–695.

Rivera, L. B., & Bergers, G. (2015b). Intertwined regulation of angiogenesis and immunity by myeloid cells. *Trends Immunol 36*(4), 240–249.

Rivera, L. B., Meyronet, D., Hervieu, V., Frederick, M. J., Bergsland, E., & Bergers, G. (2015). Intratumoral myeloid cells regulate responsiveness and resistance to antiangiogenic therapy. *Cell Rep 11*(4), 577–591.

Roberts, N., Kloos, B., Cassella, M., Podgrabinska, S., Persaud, K., Wu, Y., ... Skobe, M. (2006). Inhibition of VEGFR-3 activation with the antagonistic antibody more potently suppresses lymph node and distant metastases than inactivation of VEGFR-2. *Cancer Res 66*(5), 2650–2657.

Robinson, S. D., Reynolds, L. E., Wyder, L., Hicklin, D. J., & Hodivala-Dilke, K. M. (2004). Beta3-integrin regulates vascular endothelial growth factor-A-dependent permeability. *Arterioscler Thromb Vasc Biol 24*(11), 2108–2114.

Rockwell, P., Neufeld, G., Glassman, A., Caron, D., & Goldstein, N. (1995). *In vitro* neutralization of vascular endothelial growth factor activation of Flk-1 by a monoclonal antibody. *Mol Cell Differ 3*(1), 91–109.

Ruch, C., Skiniotis, G., Steinmetz, M. O., Walz, T., & Ballmer-Hofer, K. (2007). Structure of a VEGF-VEGF receptor complex determined by electron microscopy. *Nat Struct Mol Biol 14*(3), 249–250.

Sakurai, M. K., Lee, S., Arsenault, D. A., Nose, V., Wilson, J. M., Heymach, J. V., & Puder, M. (2007). Vascular endothelial growth factor accelerates compensatory lung growth after unilateral pneumonectomy. *Am J Physiol Lung Cell Mol Physiol 292*(3), L742–L747.

Semenza, G. (2007). Regulation of tissue perfusion in mammals by hypoxia-inducible factor 1. *Exp Physiol 92*(6), 988–991.

Semenza, G. (2013). HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J Clin Invest 123*(9), 3664–3671.

Sandler, A., Gray, R., Perry, M. C., Brahmer, J., Schiller, J. H., Dowlati, A., ... Johnson, D. H. (2006). Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med 355*(24), 2542–2550.

Senger, D. R., Galli, S. J., Dvorak, A. M., Perruzzi, C. A., Harvey, V. S., & Dvorak, H. F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science 219*(4587), 983–985.

Senger, D. R., Perruzzi, C. A., Feder, J., & Dvorak, H. F. (1986). A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. *Cancer Res 46*(11), 5629–5632.

Sennino, B., Ishiguro-Oonuma, T., Wei, Y., Naylor, R. M., Williamson, C. W., Bhagwandin, V., ... McDonald, D. M. (2012). Suppression of tumor invasion and metastasis by concurrent inhibition of c-Met and VEGF signaling in pancreatic neuroendocrine tumors. *Cancer Discov 2*(3), 270–287.

Shaheen, R. M., Ahmad, S. A., Liu, W., Reinmuth, N., Jung, Y. D., Tseng, W. W., ... Ellis, L. M. (2001a). Inhibited growth of colon cancer carcinomatosis by antibodies to vascular endothelial and epidermal growth factor receptors. *Br J Cancer 85*(4), 584–589.

Shaheen, R. M., Tseng, W. W., Vellagas, R., Liu, W., Ahmad, S. A., Jung, Y. D., ... Ellis, L. M. (2001b). Effects of an antibody to vascular endothelial growth factor receptor-2 on survival, tumor vascularity, and apoptosis in a murine model of colon carcinomatosis. *Int J Oncol 18*(2), 221–226.

Shaked, Y., Bertolini, F., Man, S., Rogers, M. S., Cervi, D., Foutz, T., ... Kerbel, R. S. (2005). Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell 7*(1), 101–111.

Shaked, Y., Ciarrocchi, A., Franco, M., Lee, C. R., Man, S., Cheung, A. M., ... Kerbel, R. S. (2006). Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. *Science 313*(5794), 1785–1787.

Shaked, Y., Henke, E., Roodhart, J. M., Mancuso, P., Langenberg, M. H., Colleoni, M., ... Kerbel, R. S. (2008). Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell 14*(3), 263–273.

Shaked, Y., McAllister, S., Fainaru, O., & Almog, N. (2014). Tumor dormancy and the angiogenic switch: possible implications of bone marrow- derived cells. *Curr Pharm Des 20*(30), 4920–4933.

Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L., & Schuh, A. C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature 376*(6535), 62–66.

Shen, J., Vil, M. D., Prewett, M., Damoci, C., Zhang, H., Li, H., ... Zhu, Z. (2009). Development of a fully human anti-PDGFRbeta antibody that suppresses growth of human tumor xenografts and enhances antitumor activity of an anti-VEGFR2 antibody. *Neoplasia 11*(6), 594–604.

Shen, J., Vil, M. D., Zhang, H., Tonra, J. R., Rong, L. L., Damoci, C., ... Zhu, Z. (2007). An antibody directed against PDGF receptor beta enhances the antitumor and the anti-angiogenic activities of an anti-VEGF receptor 2 antibody. *Biochem Biophys Res Commun 357*(4), 1142–1147.

Shibuya, M. (2013a). Vascular endothelial growth factor and its receptor system: Physiological functions in angiogenesis and pathological roles in various diseases. *J Biochem 153*(1), 13–19.

Shibuya, M. (2013b). VEGFR and type-V RTK activation and signaling. *Cold Spring Harb Perspect Biol 5*(10), a009092.

Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J., & Klagsbrun, M. (1984). Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. *Science 223*(4642), 1296–1299.

Sho, M., Akashi, S., Kanehiro, H., Hamada, K., Kashizuka, H., Ikeda, N., ... Nakajima, Y. (2005). Function of the vascular endothelial growth factor receptors Flt-1 and Flk-1/KDR in the alloimmune response in vivo. *Transplantation 80*(6), 717–722.

Shojaei, F., Wu, X., Malik, A. K., Zhong, C., Baldwin, M. E., Schanz, S., ... Ferrara, N. (2007). Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol 25*(8), 911–920.

Shrimali, R. K., Yu, Z., Theoret, M. R., Chinnasamy, D., Restifo, N. P., & Rosenberg, S. A. (2010). Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res 70*(15), 6171–6180.

Shweiki, D., Itin, A., Soffer, D., & Keshet, E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature 359*(6398), 843–845.

Singh, M., Couto, S. S., Forrest, W. F., Lima, A., Cheng, J. H., Molina, R., ... Johnson, L. (2012). Anti-VEGF antibody therapy does not promote metastasis in genetically engineered mouse tumour models. *J Pathol 227*(4), 417–430.

Sitohy, B., Nagy, J. A., Jaminet, S. C., & Dvorak, H. F. (2011). Tumor-surrogate blood vessel subtypes exhibit differential susceptibility to anti-VEGF therapy. *Cancer Res 71*(22), 7021–7028.

Skobe, M., Rockwell, P., Goldstein, N., Vosseler, S., & Fusenig, N. E. (1997). Halting angiogenesis suppresses carcinoma cell invasion. *Nat Med 3*(11), 1222–1227.

Smith, N. R., Baker, D., Farren, M., Pommier, A., Swann, R., Wang, X., ... Barry, S. T. (2013). Tumor stromal architecture can define the intrinsic tumor response to VEGF-targeted therapy. *Clin Cancer Res 19*(24), 6943–6956.

Sorensen, A. G., Emblem, K. E., Polaskova, P., Jennings, D., Kim, H., Ancukiewicz, M., ... Jain, R. K. (2012). Increased survival of glioblastoma patients who respond to antiangiogenic therapy with elevated blood perfusion. *Cancer Res 72*(2), 402–407.

Soriano, P. (1994). Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev 8*(16), 1888–1896.

Stantz, K. M., Cao, M., Cao, N., Liang, Y., & Miller, K. D. (2011). Monitoring the longitudinal intra-tumor physiological impulse response to VEGFR2 blockade in breast tumors using DCE-CT. *Mol Imaging Biol 13*(6), 1183–1195.

Stockler, M. R., Hilpert, F., Friedlander, M., King, M. T., Wenzel, L., Lee, C. K., ... Pujade-Lauraine, E. (2014). Patient-reported outcome results from the open-label phase III AURELIA trial evaluating bevacizumab-containing therapy for platinum-resistant ovarian cancer. *J Clin Oncol 32*(13), 1309–1316.

Stoelcker, B., Echtenacher, B., Weich, H. A., Sztajer, H., Hicklin, D. J., & Mannel, D. N. (2000). VEGF/Flk-1 interaction, a requirement for malignant ascites recurrence. *J Interf Cytokine Res 20*(5), 511–517.

Swartz, A. M., Li, Q. J., & Sampson, J. H. (2014). Rindopepimut: a promising immunotherapeutic for the treatment of glioblastoma multiforme. *Immunotherapy 6*(6), 679–690.

Sweeney, P., Karashima, T., Kim, S. J., Kedar, D., Mian, B., Huang, S., ... Dinney, C. P. (2002). Anti-vascular endothelial growth factor receptor 2 antibody reduces tumorigenicity and metastasis in orthotopic prostate cancer xenografts via induction of endothelial cell apoptosis and reduction of endothelial cell matrix metalloproteinase type 9 production. *Clin Cancer Res 8*(8), 2714–2724.

Tabernero, J., Yoshino, T., Cohn, A. L., Obermannova, R., Bodoky, G., Garcia-Carbonero, R., ... RAISE Study Investigators (2015). *Lancet Oncol 16*(5), 499–508.

Tam, B. Y., Wei, K., Rudge, J. S., Hoffman, J., Holash, J., Park, S. K., ... Kuo, C. J. (2006). VEGF modulates erythropoiesis through regulation of adult hepatic erythropoietin synthesis. *Nat Med 12*(7), 793–800.

Tammela, T., & Alitalo, K. (2010). Lymphangiogenesis: Molecular mechanisms and future promise. *Cell 140*(4), 460–476.

Tammela, T., Zarkada, G., Wallgard, E., Murtomaki, A., Suchting, S., Wirzenius, M., ... Alitalo, K. (2008). Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. *Nature 454*(7204), 656–660.

Tang, T. C., Man, S., Lee, C. R., Xu, P., & Kerbel, R. S. (2010). Impact of metronomic UFT/cyclophosphamide chemotherapy and antiangiogenic drug assessed in a new preclinical model of locally advanced orthotopic hepatocellular carcinoma. *Neoplasia 12*(3), 264–274.

Taylor, P. D., Wilson, H., Hillier, S. G., Wiegand, S. J., & Fraser, H. M. (2007). Effects of inhibition of vascular endothelial growth factor at time of selection on follicular angiogenesis, expansion, development and atresia in the marmoset. *Mol Hum Reprod 13*(10), 729–736.

Terme, M., Pernot, S., Marcheteau, E., Sandoval, F., Benhamouda, N., Colussi, O., ... Taieb, J. (2013). VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. *Cancer Res 73*(2), 539–549.

Tille, J. C., Wang, X., Lipson, K. E., McMahon, G., Ferrara, N., Zhu, Z., ... Pepper, M. S. (2003). Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates VEGF-C(deltaNdeltaC)- and VEGF-A-induced angiogenesis in vitro. *Exp Cell Res 285*(2), 286–298.

Tong, R. T., Boucher, Y., Kozin, S. V., Winkler, F., Hicklin, D. J., & 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*(11), 3731–3736.

Tonra, J. R., Deevi, D. S., Corcoran, E., Li, H., Wang, S., Carrick, F. E., & Hicklin, D. J. (2006). Synergistic antitumor effects of combined epidermal growth factor receptor and vascular endothelial growth factor receptor-2 targeted therapy. *Clin Cancer Res 12*(7 Pt 1), 2197–2207.

Torres Filho, I. P., Leunig, M., Yuan, F., Intaglietta, M., & Jain, R. K. (1994). Noninvasive measurement of microvascular and interstitial oxygen profiles in a human tumor in SCID mice. *Proc Natl Acad Sci 91*(6), 2081–2085.

Truman, J. P., Garcia-Barros, M., Kaag, M., Hambardzumyan, D., Stancevic, B., Chan, M., ... Haimovitz-Friedman, A. (2010). Endothelial membrane remodeling is obligate for anti-angiogenic radiosensitization during tumor radiosurgery. *PLoS One 5*(9).

Tung, J. J., Tattersall, I. W., & Kitajewski, J. (2012). Tips, stalks, tubes: Notch-mediated cell fate determination and mechanisms of tubulogenesis during angiogenesis. *Cold Spring Harb Perspect Med 2*(2), a006601.

Tvorogov, D., Anisimov, A., Zheng, W., Leppanen, V. M., Tammela, T., Laurinavicius, S., ... Alitalo, K. (2010). Effective suppression of vascular network formation by combination of antibodies blocking VEGFR ligand binding and receptor dimerization. *Cancer Cell 18*(6), 630–640.

Van Cutsem, E., Tabernero, J., Lakomy, R., Prenen, H., Prausova, J., Macarulla, T., ... Allegra, C. (2012). Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol 30*(28), 3499–3506.

Van der Veldt, A. A., Lubberink, M., Bahce, I., Walraven, M., de Boer, M. P., Greuter, H. N., ... Smit, E. F. (2012). Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. *Cancer Cell 21*(1), 82–91.

Van, B. G., Yang, A. D., Dallas, N. A., Gray, M. J., Lim, S. J., Xia, L., ... Ellis, L. M. (2008). Effect of molecular therapeutics on liver regeneration in a murine model. *J Clin Oncol 26*(11), 1836–1842.

Verheij, M., Vens, C., & van Triest, B. (2010). Novel therapeutics in combination with radiotherapy to improve cancer treatment: rationale, mechanisms of action and clinical perspective. *Drug Resist Updat 13*(1–2), 29–43.

Verheul, H. M., Hammers, H., van Erp, K., Wei, Y., Sanni, T., Salumbides, B., ... Pili, R. (2007). Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. *Clin Cancer Res 13*(14), 4201–4208.

Verhoeff, J. J., Stalpers, L. J., Van Noorden, C. J., Troost, D., Ramkema, M. D., van Bree, C., ... van Furth, W. R. (2009). Angiogenesis inhibitor DC101 delays growth of intracerebral glioblastoma but induces morbidity when combined with irradiation. *Cancer Lett 285*(1), 39–45.

Vosseler, S., Mirancea, N., Bohlen, P., Mueller, M. M., & Fusenig, N. E. (2005). Angiogenesis inhibition by vascular endothelial growth factor receptor-2 blockade reduces stromal matrix metalloproteinase expression, normalizes stromal tissue, and reverts epithelial tumor phenotype in surface heterotransplants. *Cancer Res 65*(4), 1294–1305.

Vredenburgh, J. J., Desjardins, A., Herndon, J. E., II, Marcello, J., Reardon, D. A., Quinn, J. A., ... Friedman, H. S. (2007). Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J Clin Oncol 25*(30), 4722–4729.

Watanabe, H., Mamelak, A. J., Wang, B., Howell, B. G., Freed, I., Esche, C., ... Sauder, D. N. (2004). Anti-vascular endothelial growth factor receptor-2 (Flk-1/KDR) antibody suppresses contact hypersensitivity. *Exp Dermatol 13*(11), 671–681.

Wicki, A., Rochlitz, C., Orleth, A., Ritschard, R., Albrecht, I., Herrmann, R., ... Mamot, C. (2012). Targeting tumor-associated endothelial cells: Anti-VEGFR2 immunoliposomes mediate tumor vessel disruption and inhibit tumor growth. *Clin Cancer Res 18*(2), 454–464.

Wilke, H., Muro, K., Van Cutsem, E., Oh, S. C., Bodoky, G., Shimada, Y., ... RAINBOW Study Group (2014). Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomized phase 3 trial. *Lancet Oncol 15*(11), 1224–1235.

Willett, C. G., Duda, D. G., di Tomaso, E., Boucher, Y., Ancukiewicz, M., Sahani, D. V., ... Jain, R. K. (2009). Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. *J Clin Oncol 27*(18), 3020–3026.

Winkler, F., Kozin, S. V., Tong, R. T., Chae, S. S., Booth, M. F., Garkavtsev, I., ... Jain, R. K. (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*(6), 553–563.

Xu, W. W., Li, B., Lam, A. K., Tsao, S. W., Law, S. Y., Chan, K. W., ... Cheung, A. L. (2015). Targeting VEGFR1- and VEGFR2-expressing non-tumor cells is essential for esophageal cancer therapy. *Oncotarget 6*(3), 1790–1805.

Yang, Y., Zhang, Y., Cao, Z., Ji, H., Yang, X., Iwamoto, H., ... Cao, Y. (2013a). Anti-VEGF- and anti-VEGF receptor-induced vascular alteration in mouse healthy tissues. *Proc Natl Acad Sci U S A 110*(29), 12018–12023.

Yang, Y. J., Choi, J. S., & Choi, J. W. (2015). Antiangiogenic therapy impedes infiltration by CD4+ and CD8+ cells into an early colon tumor. *J Cancer Prev 20*(2), 129–135.

Yang, X., Zhang, Y., Yang, Y., Lim, S., Cao, Z., Rak, J., & Cao, Y. (2013b). Vascular endothelial growth factor-dependent spatiotemporal dual roles of placental growth factor in modulation of angiogenesis and tumor growth. *Proc Natl Acad Sci U S A 110*(34), 13932–13937.

Yasuda, S., Sho, M., Yamato, I., Yoshiji, H., Wakatsuki, K., Nishiwada, S., ... Nakajima, Y. (2013). Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo. *Clin Exp Immunol 172*(3), 500–506.

Yi, D., Hua, T. X., Lin, H. Y., Kui, C. L., Ning, L. X., & Wang, Z. Z. (2011). Antitumor treatment efficacy by targeting epidermal growth factor receptor and vascular endothelial growth factor receptor-2 in an orthotopic human glioblastoma model. *J Neuro-Oncol 104*(1), 93–101.

Yoshiji, H., Kuriyama, S., Hicklin, D. J., Huber, J., Yoshii, J., Ikenaka, Y., ... Fukui, H. (2001). The vascular endothelial growth factor receptor KDR/Flk-1 is a major regulator of malignant ascites formation in the mouse hepatocellular carcinoma model. *Hepatology 33*(4), 841–847.

Yoshiji, H., Kuriyama, S., Yoshii, J., Ikenaka, Y., Noguchi, R., Hicklin, D. J., ... Fukui, H. (2002). Synergistic effect of basic fibroblast growth factor and vascular endothelial growth factor in murine hepatocellular carcinoma. *Hepatology 35*(4), 834–842.

Yu, J. L., Rak, J. W., Coomber, B. L., Hicklin, D. J., & Kerbel, R. S. (2002). Effect of p53 status on tumor response to antiangiogenic therapy. *Science 295*(5559), 1526–1528.

Yu, L., Wu, X., Cheng, Z., Lee, C. V., LeCouter, J., Campa, C., ... Ferrara, N. (2008). Interaction between bevacizumab and murine VEGF-A: A reassessment. *Invest Ophthalmol Vis Sci 49*(2), 522–527.

Yuen, D., Pytowski, B., & Chen, L. (2011). Combined blockade of VEGFR-2 and VEGFR-3 inhibits inflammatory lymphangiogenesis in early and middle stages. *Invest Ophthalmol Vis Sci 52*(5), 2593–2597.

Zebrowski, B. K., Yano, S., Liu, W., Shaheen, R. M., Hicklin, D. J., Putnam, J. B., Jr., & Ellis, L. M. (1999). Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. *Clin Cancer Res 5*(11), 3364–3368.

Zhang, Y., Han, Q., Ru, Y., Bo, Q., & Wei, R. H. (2015). Anti-VEGF treatment for myopic choroid neovascularization: From molecular characterization to update on clinical application. *Drug Des Devel Ther 9*, 3413–3421.

Zhang, L., Hannay, J. A., Liu, J., Das, P., Zhan, M., Nguyen, T., ... Lev, D. (2006). Vascular endothelial growth factor overexpression by soft tissue sarcoma cells: Implications for tumor growth, metastasis, and chemoresistance. *Cancer Res 66*(17), 8770–8778.

Zhang, L., Yu, D., Hicklin, D. J., Hannay, J. A., Ellis, L. M., & Pollock, R. E. (2002). Combined anti-fetal liver kinase 1 monoclonal antibody and continuous low-dose doxorubicin inhibits angiogenesis and growth of human soft tissue sarcoma xenografts by induction of endothelial cell apoptosis. *Cancer Res 62*(7), 2034–2042.

Zhu, Z., Hattori, K., Zhang, H., Jimenez, X., Ludwig, D. L., Dias, S., ... Rafii, S. (2003). Inhibition of human leukemia in an animal model with human antibodies directed against vascular endothelial growth factor receptor 2. Correlation between antibody affinity and biological activity. *Leukemia 17*(3), 604–611.

Zhu, A. X., Park, J. O., Ryoo, B. Y., Yen, C. J., Poon, R., Pastorelli, D., ... Investigators, R. T. (2015). Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): A randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol 16*(7), 859–870.

Zhu, Z., Rockwell, P., Lu, D., Kotanides, H., Pytowski, B., Hicklin, D. J., ... Witte, L. (1998). Inhibition of vascular endothelial growth factor-induced receptor activation with anti-kinase insert domain-containing receptor single-chain antibodies from a phage display library. *Cancer Res 58*(15), 3209–3214.

Zimmermann, R. C., Xiao, E., Husami, N., Sauer, M. V., Lobo, R., Kitajewski, J., & Ferin, M. (2001). Short-term administration of antivascular endothelial growth factor antibody in the late follicular phase delays follicular development in the rhesus monkey. *J Clin Endocrinol Metab 86*(2), 768–772.