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Targeting the Angiopoietin-2/Tie-2 axis in conjunction with VEGF signal interference

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Abstract

Anti-angiogenic therapies target the tumor vasculature, impairing its development and growth. It was hypothesized over 40 years ago by the late Judah Folkman and Julie Denekamp that depriving a tumor of oxygen and nutrients, by targeting the tumor vasculature, could have therapeutic benefits. Identification of growth factors and signaling pathways important in angiogenesis subsequently led to the development of a series of anti-angiogenic agents that over the past decade have become part of the standard of care in several disease settings. Unfortunately not all patients respond to the currently available anti-angiogenic therapies while others become resistant to these agents following prolonged exposure. Identification of new pathways that may drive angiogenesis led to the development of second-generation anti-angiogenic agents such as those targeting the Ang-2/Tie2 axis. Recently, it has become clear that combination of first and second generation agents targeting the blood vessel network can lead to outcomes superior to those using either agent alone. The present review focuses on the current status of VEGF and Ang-2 targeted agents and the potential utility of using them in combination to impair tumor angiogenesis.

Keywords

Ang-2 targeting; Angiogenesis; Anti-angiogenic therapy; VEGF targeting; Combination therapy

Introduction

The formation of new blood vessels from pre-existing ones, angiogenesis, is a physiological event that occurs during embryonic vascular development as well as in adult vasculature as part of wound healing, reproduction and the menstrual cycle [1]. Angio-genesis also is an important process in pathological conditions including cancer, diabetic retinopathy, atherosclerosis and rheumatoid arthritis [2]. In cancer, it is a fundamental process involved in tumor development, progression and dissemination [3–5]. As a consequence, key growth factors in angiogenesis have been exploited for anti-cancer therapies [3,6,7].

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Conflict of interest

The authors declare no conflict of interests.

Tumor vasculature is distinct from normal vasculature. Normal vessels are quiescent with minimal endothelial cell proliferation, tight endothelial–endothelial cell and endothelial–peri-endothelial cell (e.g. smooth muscle cells and pericytes) contacts to regulate vessel permeability [8]. Tumor vessels, on the other hand, have highly proliferative endothelial cells with loose to minimal endothelial– endothelial and endothelial–peri-endothelial cell contacts yielding a leaky vascular phenotype [9,10]. Yet despite being highly active, the leaky tumor vasculature is unable to provide a tumor with its oxygen and nutritional needs leading to aberrant tumor microenvironments that negatively impact the ability to control the cancer with conventional anticancer therapies [10,11]. Given a tumor's dependence on angiogenesis, agents targeting the ability of tumors to initiate new vessel formation rapidly advanced into the clinical realm of cancer management.

Today there are ten agents with direct anti-angiogenic activity (primarily antibody and small molecule inhibitors) that have been approved by the FDA for the treatment of solid tumors (Table 1). Although outcomes have been generally encouraging, patients often fail to respond or become resistant to the currently available therapies [12,13]. Consequently, efforts to develop optimal approaches to realize the true potential of vascular-targeted therapies as cancer treatment are actively being pursued. Although a number of angiogenesis targeting approaches are under active preclinical and clinical development (discussed later), the focus of this review is on the status of inhibitors that target the VEGF and Ang-2 axes. Due to the unique mode of action and apparent interplay between VEGF and Ang-2 pathways there has been tremendous recent effort to combine these two modalities to achieve enhanced treatment outcomes. The rationale for such combination and advances in therapies will be discussed in this review.

Ang-2/Tie2 signaling disrupts normal vasculature

The angiopoietin/Tie system was identified nearly two decades ago [14–18]. It is an endothelial cell specific system that is responsible for the maintenance and disruption of normal vasculature [19]. It is composed of three ligands in humans, Ang-1, -2, and -4; Ang-1 and Ang-2 are the best characterized of the three and will be discussed here. The angiopoietin/Tie axis is essential for vasculogenesis, normal vascular development, as well as angiogenesis; however vasculogenesis will not be discussed in depth in this review (for reviews see [19–21]).

Tyrosine kinase with Ig and EGF homology domains-2 (Tie2) is a transmembrane tyrosine kinase receptor and is expressed by both vascular and lymphatic endothelial cells [14,16]. Tie1 remains an orphan receptor with no known direct ligand binding. Ang-1 and Ang-2 are both secreted proteins with similar structures and 60% amino acid sequence homology. They have however, opposing functions upon binding the Tie2 receptor [18]. Ang-1 paracrine signaling to Tie2, from peri-endothelial cells, maintains a mature-quiescent vessel with tight endothelial–endothelial and endothelial–periendothelial cell interactions, shown in Fig. 1A [23,24]. In the adult, Ang-1 is found in various tissues and secretion occurs continuously at low levels; signaling mechanisms by Ang-1 mediated Tie2 phosphorylation involves Rho and mDia leading to inhibition of VEGF/VEGFR-2 mediated vascular permeability through src [25]. The endothelium-specific vascular endothelial–phosphotyrosine phosphatase (VE-

PTP) has also been shown to mediate cross talk between the Ang-1/Tie2 and VEGF/VEGFR pathways [26]. Ang-1 phosphorylation of Tie2 stimulates pro-survival pathways, such as PI3K-AKT and MAPK/ERK, as well as represses pro-inflammatory pathways through NF-κβ [27–29] indicating that Ang-1 can promote a stable, quiescent vasculature through various signaling modalities.

Ang-2 is stored in Weibel–Palade bodies in endothelial cells and secreted upon microenvironmental changes [30–33]. Contrary to Ang-1/Tie2 signaling Ang-2 autocrine signaling via the Tie2 receptor results in loss of cell–cell contacts and vascular destabilization (Fig. 1B) [18,34]. Consequently Ang-2 is usually referred to as the Tie2 antagonist [18,35–37]. However, Ang-2 may also act as a Tie2 agonist in a context dependent manner [38,39]. Furthermore the ratios of Ang-1 to Ang-2 as well as Tie1 to Tie2 in the microenvironment can affect signaling activities through Tie2, clearly making this system highly complex [40]. In general Ang-1 appears to prefer Tie2 homodimer interactions as the presence of Tie1 can hinder Ang-1 mediated signaling, while the absence of Tie1 can enhance Ang-1 mediated signaling through Tie2 [40]. Tie 1 and Tie2 receptors have been shown to form heterodimers, and even though no ligands are known to bind Tie1, the receptor can interact with Ang2 through the heterodimers [39,41]. However, the presence or absence of Tie1 does not alter Ang-2's interaction with Tie2 nor the antagonistic characteristics of Ang-2 toward Ang-1 [40].

Ang-2 binding to Tie2 homodimers can lead to agonistic interactions similar to Ang-1/Tie2 interactions, mainly as shown in the lymphatic vasculature where Tie1 is sparsely expressed [38]. Tie1 is commonly expressed in blood endothelial cells and has been shown to be upregulated in the tumor microenvironment [42]. Elevated Tie1 expression can increase Tie1/Tie2 heterodimer formation resulting in increased Ang-2 binding to the receptors which antagonizes Ang-1 interactions and results in vessel destabilization [39,41]. However, current evidence suggests that Ang-2's antagonistic role cannot be solely attributed to Tie1 involvement, therefore making this axis highly unique and complex. Ang-1's inability but Ang-2's ability to interact with both Tie2 homodimers and Tie1/ Tie2 heterodimers is a consequence of a difference of three amino acids (proline, glutamine, arginine) in the protein sequence outside the receptor binding site of Ang-2 as compared to Ang-1 (threo-nine, alanine, glycine) [43].

VEGF/VEGFR signaling stimulates new vessel growth

The VEGF/VEGFR system was identified three decades ago [44,45]. The Vascular Endothelial Growth Factor (VEGF) family has several members including VEGF-A, -B, -C, -D, -E and placental growth factor (PlGF) that bind to various transmembrane tyrosine kinase VEGF receptors VEGFR-1, -2, and -3. The following discussion will focus on the major angiogenic factor, VEGF-A, and its receptors VEGFR-1 and VEGFR-2. VEGF-A has several isoforms resulting from alternative splicing; the most abundant isoform being VEGF-A_{165} consisting of 165 amino acids and will be referred to as VEGF for the rest of this review. VEGF binds both VEGFR-1 and VEGFR-2 with higher affinity for VEGFR-1 but lower kinase activity than binding to VEGFR-2 [46,47].

VEGF has numerous effects on the endothelial cells of the vasculature; it acts as a pro-survival factor and stimulator of endothelial cell proliferation, invasion and migration ultimately leading to new vessel growth (Fig. 1C) [46]. VEGF signaling enhances endothelial cell survival by activation of the PI3K-Akt pathway and upregulation of anti-apoptotic proteins such as Bcl-2 [48,49]. VEGF is also known as vascular permeability factor for its ability to loosen endothelial cell–cell contacts through mediating the loss of VE-cadherin via activation of src signaling [50,51]. Effects on endothelial cell proliferation is mainly through activation of Erk1/2 signaling and endothelial cell migration through activation of focal adhesion kinase (FAK) as well as induction of several extracellular matrix degrading proteins including metalloproteinases, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (TTPA) [46,52]. It should be noted that many other growth factors and their receptors, including fibroblast growth factor (FGF/FGFR), epidermal growth factor (EGF/EGFR), insulin-like growth factor (IGF/ IGFR), and platelet derived growth factor (PDGF/PDGFR), similarly exert stimulating effects on endothelial cells; however their review is outside the scope of the present article (for excellent comprehensive reviews of those factors see [53–57].

Along with its survival, migratory and proliferative effects, VEGF also interacts with the Notch-Delta like 4 (Dll4) pathway affecting tip and stalk cell selection as new vessels sprout [58]. In general, tip cells are migratory and follow pro-angiogenic signaling cues originating from the tumor while stalk cells are proliferative and produce the newly growing vessel [58].

Pro-angiogenic factors, VEGF and Ang-2, in the tumor microenvironment

Solid tumors require active angiogenesis for their development and growth. Tumor cells have the ability to secrete key proangiogenic factors to directly stimulate this process. Although the present discussion is focused on two primary factors, VEGF and Ang-2, found in the tumor microenvironment, it is clear that many other factors can affect tumor angiogenesis and are also being explored as therapeutic targets (for review see [59–65]. VEGF is secreted by virtually all solid tumor cells although some tumor types may secrete heightened levels compared to others. Elevated levels of VEGF have been correlated to disease progression and prognosis in various cancers including colorectal, lung, hepatocellular, breast, pancreatic and gastrointestinal cancers [66–71].

In general, heightened secretion of VEGF by tumor cells is a response to hypoxic conditions inherent to all developing solid tumors. As the tumor grows, cells existing further than the oxygen diffusion distance (70–100 μm) from blood vessels become hypoxic [72]. Hypoxia Inducible Factor-1α (HIF-1α) is stabilized under low oxygen conditions and dimerizes with HIF-1β; the HIF complex then trans-locates to the nucleus and binds to the VEGF promoter and increases VEGF transcription [73,74]. Elevated VEGF levels, in turn, stimulate endothelial cells of nearby vessels to initiate new vessel growth to the tumor [47,75].

Ang-2 serum levels in patients with solid tumors are typically elevated compared to measurements in healthy individuals [76,77] and increase with disease progression [78–82]. Additionally, Ang-2 levels in the tumor microenvironment have been correlated with increased recruitment of Tie2 expressing macrophages (TEMs) that correlate with pro-

angiogenic activity [83]. Unlike VEGF, the specific mechanisms for Ang-2 elevation in the microenvironment are unclear. Our preclinical studies indicate that renal cell carcinoma cells (Caki-1, Caki-2, 786-0, A498) do not secrete Ang-2 directly under aerobic nor hypoxic conditions but that they do secrete factors, currently unidentified, that can stimulate the release of Ang-2 from the endothelial cells (Biel NM and Siemann DW, unpublished 2013); suggesting that the presence of Ang-2 and VEGF in the tumor microenvironment occurs through distinct but perhaps complementary actions. Still, the presence of both VEGF and Ang-2 in the tumor microenvironment and their respective importance in angiogenesis has made them attractive targets as anti-angiogenic cancer therapy.

First generation anti-angiogenic agents: targeting the VEGF signaling pathway

Agents targeting the VEGF pathway exert their effects by inhibition of endothelial cell proliferation, migration and tube formation (Fig. 2). The first FDA approved anti-angiogenic agent was Bevacizumab (Avastin®; Genetech/Roche) a humanized monoclonal antibody against VEGF-A. Initially approved in 2004 for metastatic colorectal cancer and is now approved for use in a number of oncologic settings. Sorafenib (Nexavar®, Bayer/Onyx), a multi-tyrosine kinase small molecule inhibitor, was approved in 2005 for the treatment of renal cell carcinoma. The following year the small molecule inhibitor Sunitinib (Sutent®, Pfizer) was approved for renal cell carcinoma.

Small molecule tyrosine kinase inhibitors Pazopanib (Votrient®, GlaxoSmithKline), Vandetanib (Caprelsa®, AstraZeneca), Cabozantinib (Cometriq®, Exelixis) and Axitinib (Inlyta®, Pfizer) were approved for renal cell carcinoma and thyroid cancers. The VEGF-Trap Ziv-Aflibercept (Zaltrap®, Regeneron) has been approved for metastatic colorectal cancer and the most recent FDA approval was the human monoclonal VEGFR-2 antibody Ramucirumab (Cyramza®, Eli Lilly) for stomach cancer (Table 1). Many other agents are currently in clinical trials in a wide range of oncologic settings. To date, Bevacizumab has proven to be most successful having been approved for use in the treatment of glioblastoma, metastatic colorectal, non-small cell lung cancer, renal cell carcinoma and until recently metastatic breast cancer [84–86].

Although these agents show minimal direct antitumor effects when administered alone, they can enhance the impairment of tumor growth when combined with conventional anticancer therapies [87–89]. Such combination treatments also resulted in somewhat greater chemotherapy and radiation therapy induced tumor cell kill. It's been proposed that anti-angiogenic treatments yield a window of vascular normalization that leads to improved chemotherapeutic drug delivery to the tumor core as well as a reduction in tumor hypoxia resulting in better cell kill by radiation therapy [87,90,91]. However, tumor oxygenation status following anti-angiogenic agent therapy can vary greatly with changes ranging from decreased oxygenation to no change in oxygenation to increased oxygenation having been observed post treatment [88].

Second generation anti-angiogenic agents: targeting the Ang-2 signaling pathway

Unlike the VEGF axis targeted agents, which predominantly affect endothelial cell proliferation, migration and tube formation, the inhibition of the Ang-2/Tie2 axis is more complex and multifactorial. For example, Ang-2 inhibition impairs endothelial and smooth muscle cell contact loss leading to reduced tumor vasculature and increased vessel normalization. Ang-1 inhibition alone has little effect on the tumor vasculature. However, combined Ang-2 and Ang-1 signal inhibition leads to reduced tumor vasculature without vessel normalization. These findings suggest that normalization of the vasculature is Ang-1 dependent and Ang-2 blockade allows for enhanced Ang-1/Tie2 interactions. While the reduction of tumor vessels is Ang-1 independent, the maintenance of tumor vessel reduction in the presence of both Ang-1 and Ang-2 blockade most likely accounts for the fact that Ang-2 can interact with integrins, especially on sprouting tip cells devoid of Tie2 [92–94]. Furthermore, there is evidence that Ang-2 blockade reduces the recruitment of tumor-associated macrophages (TAMs) that express Tie2, and aid angiogenesis [83,95]. This in turn may significantly reduce angio-genesis [96].

Consequently, Ang-2 targeted agents may promote both the maintenance of a normal vascular phenotype (Fig. 3) and reduction of vessel sprouts. The recognized importance of Ang-1 and Ang-2 in vascular integrity has resulted in great interest in the possibility of targeting the Ang-2/Tie2 axis to inhibit tumor angiogenesis; both for the development of novel antiangiogenic agents and as a means to circumvent the lack of response or adaptive resistance that has been reported with VEGF directed agents [12,13,97]. A variety of approaches have been undertaken (Table 1).

The Ang-2 specific antibody LC06 (Roche) primarily serves as the Ang-2 specific arm of the Ang-2-VEGF CrossMab, to be discussed later. Zhang et al. demonstrated anti-tumor effects of an Ang-2 specific single-chain antibody (scFv-Ang-2) [98]. DX-2240 (Sanofi-Aventis) is a human monoclonal antibody targeting the Tie1 receptor based on the interaction of Ang-2 with Tie1/Tie2 heterodimers in vessel destabilization. Recently, D'Amico et al. have shown direct evidence for the benefit of targeting the Tie1 receptor using a Tie1 deficient endothelial cell specific mouse model [99].

There are three fully human monoclonal antibodies against Ang-2 that are being evaluated in phase I clinical trials, MEDI3617 (MedImmune/AstraZeneca), Nesvacumab (REGN910; Regeneron/ Sanofi) and AMG780 (Amgen) [100–102]. CVX-060 (Pfizer) is a monospecific Ang-2 CovX-Body that was evaluated in phase I clinical trials [103]. Trebananib (AMG386), the Ang-1/Ang-2 peptibody, is the currently most clinically advanced agent having entered phase III trials in ovarian cancer as well as being evaluated in several phase I/II trials in a variety of oncologic settings, particularly in combination with conventional chemotherapeutic agents.

Challenges of current anti-angiogenic therapies

Despite the strong scientific evidence supporting the use of blood vessel targeted agents, resistance to these agents can develop [12,13,97,104]. Furthermore lack of improvement in overall patient survival has curtailed their use; as in the case of the removal of Bevacizumab for the treatment of metastatic breast cancer [13,84,86,97]. Tumors can develop acquired resistance to anti-angiogenic therapies by upregulation of pathways in angiogenesis that are not inhibited by the therapy [12]. Even though VEGF is a major driver of angiogenesis there are other growth factors such as basic FGF, interleukin-8 (IL-8), PlGF, PDGF, and IGF that can compensate in the absence of VEGF pathway activation [12,59]. The lack of tumor responses and potential to l for compensatory mechanisms that could lead to treatment resistance clearly emphasize the continued need to develop more effective anti-angiogenic therapeutic strategies in order to fulfill the promise of vascular-targeted therapeutic strategies.

Currently several multikinase inhibitors targeting FGFRs, VEGFRs and RDGFRs are undergoing active preclinical and clinical development [61]. Notch-Delta-like 4 (Dll4) targeted agents are also being evaluated and studies suggest that Dll4 inhibition could be beneficial in VEGF resistant tumors [62,105,106]. The future of PlGF inhibitors is somewhat less clear as recent studies have suggested that such agents may offer little benefit alone or in combination [64,107]. Selective PDGFR inhibitors also are in development as anti-angiogenic agents. However, it is worth noting that many of the small molecule tyrosine kinase inhibitors approved by the FDA also target PDGFR (Table 1) [108–110]. Inhibitors of IGF are being actively pursued as well but the complexity of this signaling axis has somewhat limited the ability to achieve maximal therapeutic potential [65,111].

The most common side effects associated with anti-angiogenic therapy are hypertension and occasional bleeding along with impaired physiological angiogenesis such as wound healing, reproduction and fetal development [112–114]. These side effects limit the population of patients that can be treated and affect the dosing and scheduling of agents that are administered. However, the removal of these agents from the patients' therapeutic regimen quickly restores physiological angiogenesis, making it feasible to remove or resume treatment before or after major surgeries.

Combination of Ang-2 and VEGF axis inhibitors

The increased understanding of the angiopoietin system coupled with the development of selective Ang-2/Tie2 axis targeted agents has led to the possibility of combining such agents with anti-VEGF therapies. Since effective tumor angiogenesis requires (a) destabilization of normal vasculature (mediated by the Ang-2/ Tie2 axis) followed by (b) endothelial cell activation by proangiogenic factors (such as VEGF), the combination of therapies targeting both steps ought to provide enhanced impairment of the angiogenesis process. Indeed, several preclinical studies have shown the benefit of combining Ang-2 and VEGF targeted agents; in general, targeting both pathways shows superior anti-angiogenic and antitumor effects compared to treatments with either Ang-2 or VEGF targeted agents alone (Fig. 4) [101,102,115,116].

There are several ongoing clinical trials evaluating the combination of Ang-2 and VEGF targeted agents. Trebananib is being evaluated in a number of phase II trials in combination with the leading anti-VEGF agents, Bevacizumab, Pazopanib, Sorafenib, and Sunitinib in various solid tumor settings. The Ang-2 specific monoclonal antibodies, MEDI3617 and Nesvacumab (REGN910), are being evaluated in phase I trials with Bevacizumab and Aflibercept respectively (Table 2). There was a recently completed phase I/II clinical trial with the monospecific Ang-2 CovX-Body (CVX-060; Pfizer) in combination with Sunitinib. A phase II trial in combination with Axitinib was terminated due to adverse toxicities; new strategies for dosing and scheduling are being evaluated.

Given the complementary effects of anti-Ang-2 and anti-VEGF therapies, there has also been interest in developing agents that simultaneously target both axes. Regorafenib (Stivarga®, Bayer AG) is a small molecule inhibitor that was approved for metastatic colorectal cancer in 2012 and for gastrointestinal cancer in 2013. Although it is reported to have both VEGFR-1, -2, -3 and Tie2 activity, the former is more potent with little impact on Tie2 phosphorylation [117]. Currently, there are 57 reported clinical trials with this agent in patients in all phases and with various tumor types used alone or in combination with chemotherapy. The Ang-2/ VEGF-A binding peptide antibody fusion protein (CVX-241, Pfizer) was evaluated in phase I clinical trials (NCT01004822). This trial was terminated in 2011 due to shorter half life of VEGF-A binding than expected. In addition, several small molecule agents with dual action against both VEGFR and Tie2 receptor tyrosine kinase activity have been developed. CEP-11981 (Cephalon, Inc) has completed a phase I clinical trial (NCT00875264) in 2012 with no other trials active. ARRY-614 (Array BioPharma, Inc) is currently being evaluated in phase I clinical trials in myelodysplastic syndromes (NCT00916227, NCT01496495). The agent ACTB-1003 (ACT Biotech/Bayer AG) has also been reported to have Tie2 activity; however this agent is advertised as an FGFR/PI3K inhibitor.

Another strategy to combine Ang-2 and VEGF targeting into one agent comes from the chimeric decoy receptor, DAAP, and the Ang-2/VEGF-A bispecific antibody (Ang-2-VEGF-A CrossMab). DAAP was evaluated in preclinical studies and showed greater antitumor effects than treatment with either VEGF-Trap or Tie2-Fc alone [118]. However no follow-up studies have been reported since the initial publication. The Ang-2-VEGF-A CrossMab (RO5520985/RG7221, Roche) is a cross between VEGF-A based on Bevacizumab and the Ang-2 specific human IgG1, LC06. It showed antitumor, anti-angiogenic and anti-metastatic effects in xenograft models and has recently completed phase I clinical trial evaluation (NCT01688206). Phase II evaluations in colorectal cancer are planned [119,120].

Future considerations for Ang-2 and VEGF anti-angiogenic therapy

Currently, the Angiopoietin/Tie2 pathway inhibitors are being evaluated in combination with chemotherapeutic agents and/or VEGF targeted anti-angiogenic agents [100]. While such combinations are logical and meritorious, there are additional possibilities for the application of Angiopoietin/Tie-2 axis targeting agents that have yet to be explored.

Radiation therapy

Radiation therapy is a standard form of therapy for many malignancies [121]. Hypoxic tumor microenvironments which are common in most solid tumors can negatively impact the curative potential of this therapy because tumor cells lacking oxygen are radiation resistant [122]. Several studies have shown that combining radiation therapy with anti-angiogenic agents, primarily anti-VEGF agents [123,124], results in superior tumor responses but the timing of the agents is crucial for favorable outcomes [125,126]. To date no clinical studies combining radiation with anti-Ang-2 therapies have been reported. Given the positive anti-angiogenic effects reported for Ang-2/Tie2 axis targeting agents, their therapeutic potential when applied with radiation treatment ought to be considered. Additionally, with the apparent beneficial effects of combining anti-VEGF and anti-Ang-2 therapeutics, the future evaluation of such a combination in conjunction with radiation therapy would appear logical.

Vascular disrupting agents

An alternative strategy to enhance the anti-vascular effects in tumors would be the combination of anti-angiogenic agents with vascular disrupting agents (VDAs) [127,128] given the distinctively different modes of action of these classes of agents. VDAs damage existing tumor vasculature rather than inhibit the formation of new blood vessels as do anti-angiogenics. VDAs selectively target the expanding tumor vascular network by exerting their effects against proliferating endothelial cells, a phenomenon that is absent in normal vasculature [129,130]. VDAs have been shown to be beneficial in preclinical settings both in combination with anti-VEGF agents as well as conventional anti-cancer therapies [131,132]. In the clinic, the combination of Bevacizumab plus Combretastatin has recently been reported to yield promising results in ovarian cancer [133]. Taking into consideration the complementary anti-angiogenic actions of Ang-2 and VEGF targeted agents alongside the complementary anti-vascular actions of VDAs and anti-angiogenics, a combination of such agents also is feasible. The study of Welford and colleagues [134] which showed superior outcomes when combining the VDA Combretastatin with the CXCR4 inhibitor AMD3100 which depletes Tie2 expressing macrophages in the tumor microenvironment lends support to a VDA plus anti-Ang-2 targeting strategy.

Concluding remark

Despite significant advances during the past 40 years, the true potential of treatment strategies targeting the tumor vascular network may not as yet have been realized. Vascular involvement in tumor development and growth is a complex interaction of various pathways. The ability to identify the appropriate pro-angiogenic axes in individual patients will lead to better selection of targeting agents to yield maximal efficacy. Furthermore, better understanding of the intricacies of the tumor vasculature and the development of novel tumor vessel directed targeting agents when applied alone or in combination or in conjunction with conventional anticancer therapies such as radiation or chemotherapy may ultimately achieve the goal of improving treatment outcomes.

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Highlights

- Anti-angiogenic therapies such as Ang-2 and VEGF targeted agents have been heavily pursued in oncologic settings.
- Angiopoietin/Tie2 system plays a role in vascular destabilization.
- VEGF/VEGFR system plays a role in endothelial cell survival, proliferation, and migration.
- The combination of Ang-2 and VEGF targeted agents have recently shown to be complimentary and superior to either pathway targeted agent alone.
- Recently, development of dual Ang-2 and VEGF targeted agents have been developed.

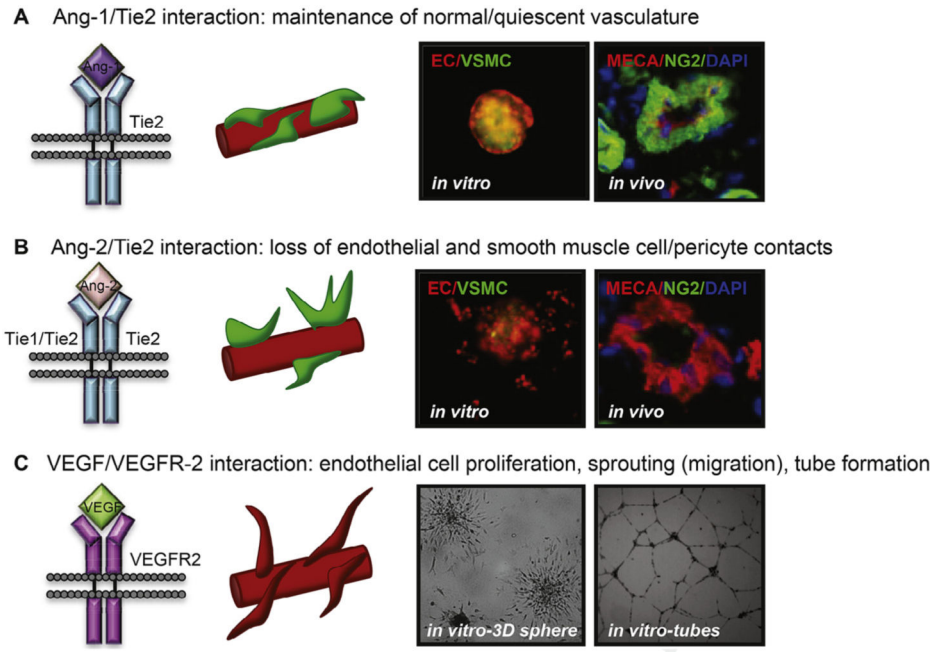

Fig. 1.

The Angiopoietin/Tie2 and VEGF/VEGFR signaling axis in angiogenesis. (A) Ang-1/Tie2 interaction maintains normal-quiescent vasculature with tight endothelial– endothelial and endothelial–smooth muscle cell/pericyte contacts as demonstrated by an *in vitro*, unstimulated, co-culture endothelial and smooth muscle cell sphere and *in vivo* normal vasculature with smooth muscle cell coverage. (B) Ang-2/Tie2 interaction with Tie2 homodimers or heterodimers results in loss of endothelial–endothelial and endothelial–smooth muscle cell contacts (i.e. destabilization of the vasculature) as demonstrated by an *in vitro*, PMA (stimulant of Ang-2 secretion) stimulated, co-culture sphere and *in vivo* vasculature from tumor tissue with loss of smooth muscle cell coverage. (C) VEGF/VEGFR-2 interaction stimulates endothelial cell survival, proliferation and sprouting of new vessels. Endothelial cell sprouting is illustrated by the collagen embedded 3D HUVEC spheres (image taken at 24 hrs) and tube formation by matrigel plated HUVEC (image taken at 12 hrs). EC: endothelial cell (HUVEC); VSMC: vascular smooth muscle cell (HUASMC); MECA: endothelial cell marker; NG2: peri-endothelial cell marker (ex. smooth muscle cell, pericyte); DAPI: nuclear marker (modified from *Microvasc. Res.* 2012: 83 (3): 290–297. Molnar N. and Siemann D.W., fig. 3D [22]; and Biel N.M. and Siemann D.W., unpublished results).

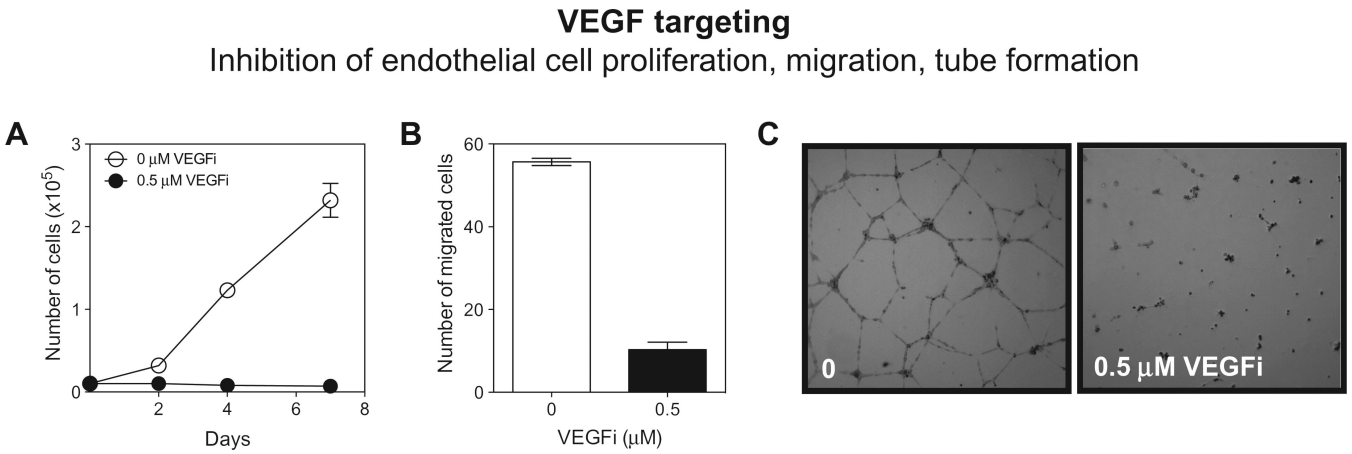

Fig. 2.

VEGF axis targeting in angiogenesis. Interference of the VEGF/VEGFR-2 signaling axis leads to inhibition of human umbilical vein endothelial cell (HUVEC) proliferation. (A) Transwell migration at 24 hrs (B) and tube formation on matrigel at 24 hrs ± VEGFi (Cediranib/AZD2171, AstraZeneca) (C) (Biel N.M. and Siemann D.W., unpublished results).

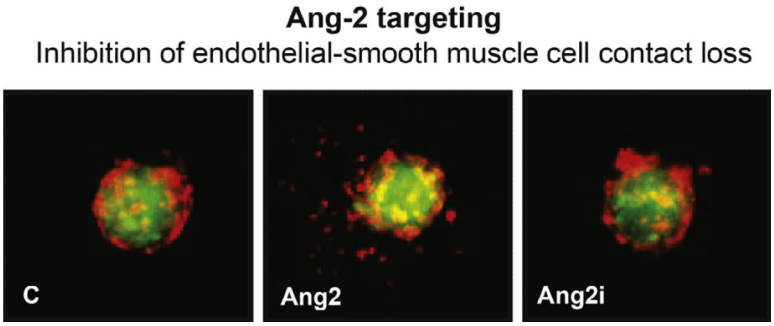

Fig. 3.

Ang-2 axis targeting in angiogenesis. Interference with Ang-2/Tie2 interaction inhibits the Ang-2 dependent loss of endothelial and smooth muscle cell contacts. Red: human umbilical vein endothelial cells (HUVEC); Green: human umbilical artery smooth muscle cells (HUASMC); Ang2i: MEDI3617 (MedImmune) (modified from *Microvasc. Res.* 2012: 83 (3): 290–297. Molnar N. and Siemann D.W., fig. 2B [22]). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

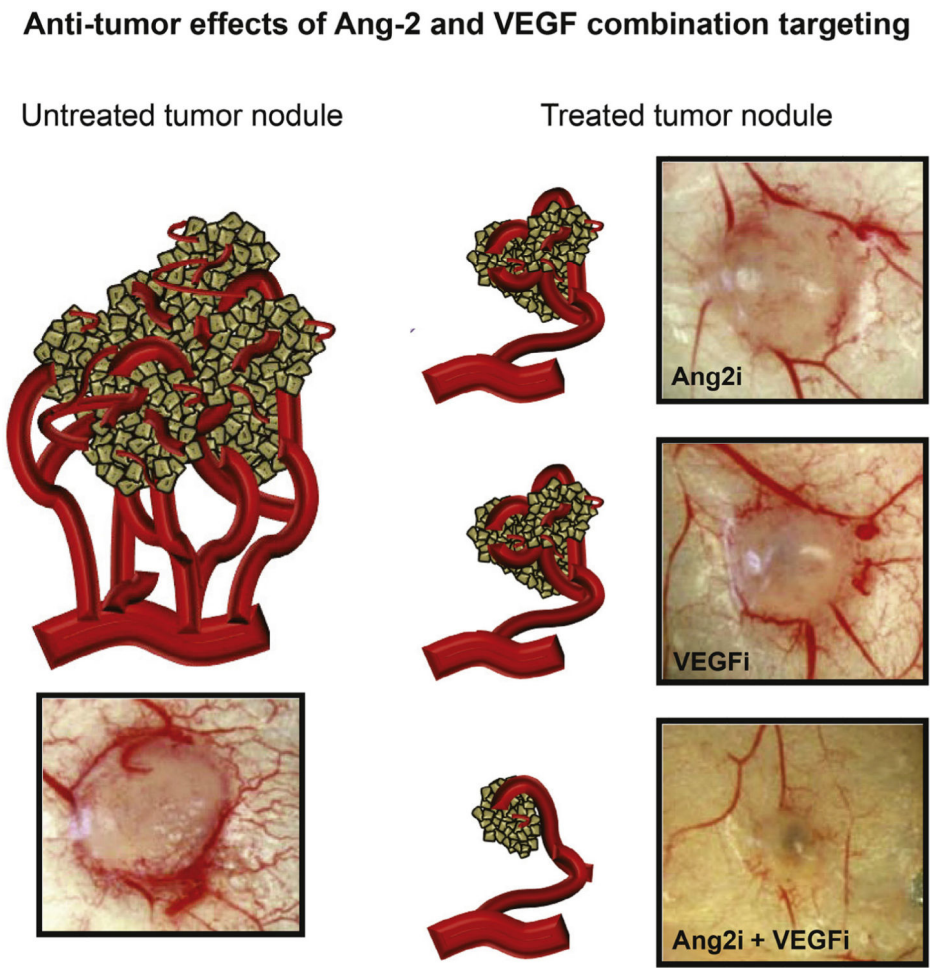

Fig. 4.

Combination of Ang-2 and VEGF targeting *in vivo*. Both Ang-2 and VEGF targeting agents inhibit tumor angiogenesis and reduce tumor growth but the combination of these therapies results in greater anti-angiogenic and anti-tumor effects. Ang2i: MEDI3617; VEGFi: Sunitinib (modified from *J. Cancer Ther.* 2013: 4: 1–6. Molnar N. and Siemann D.W., fig. 2A [115]).

Table 1

VEGF and Ang-2 axis targeted agents in the clinic and preclinical settings. (Due to the scope of this review, this table only shows the VEGF/VEGFR axis targeted agents that have been FDA approved.).

Agent	Target	Clinical Status	Tumor Setting
VEGF Targeted			
Bevacizumab (Avastin®)	VEGF	FDA Approved	mCRC, RCC, NSCLC, Glioblastoma
Sorafenib (Nexavar®)	VEGFR, PDGFR, Raf, RET, cKIT, Flt3	FDA Approved	RCC, HCC
Sunitinib (Sutent®)	VEGFR, PDGFR, Flt3, RET, CSF-1	FDA Approved	RCC, GIST, Pancreatic cancer
Pazopanib (Votrient®)	VEGFR, PDGFR, cKIT	FDA Approved	RCC, Sarcoma
Vandetanib (Caprelsa®)	VEGFR, EGFR, RET	FDA Approved	Medullary thyroid cancer
Cabozantinib (Cometriq®)	cMET, VEGFR-2	FDA Approved	Medullary thyroid cancer
Axitinib (Inlyta®)	VEGFR, PDGFR, cKIT	FDA Approved	RCC
Ziv-Aflibercept (Zaltrap®)	VEGF	FDA Approved	mCRC
Ramucirumab (Cyramza®)	VEGFR-2	FDA Approved	Stomach cancer
Angiopoietin Targeted			
Trebananib (AMG386)	Ang-1/Ang-2	Phase III	Ovarian cancer
		Phase I	AML, CNS
AMG780	Ang-2	Phase I	Solid tumors
MEDI3617	Ang-2	Phase I	Solid tumors
Nesvacumab (REGN910)	Ang-2	Phase I	Solid tumors
CVX-060	Ang-2	Phase I	Solid tumors
DX-2240	Tie1	Preclinical	
scFv-Ang-2	Ang-2	Preclinical	
LC-06	Ang-2	Preclinical	
VEGF and angiopoietin targeted			
Regorafenib (Stivarga®)	VEGFR-2, PDGFR, Tie2, KIT, RET, Raf	FDA Approved	mCRC, GIST
CEP-11981	Tie2, VEGFR	Phase I	Solid tumors
ARRY-614	Tie2, VEGFR-2, p38, Abl	Phase I	Myelodysplastic syndromes
CVX-241	Ang-2, VEGF	Phase I	Solid tumors
RO5520985/RG7221	Ang-2-VEGF CrossMab	Phase I	Solid tumors
ACTB-1003	Tie2, VEGFR-2, FGFR-1, RSK, S6K ***A***	Preclinical	
DAAP	Ang-2, VEGF	Preclinical	

mCRC: metastatic colorectal cancer, RCC: renal cell carcinoma, NSCLC: non-small cell lung cancer, HCC: hepatocellular carcinoma, GIST: gastrointestinal stromal tumor, AML: acute myeloid leukemia, CNS: central nervous system tumor.

Table 2

Clinical trials with Ang-2 and VEGF-axis combination therapies (active and/or recruiting).

Angiopoietin-targeted agent	VEGF-targeted Agent	Tumor Setting	Clinical Trial
Phase I			
MEDI3617	Bevacizumab	Solid tumors	NCT01248949
Nesvacumab (REGN910)	Ziv-Aflibercept	Solid tumors	NCT01688960
Phase II			
Trebananib	Bevacizumab	mCRC	NCT01249521
Trebananib	Bevacizumab	RCC	NCT01664182
	Pazopanib		
	Sorafenib		
	Sunitinib		
Trebananib	Bevacizumab	Glioblastoma	NCT01609790
Trebananib	Bevacizumab	Metastatic breast cancer	NCT00511459
Trebananib	Sorafenib	HCC	NCT00872014
Trebananib	Sunitinib	RCC	NCT00853372
Trebananib	Sorafenib	RCC	NCT00467025

mCRC: metastatic colorectal cancer, RCC: renal cell carcinoma, HCC: hepatocellular carcinoma.