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Potential lymphangiogenesis therapies: learning from current anti-angiogenesis therapies - A review

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Abstract

In recent years, lymphangiogenesis, the process of lymphatic vessel formation from existing lymph vessels, has been demonstrated to have a significant role in diverse pathologies, including cancer metastasis, organ graft rejection, and lymphedema. Our understanding of the mechanisms of lymphangiogenesis has advanced on the heels of studies demonstrating vascular endothelial growth factor C (VEGF-C) as a central pro-lymphangiogenic regulator and others identifying multiple lymphatic endothelial biomarkers. Despite these breakthroughs and a growing appreciation of the signaling events that govern the lymphangiogenic process, there are no FDA-approved drugs that target lymphangiogenesis. In this review, we reflect on the lessons available from the development of anti-angiogenic therapies (26 FDA-approved drugs to date), review current lymphangiogenesis research including nanotechnology in therapeutic drug delivery and imaging, and discuss molecules in the lymphangiogenic pathway that are promising therapeutic targets.

Keywords

Angiogenesis; Lymphangiogenesis

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1 Introduction

Considered the “forgotten” circulation in health and systemic diseases [1], the lymphatic system is often perceived as an extensive, passive conduit of fluid and cells that regulates fluid homeostasis, nutrient absorption in the gastrointestinal tract, and immune surveillance throughout the body. [2] Utilizing knowledge of lymphatic endothelial cell (LEC) markers, including lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), prospero-related homeobox 1 (Prox-1), and podoplanin, investigators characterized an otherwise invisible lymphatic network in animal models and humans and came to understand the complex role of lymphangiogenesis in the context of various pathologies. [3,4] For example, tumor cells can release pro-angiogenic and pro-lymphangiogenic factors that direct the formation of lymphatic and blood vessels, via which metastasis occurs. In chronic inflammatory conditions like psoriasis, lymphatic vessels suppress inflammation by promoting the drainage of inflammatory mediators and fluids. [3]

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In 1971, Judah Folkman proposed that tumorigenesis often advances due to the co-option of angiogenesis. [5] Since then the clinical use of anti-angiogenic therapies has rapidly developed from preclinical stages. Studies in the 1990’s used monoclonal antibodies against vascular endothelial growth factor (VEGF) to demonstrate that tumor growth can be suppressed by neutralizing VEGF. [6] In 2004, the U.S. Food and Drug Administration (FDA) approved a first-in-class anti-VEGF monoclonal antibody, *bevacizumab* (brand name *Avastin*), for the treatment of metastatic carcinoma of the colon and rectum, and later approved the drug for other indications including non-small cell lung cancer [7] and other cancers (Figure I). Bevacizumab acts by binding to VEGF-A preventing it from activating VEGF receptor 2 (VEGFR2), which is a key player in the pro-angiogenic pathway. [8] *Aflibercept*, *sorafenib*, *sunitinib*, *axitinib*, *nintedanib*, *regorafenib*, *pazopanib*, *cabozantinib*, *vandetanib* and *thalidomide* are among the 26 anti-angiogenic agents that are now approved for the treatment of various cancers (Table I). Another anti-angiogenic compound, endostatin, which is FDA-approved in the US and other countries, is important due to its widespread anti-angiogenic effects. All of these drugs target various members of signaling pathways that are involved in angiogenesis, and they do this in a variety of ways. [9] For example, while bevacizumab targets the ligand to prevent ligand-dependent activation of a key receptor tyrosine kinase, targeting the receptor to prevent either ligand binding (ramucirumab) or receptor dimerization (pertuzumab) is an additional therapeutically beneficial strategy. On the other hand, endostatin, which is a 20-kDa C-terminal fragment of type-XVIII collagen, causes tumor regression by suppressing endothelial cell migration, thereby inducing apoptosis in cells. Altered endostatin levels have been observed in a variety of diseases, indicating that it acts through several other mechanisms, both anti-angiogenic and anti-lymphangiogenic, that are not yet completely understood, and thus, further research is required to better understand its various effects. [10]

Interest in targeting lymphangiogenesis in pathologies was delayed compared to that related to angiogenesis, due to difficulties in characterizing and imaging lymphatic vessels. The

discovery of pro-lymphangiogenic factors VEGF-C in 1996 [11] and VEGF-D in 1997[12] led to the initial interest in lymphangiogenesis. Then the characterization of LYVE-1, [13] Prox-1, [14] podoplanin, [15] VEGFR3 [16] and other lymphatic-specific biomarkers in the late 1990s brought more attention to this space (Figure II). Akin to the enthusiasm for anti-angiogenic therapies, the potential for anti-lymphangiogenic therapies created considerable excitement. Despite a growing understanding of lymphangiogenesis biology, there has yet to be a single drug on the market that targets lymphangiogenesis for treatment of conditions that involve lymphatic vessels, including cancer metastasis and organ graft rejection.

Unfortunately, despite promising results in murine models for the treatment of cancer, organ graft rejection, and other conditions, many of the 26 approved drugs targeting angiogenesis have fallen short of expectations in the clinic. For example, bevacizumab is largely used only in combination with other drugs. While the progression-free survival (PFS) of patients treated with bevacizumab as first-line therapy has shown marked improvement in many phase III studies, overall survival (OS) failed to differ significantly between experimental and control groups [17–24]. Even as a second-line treatment, bevacizumab has failed to show improvement in PFS. [25–29] Many groups have challenged the efficacy of anti-angiogenic drugs, [30–35] urging the scientific community to find alternative targets for diseases that involve vasculature and lymphangiogenesis has become a key topic in such discussions. [36–40]

The growing appreciation for the potential therapeutic benefits of anti-lymphangiogenic drugs has not yet been translated clinically. A search for clinical trials using the key words “angiogenesis” and “cancer” returned nearly 317 studies, whereas a search of “lymphangiogenesis” and “cancer” returned only 3 studies (Figure III). We reported in 2016 that a PubMed search for “angiogenesis” returned nearly 80,000 reports at that time, whereas a search for “lymphangiogenesis” returned approximately 2,635 entries at that time. [4] A more recent (2017) similar search showed that angiogenesis research continues to accelerate beyond the pace of lymphangiogenesis research (a search for “angiogenesis” yielded 94,542 results, whereas a search for “lymphangiogenesis” yielded a total of 3,163 results to date). This review describes the challenges encountered in the course of developing anti-angiogenic therapies, summarizes the principles of lymphangiogenesis, and identifies potential drug targets for lymphangiogenesis-related diseases.

2 Anti-angiogenesis therapies: Successes and lessons learned

As Folkman predicted in 1971,[5] inhibition of angiogenesis, which deprives cells within a tumor of oxygen and other nutrients, is a viable anti-cancer therapy. Signal transduction of VEGFs and their receptors activate the formation of new blood vessels that supply oxygen and nutrients to tumors and are the target of 50% of FDA-approved anti-angiogenic therapies (Table 1).[41] Bevacizumab remains the most successful anti-angiogenic therapy developed to date. This drug is an anti-VEGF-A monoclonal antibody (mAb) that does not interfere with other signaling pathways. [42] Clinical trials of bevacizumab concluded that it is well tolerated with less toxicity and can be used in combination with chemotherapy without any synergistic toxicity. [43,44] Previously known as rhuMAb, it binds to VEGF-A. [45] Notably, even with the success of mAb therapies such as bevacizumab, the next stage of

anti-angiogenic mAb therapy may take the form of nanobodies, small antigen-binding fragments derived from mAbs that can overcome the limited diffusion capabilities of their larger counterparts and thus better access various tissues including dense tumors. [46] Sunitinib, another approved anti-angiogenic drug, targets the tyrosine kinases VEGFRs and platelet-derived growth factor receptor (PDGFR) to reduce tumor neovascularization. Sunitinib, unlike bevacizumab, has a broad spectrum of activity against multiple tyrosine kinase pathways. [47] It is particularly effective in treating renal cell carcinoma and gastrointestinal stromal tumors. [48] Sunitinib is an oxindol molecule that binds with the intracellular ATP-binding sites of a wide range of receptor tyrosine kinases including VEGFR, PDGFR, KIT, and FLT3. [48,49] In this way, it inhibits angiogenesis by interfering with the signaling pathway of VEGF.

These VEGF pathway-targeting drugs represent, in theory, a promising therapeutic approach, because they block a well-characterized, major regulatory pathway for blood vessel growth. Favorable results for anti-angiogenic therapies in many murine models, which often demonstrate reduced metastatic activity and increased survival rates, have boosted investment into the angiogenesis space. However, most of these drugs have failed to meet expectations in human patients, supporting the notion that animal models and/or the clinical trial process may not accurately predict the efficacy of a given therapy in the “real world” setting. [50] The anti-angiogenic therapies developed so far show the maximal benefit for patients when used in a combination with agents that also target the cancer itself. [51–55] Thus, targeting the VEGF-A/VEGFR2 pathway alone is not enough, and there is a demand for additional therapies against other molecular pathways contributing to angiogenesis. This is outside the scope of this paper. For a more comprehensive review on the molecular mechanisms of angiogenesis and the limitations of current anti-angiogenic therapies, readers should consult the following reference [56]. More importantly, patients may benefit from anti-angiogenic and lymphangiogenesis-targeting combination therapies, since lymphangiogenesis is known to be involved in the pathobiology of several diseases (discussed later in this review) that also present with aberrant angiogenesis for which single VEGF-A/VEGFR2 inhibitors have been shown to be insufficient. Likewise, as is most likely the case for VEGF-A- and VEGFR2-targeting therapies that have shown limited clinical efficacy [56], lymphangiogenesis-targeting therapies developed against VEGF-C or VEGFR3 alone may be likely to face the same problems such as resistance. [57]

Nevertheless, the development and application of anti-angiogenic therapies has provided key insights into the relevant pathogenesis, particularly of tumors. For instance, preventing angiogenesis is likely to intensify and/or prolong hypoxia, which triggers a plethora of alternative strategies for tumor cells to survive, including vascular mimicry, vascular co-option, and metastasis. [50,58–65] Mathematical modeling of the consequences of suppressing oxygen availability predicts this very outcome, due to the natural selection of cells that can tolerate blood deficiency and are then driven to escape nutrient-starved regions. [66] Such responses are driven at least in part by hypoxia-induced stabilization of hypoxia-inducible factor 1 α (HIF-1 α), a transcription factor that increases the expression of many genes, including VEGF, matrix metalloproteinases (MMPs), fibroblast growth factor 2 (FGF2), angiopoietin-1, and TIE2. [67,68] Increased levels of VEGF enable the tumor to overwhelm anti-VEGF-based therapies and/or engage alternative pathways to initiate the

angiogenesis program (e.g., FGF2). A better understanding of the vascularization of cancers as well as evasion mechanisms will be crucial to the future development of anti-angiogenic therapies. [69]

3 Biomarkers

The paucity of biomarkers of the angiogenic status of a vascular bed contributes to our inability to effectively design and administer anti-angiogenic therapy. The ideal biomarker would not only indicate the disease status (similar to how abnormally elevated body temperature indicates infection), but also provides information on the drivers of pathological angiogenesis (analogous to a strep throat test which indicates the presence of group A streptococcus). Profiling the changes in signaling networks associated with and responsible for the resolution of pathological angiogenesis is one approach to identifying such biomarkers.

Just as how one biomarker for evaluating the effectiveness of anti-angiogenic therapies in metastatic cancer patients will not likely explain heterogeneity,[69] a single biomarker for pathological lymphangiogenesis will be insufficient. As discussed by Vasudev and Reynolds, the use of predictive algorithms in the future will help inform doctors and patients of the most appropriate anti-angiogenic therapies based on individual patients' parameters. These parameters may include single nucleotide polymorphisms (SNPs), serum markers, and imaging. [69] Moreover, markers characterizing the angiogenic and lymphangiogenic status of patients' tumor vasculatures would be invaluable tools.

There are currently approximately 149 phase III clinical trials studying anti-angiogenic cancer therapies (Figure IV). Among these are 20 phase II/III trials of anti-angiogenic therapies specifically for treatment of colorectal cancers only (Table II). In stark contrast, there are no ongoing phase II or III clinical trials of lymphangiogenesis-targeting therapies for colorectal cancer, or any type of cancer, despite a wealth of literature indicating the importance of aberrant lymphangiogenesis in the progression of colorectal cancer. [36] However, Nagahashi et al. state that the National Institutes of Health may not explicitly mention lymphangiogenesis in trial descriptions, and as such, there are in fact many currently ongoing or completed clinical trials studying potential anti-lymphangiogenic drugs. [36] Much needed research on the lymphangiogenic effects of not only novel but current cancer therapeutics may help build more robust data on the correlation between lymphatic interference and survival in cancer patients.

4 Lymphangiogenesis in health and disease: why we should focus on lymphangiogenesis-targeting treatments

The lymphatic system is blind-ended and unidirectional, lacking a central pump unlike the cardiovascular system, and it serves to regulate fluid homeostasis, nutrient absorption in the gastrointestinal tract, and immune surveillance throughout the body. [2] Physiological lymphangiogenesis in adults is rare, with few exceptions including in the ovaries and mammary tissues during the female reproductive cycle. [39,70] In contrast, pathologic lymphangiogenesis is found in many disease-related processes, including organ graft

rejection, cancer metastasis, lymphedema, and injury-induced corneal edema. [71] The molecular and cellular mechanisms of lymphangiogenesis involved in organ graft rejection, [72] cancer metastasis, [2,38,39,73] and lymphedema [74] are discussed in detail elsewhere. Moreover, lymphangiogenesis has an active and complex role in pathological circumstances such as inflammation, possibly including allergies and asthma. [75,76] A common factor in these diverse lymphangiogenic-related conditions is activation of the VEGF pathway, which includes ligands (VEGF-C, VEGF-D), receptors (VEGFR2 and VEGFR3), intracellular signaling enzymes (PI3K, mitogen-activated protein kinases [MAPKs]), and transcription factors (Prox-1). Lymphatic-deficient tissue like the cornea is avascular and easily accessible. This makes the cornea, in particular, an excellent platform for lymphangiogenesis research. Recently, groups have studied the corneal lymphatic response to injury and transplantation using real-time intravital imaging techniques on fluorescent reporter-tagged transgenic mice,[77,78] identified novel factors that regulate lymphangiogenesis such as aqueous humor-derived vasoactive intestinal peptide and α -melanocyte stimulating hormone,[79] and studied the role of lymphatic vessel growth in modulating the adaptive immune response to HSV-1 infection.[80]

4.1 Metastasis

Lymphatic metastasis is a major prognostic factor for different types of cancer. While there are various means for tumor cells to spread, intravasation into the lymphatic or blood circulation has been observed as the principle method by which metastases arise. In fact, due to structural advantages of the lymphatic route, tumor cell intravasation preferably occurs via the lymphatic system rather than the bloodstream.[81] Interestingly, it was once thought that almost no lymphatic vessels exist within tumors.[82] Many studies have since demonstrated the role of lymphangiogenic growth factors in tumor dissemination to regional lymph nodes. Furthermore, the lymphatic system was commonly thought to facilitate metastasis simply as a passive channel, providing a route for tumor cells to travel. However, recent advances in lymphatics research have revealed more complex and active mechanisms by which the lymphatic network interacts with cancer cells and induce tolerance of anti-tumor immune cells.[73,83] Therefore, this network has emerged as a major point of interest in oncological research.

In the current paradigm of the role of lymphangiogenesis in cancer, there are two distinct ways by which lymph vessels grow during metastasis. Intratumoral and peritumoral lymphatic vessel growth is known as “tumoral lymphangiogenesis,”[84,85] whereas “lymph node lymphangiogenesis” occurs from sentinel nodes[86,87] and is the main passage through which tumor cells travel to regional lymph nodes via the lymphatics.[2] In fact, tumor cells induce lymph node lymphangiogenesis even before their metastasis, allowing them to build their own paths to regional node sites. [88,89] Metastasis is a reliable prognostic factor for survival and cancer staging, involving evaluation of regional lymph node invasion and the severity of disease, and is important for therapeutic decision making. Evidence of lymph node lymphangiogenesis suggests that lymph nodes are primed to serve as a supportive environment for the arrival of cancer cells.[90–92] There is also good reason to pursue lymphangiogenesis targets for therapeutic applications, as some cancer patients have shown increased lymphangiogenic activity and subsequent recurrence of secondary

tumors despite anti-angiogenic therapy. It is theorized that the regression of blood vessels leads to hypoxia-induced over-expression of lymphangiogenic factors that counteractively heighten the risk of tumor metastasis.[93] Preliminary studies in mouse models show promising results by knockdown of VEGFC or sVEGFR2 in mammary cancer[94] and lung cancer.[95] To date, however, no anti-lymphangiogenic cancer therapies have been approved by the FDA due to our limited knowledge of the complex interactions between anti-angiogenic drugs, lymphangiogenesis, and cancer metastasis.

4.2 Organ graft rejection

Despite improved human leukocyte antigen (HLA)-matching criteria and modern immunosuppressive treatments, the incidence of immune-mediated graft rejection in organ transplantation remains high. Lymphangiogenesis is a central mechanism generating lymphatic vessels, which act as the “afferent” highway for antigen-presenting cells (APCs) and soluble antigenic material to travel to regional lymph nodes, while blood vessels act as “efferent” highways by which effector cells travel from lymph nodes to reject allografts. [96] In fact, every solid organ transplantation is accompanied by angiogenesis and lymphangiogenesis to varying extents, and lymphangiogenesis-mediated graft rejection has been observed after corneal, kidney, lung, and heart transplantations.

Among the various organs that are routinely transplanted, the cornea serves as an excellent model for understanding vascular growth in the pre- and post-operative periods due to its baseline clarity and avascular state. The lack of vasculature is vital to maintaining corneal transparency. The 2-year rejection rate for corneal transplantation cases is about 10% in low-risk patients who demonstrate little to no vasculature in the cornea prior to surgery. [97] However, this rate of rejection is 50% if the host corneal bed is vascularized. [98] Furthermore, it has been experimentally shown in mice that even low-risk transplantations can end in rejection when post-operative vascularization is induced. While the mechanisms remain unclear, it is hypothesized that newly formed blood and lymphatic vessels play a collaborative role in recruiting mononuclear phagocytes to promote an immune response. In one study, sorafenib, a multi-tyrosine kinase inhibitor with anti-angiogenic and anti-lymphangiogenic properties, showed promise for improving corneal graft survival in a high-risk corneal transplantation mouse model. [99] Investigations of anti-lymphangiogenic molecules have only recently picked up momentum and have already demonstrated their clinical potential in lymphatics-related pathologies. However, while much lymphangiogenesis research has been conducted in the cornea, lymphangiogenic mechanisms in other organ systems remain less clear. For example, some studies purport the favorability of cortical and perivascular lymphatic vessels in renal allograft survival and function, suggesting that lymphatic vessels may initially play a role in clearing inflammatory cytokines and cells that would otherwise lead to fibrosis and compromise allograft kidney function [100,101]. However, long-term renal allograft fibrosis caused by chronic inflammation that ultimately leads to rejection may be mediated by increased lymphangiogenesis. [102,103]

4.3 Lymphedema

A disruption in the lymphatic structure can result in the accumulation of protein and biochemical metabolites in the interstitial space, causing an increase in the tissue colloid osmotic pressure and accumulation of fluid. This condition is known as lymphedema, and severe forms are characterized by fibroadipose deposition. [104] Lymphedema can lead to significantly decreased quality of life due to physical disfigurement and disablement. Furthermore, chronic lymphedema increases the risk of angiosarcoma, also called Stewart-Treves Syndrome.[1,36] The pathophysiology is proposed to be a result of impaired lymphangiogenesis; despite this, therapeutic options for the management of lymphedema are relegated to mainly surgical or physiotherapeutic procedures with limited efficacy. This may be because our understanding of the biological mechanisms underlying lymphangiogenesis is still lacking, although it is increasing quickly, and only recently have we been able to identify candidates for therapeutic targets in the lymphatic network.

Primary lymphedema arises from dysfunctional development of the lymphatic system, often manifesting at birth but also in adolescence or later in life. [104] On the other hand, more common is secondary lymphedema, which occurs as a consequence of surgery, trauma, parasitic infection or radiotherapy. [105] Surgery and radiation cancer therapies are the leading cause of secondary lymphedema in industrialized nations. Approximately 30% of breast cancer patients experience lymphedema of the upper limb, due to the disruption of lymphatic passage to the axillary nodes. [106] Chronic inflammation with a prominent T helper 2 cell response in lymphedematous limbs is a common finding in both animal models and humans, in which the inflammatory milieu of cytokines inhibits lymphangiogenesis, impairs lymphatic function, and promotes fibrosis. [104,107,108] This complex interplay between inflammation and impaired lymphatic function is an attractive focus for research, since in such cases, treatment with the pro-lymphangiogenic factor VEGF-C, which may already be over-expressed in lymphedematous tissues, may further increase the risks of relapse and metastasis in these patients. [104,109–111] Some studies have also experimentally demonstrated that pro-inflammatory cytokines, including interleukin-8 (IL-8), can reduce lymphedema by causing inflammation and promoting lymphangiogenesis. [112]

5 Lymphangiogenesis mechanisms: major target sites

5.1 VEGF pathway

Lymphangiogenesis is regulated mainly by the VEGF/VEGFR system (Figure V), specifically ligands, VEGF-C and VEGF-D, and receptor tyrosine kinases, VEGFR2 and VEGFR3. VEGFR3 was the first to be discovered and was instrumental in the isolation of VEGF-C [11,113]. As VEGFR2 is for hemangiogenesis, VEGFR3 is the main receptor modulating lymphangiogenesis and is highly selectively expressed on endothelial cells committed to the lymphatic lineage; an exception, however, are endothelial cells of fenestrated blood vessels in some endocrine organs that also express VEGFR3. [2] VEGF-A triggers angiogenesis through its interaction with VEGFR1 and VEGFR2. Lymphangiogenesis is driven by VEGF-C and VEGF-D, which activate VEGFR3; VEGF-C also engages VEGFR2. [11] The interplay between complex factors such as the structural

differences between VEGF-C and VEGF-D and their expression levels [114], the proteolytic cleavage of these factors [39,115], and the tissue of origin all impact the binding affinities of VEGF-C and VEGF-D to VEGFR2 and VEGFR3 and thus impact the regulation of lymphangiogenesis. [114,116] Because VEGF-C is considered the main ligand for lymphangiogenesis, this review will continue discussing the role of VEGF-C; however, the role of VEGF-D in human disease is just beginning to be better understood. [117]

In a recent study, Zhong et al. introduced a Prox1-GFP/Flt1-DsRed (PGFD) mouse model that allows for live imaging of angiogenic and lymphangiogenic processes in the cornea and other organs. They found that lymphangiogenesis occurred even when angiogenesis did not, in concordance with previous studies reporting the occurrence of corneal lymphangiogenesis independently of hemangiogenesis. [118–123] Conditional deletion of VEGFR2 in endothelial cells markedly reduced lymphangiogenesis in the mouse cornea in response to VEGF-A and VEGF-C pellets, compared to that in mice without this conditional deletion, suggesting a role for blood endothelial cell VEGFR2 in lymphatic vessel formation and growth. [78] There seem to be various combinations of ligand binding affinities and kinase activities that produce a spectrum of downstream effects, both angiogenic and lymphangiogenic. Thus, before a therapeutic target can be determined, it is crucial to further characterize the specific mechanisms of the VEGF signaling system.

All VEGFRs are expressed on vascular endothelial cells at differing levels. [124] VEGFR1 expression is documented in macrophages, osteoclasts, dendritic cells, placental trophoblasts and pericytes, although its functions in these contexts have yet to be determined. [125–127] VEGFR2 is expressed mainly during early embryogenesis and re-expressed under certain pathological conditions, including tumor-induced angiogenesis. [128] Expression of VEGFR2 has also been observed in osteoblasts, neuronal cells, retinal progenitor cells, pancreatic ductal cells, megakaryocytes [128], and mesenchymal stem cells. [129] This does not include the upregulation of VEGFR3 seen in certain diseases. While VEGFR1 and VEGFR2 have shown diverse expression profiles, VEGFR3 has only been observed in a small set of cell types, including LECs, fenestrated vessels in endocrine organs, macrophages [130], osteoblasts [131], and bone-marrow mesenchymal stem cells. [129] The importance of VEGFR2 in VEGF-C-dependent responses has been investigated using VEGF-C156S, a mutant form of VEGF-C specific for VEGFR3. [132–134] VEGFR3 stimulation may induce an anti-inflammatory gene expression profile in LECs, consistent with previous findings of reduced inflammation following VEGF-C/VEGFR3 signaling. [135]

Blockade of VEGFR3 or VEGF-C may help or hinder the progression of certain pathologies, indicating that the beneficial or detrimental role of VEGFR3 signaling depends on the disease in question. Blockade of the VEGFR3 signaling pathway mitigates inflammation and thus ameliorates the phenotypes in certain animal models of disease such as graft versus host disease (GVHD), [136] corneal graft rejection, [137] dry eye disease, [138] allergic eye disease, [139] obesity, [140] the tumorigenesis of squamous cell carcinoma, [141] and pulmonary arterial hypertension (PAH). [142,143] Interestingly, in the clinic, PAH has been found to be associated with increased levels of the anti-angiogenic chemokine CXCL4 [144] and the anti-angiogenic isoform of VEGF VEGF165b, [145]

suggesting the role of suppressed angiogenesis in the etiology of PAH. Also, one preclinical study reports that VEGFR3 inhibition early in the course of disease reduces inflammation, lung arterial obliteration, and pulmonary hypertension by offsetting the increased VEGFR3 signaling that contributes to pulmonary remodeling in PAH. [143] Thus, hypothetically, treatment of PAH could involve a combination pro-angiogenic and anti-lymphangiogenic therapies.

Conversely, blockade of VEGFR3 may be detrimental in the treatment of certain diseases and exacerbate inflammation or inhibit therapeutic immune responses. For example, VEGFR3 inhibition enhances inflammation of the colon in a mouse model of inflammatory bowel disease (IBD). [146] An increased density of abnormally dilated lymphatic vessels is found in the colons of these mice, similar to findings in IBD patients.[147,148] In the aforementioned study, anti-VEGFR3 mAb therapy impaired lymphatic vessel function important for the clearance of inflammatory mediators. [146] Similarly, due to defective drainage of inflammatory cells and cytokines, VEGFR3 inhibition also exacerbates rheumatoid arthritis [149,150] and chronic airway inflammation. [151] In the context of melanoma treatment, VEGFR3 blockade interferes with the beneficial effect of checkpoint blockade immunotherapy, [152] This suggests that VEGF-C is beneficial in combination with immunotherapy through the induction of CCL21 expression in tumor-associated lymphatic vessels, which recruits naïve T cells and dendritic cells to the tumor microenvironment. At this point, it is unclear whether this is the case with other cancers. However, the latter example illustrates that potential anti-lymphangiogenic cancer therapies, especially if they are to be used with cancer immunotherapies in the future, may need to be designed to balance the competing processes of inhibiting lymphangiogenesis-associated metastasis and facilitating the recruitment of tumor-killing immune cells. All together, these studies indicate that pro- and anti-lymphangiogenic therapies that must be applied only after understanding whether lymphangiogenesis in certain diseases is an instigator of inflammation or a necessary sequela for its resolution (for recent reviews on inflammation-associated and the immunomodulatory effects of lymphangiogenesis, refer to the following references.[153,154]

An example of an inflammatory pathway encroaching on the VEGF pathway to induce lymphangiogenesis is well demonstrated by the role of TNF- α in lymphatic metastasis. Ji et al. shed light on this mechanism in which TNF- α directly stimulates lymphatic endothelial cell migration independently of VEGFR3. [155] Lymphatic vessel development is triggered by TNF- α binding to TNFR1, but TNF- α -induced lymphangiogenesis can only occur after lymphatic endothelial cell tip formation, which can only be induced by VEGFR3-mediated signaling. Hence, if VEGFR3 is blocked, TNF- α -TNFR1 signaling-induced lymphangiogenesis is also precluded. TNF- α also indirectly stimulates lymphangiogenesis by activating inflammatory macrophages that produce VEGF-C, inducing persistent stimulation of lymphangiogenesis and ultimately, tumor metastasis. [155]

5.2 EGF pathway

The epidermal growth factor receptor (EGFR) family is a group of receptor tyrosine kinases that are over-expressed in many cancer types (Figure VI) [156,157] Of the four receptors in

the family, EGFR2 (also often called human epidermal growth factor receptor 2 [HER2] or NEU) and EGFR3 lack the ability to bind EGF or show intrinsic tyrosine kinase activity, respectively, and therefore, form heterodimers with EGFR1 or EGFR3 to initiate downstream MAPK signaling. [158,159] Research has demonstrated that over-expression of EGFRs promotes cancer cell proliferation, migration, and invasion, and over-expression of VEGFs, induced by EGFR signaling in tumors, promotes angiogenesis to expand the tumor vasculature. [160–162]

This supports an intimate interplay between the VEGF and EGF pathways in promoting cancer progression through angiogenesis. A novel sorafenib derivative that targets both VEGFRs and EGFRs has been shown to be efficacious *in vitro* against prostate tumors. [163] In addition, in animal xenograft models of non-small cell lung cancer, therapies targeting both VEGFR and EGFR for tumor treatment are more effective than those targeting either receptor family alone [164,165]. Therefore, monoclonal antibodies such as cetuximab and receptor tyrosine kinase inhibitors such as erlotinib that target EGFRs represent a two-pronged approach to cancer therapy by inhibiting tumor growth directly and indirectly by blocking proliferation of the tumor vasculature (Figure VI).

Compared to that of normal endothelial cells, tumor-associated endothelial cell expression of EGFRs is abnormal. [166–168] The transfer of oncogenic EGFR produced by tumors via extracellular vesicles to endothelial cells in pathologic angiogenesis has also been demonstrated. [169,170] Despite preclinical evidence of the efficacy of EGFR inhibitors for treating angiogenesis-driven cancer progression and metastasis, clinical evidence shows only limited efficacy of EGFR-targeting anti-angiogenic therapies in humans. [161,171–175] Lymphatics have a well-supported role in tumor metastasis, and thus, understanding the role of EGFR signaling in lymphangiogenesis and the effects of EGFR inhibitors may advance the development of potentially effective anti-lymphangiogenic therapies for the prevention or treatment of metastatic cancers.

Signaling events involving EGFRs and the processes governing lymphangiogenesis are closely intertwined. EGFR and HER2 are expressed on human LECs associated with skin [176,177], while EGFR has been shown to be expressed in the intra- and peritumoral lymphatic vessels of oral squamous cell carcinoma [178] and the peritumoral lymphatics of colon cancer. [179] In addition, anti-EGFR therapy improved lung phenotypes in mice models of lymphangioleiomyomatosis [180], a destructive cystic lung disease characterized by neoplastic lesions that express lymphatic endothelial cell markers Prox-1, LYVE-1 and podoplanin [181] and producing lymphangiogenic factors VEGF-C and VEGF-D. [182] In vitro, EGF-treated human LECs exhibit enhanced migration and tube formation, while in vivo, EGF treatment leads to increased lymphatic vessel area and size, albeit to a lesser extent than VEGF-C treatment. [176] In a xenograft melanoma mouse model, EGF produced by tumors induces lymphangiogenesis, with knockdown of EGF expression decreasing tumor-associated lymphatic vessel density but not blood vessel density. [183] Frequently, tumors shed extracellular portions of transmembrane proteins through the over-expression of sheddases to modulate cell-to-cell communication [184] and increase ectodomain shedding of membrane-bound EGFs. [185] For example, A disintegrin and metalloprotease 17 (ADAM17) increases heparin-binding epidermal growth factor (HB-EGF) shedding by

LECs, resulting in increased LEC invasion, migration, and tube sprouting. [177] Silencing of ADAM17 in LECs and treatment with an EGFR inhibitor resulted in both decreased motility and sprouting. [177] Finally, dual inhibition of transforming growth factor beta (TGF- β) and EGFR/HER2 using lapatinib suppresses the growth and metastasis of pancreatic ductal adenocarcinoma, which expresses many lymphangiogenic factors. [186] Although there are promising results on the interaction between EGFR signaling and lymphangiogenesis, the detailed mechanisms have yet to be elucidated, and thus far, there have been no clinical trials specifically studying the pro-/anti-lymphangiogenic effects of EGFR inhibitors on lymphangiogenesis-related disorders or cancer metastasis.

5.3 FGF and PDGF pathways

FGFs and platelet-derived growth factors (PDGFs) induce both angiogenesis and lymphangiogenesis. [187] In the cornea, FGF2 (also known as basic fibroblast growth factor, bFGF) increases expression of VEGF-A, VEGF-C, and VEGF-D, thereby indirectly increasing angiogenesis via VEGFR2 signaling and lymphangiogenesis via VEGFR3 signaling. [188,189] One study showed that while both FGF2 and VEGFs induce blood and lymphatic vessel growth consistent with previous findings, FGF2 more potently induces lymphatic vessel growth in the cornea. [190] FGF2 is the main driver of corneal neovascularization after herpes simplex virus 1 (HSV-1) infection, and consequently, neutralization of FGF2 reduces both corneal angiogenesis and lymphangiogenesis. [191] In the tumor microenvironment, FGF2 indirectly promotes LEC proliferation and migration via FGFR1 signaling. [192] This interaction may be fortified by LYVE-1, as glycosylated LYVE-1 binds FGF2 tightly, and in fact, soluble LYVE-1 inhibits FGF2-mediated lymphangiogenesis. [193] However, inhibition of FGF2 in targeted therapies must be executed carefully to avoid possible toxicities; for example, FGF2 is involved in neurogenesis and its inhibition may impair nerve regeneration. [80,194] Furthermore, FGF2 and VEGF-C were shown to work synergistically to promote tumor growth and metastasis to the lung via blood vessels and to sentinel lymph nodes via lymphatic vessels in a mouse model of fibrosarcoma. [192] Together, these studies support the advantage of combination therapies that inhibit both FGFR and VEGFR-3 signaling to therapeutically target pathologic lymphangiogenesis.

The FGF/FGFR pathway may also be an attractive target for anti-angiogenic cancer therapies, as it may be a potential escape mechanism for cancers refractory to anti-VEGF-A monotherapies. [195] Therefore, potential VEGF-C/VEGFR3 anti-lymphangiogenesis therapies for metastatic cancers can also benefit from anti-FGF2/FGFR compounds. In addition, while the family of PDGF molecules, PDGF-AA, PDGF-BB, and PDGF-AB, can all induce angiogenesis equally, PDGF-BB and PDGF-AB more potently stimulate lymphangiogenesis by binding to PDGFR β compared with PDGF-AA, which engages PDGFR α exclusively. [196,197] PDGF-BB-mediated lymphangiogenesis in the cornea occurs independently of VEGFR3 and TIE2 and facilitates tumor growth and metastasis *in vivo*. [197] Interestingly, FGF2 upregulates PDGFR α and PDGFR β and potentiates angiogenic signaling of PDGF-BB in a mouse model of corneal angiogenesis. [198] Conversely, stimulating both FGF and PDGF-BB signaling pathways results in a more potent angiogenic effect than VEGF stimulation alone, for these molecules may act as

chemoattractants for macrophages that facilitate blood endothelial tip migration and anastomosis. [199] It is well documented that macrophages can induce lymphangiogenesis both directly through trans-differentiation and indirectly via the production of VEGF-C and VEGF-D. [118–121] However, there are no recent studies investigating the potential synergistic effect of PDGF-BB and FGF2 in lymphangiogenesis, despite plenty of research validating their pro-lymphangiogenic effects alone. Interestingly, a recent study by Liu et al. showed that metastasis of tumor cells is enhanced by PDGF-BB and FGF2 treatment of cancer-associated fibroblasts. [200] Such findings provide additional motivation for studies on the interaction between blood endothelial cells, LECs, fibroblasts, other stromal cells, and tumor cells within the tumor microenvironment to understand their multifaceted interactions in cancer metastasis. Therefore, potential VEGF-C/VEGFR3 anti-lymphangiogenesis therapies for metastatic cancers may also benefit from anti-FGF-2/FGFR and anti-PDGF-BB/PDGFR β compounds.

6 Current lymphangiogenesis research

The biological regulation of angiogenesis and lymphangiogenesis is vast, involving hundreds of regulatory molecules. Thus, much effort is focused on studying molecules that act beyond the aforementioned growth factors and could have clinical applicability. For instance, therapeutic agents that target the angiopoietin (Ang)-TIE pathway are currently in clinical development for oncological and ophthalmological applications. [201] Ang-1 and Ang-2 have a direct promoting effect on cervical cancer migration and invasion, and their down-regulation has predictably attenuated tumor growth. [202] The same study found that TIE2 expression in cervical tumor cells is associated with significantly poor prognosis. Furthermore, treatment with Ang-2 inhibitors significantly improves outcomes of corneal grafts. [203]

A recent report indicates that fatty acid β -oxidation is required for lymphangiogenesis. Lymphangiogenesis is impaired in mice that lack carnitine palmitoyltransferase 1A (CPT1A), a rate-controlling enzyme in fatty acid oxidation. [204] A decrease in acetyl coenzyme A, a consequence of CPT1A knockout, leads to decreased acetylation of histones at lymphangiogenic genes. Furthermore, CPT1A is upregulated by Prox-1. Wong et al. suggested that there may be underappreciated therapeutic potential in lowering fatty acid oxidation to suppress these genes. In the case of lymphedema, supplementation with metabolites such as acetate may promote lymphangiogenesis instead. [204]

One study demonstrated that toluquinol, a marine fungus metabolite, suppresses lymphangiogenesis by interfering with VEGF-C–induced VEGFR3 phosphorylation *in vivo*, *ex vivo* and *in vitro*. [205] In these experiments, toluquinol is a more potent inhibitor of lymphangiogenesis than of angiogenesis. Several other drugs have been shown previously to reduce lymphangiogenesis by blocking VEGFR3, including deguelin [206,207], liposomal honokiol [208], Ki23057 [209], etodolac [210], MMI270 [211], CSDA [212], and norcantharidin [213]. More recently, fucoidan, a fucose-enriched sulfated polysaccharide, was shown to have dose-dependent depressive-effects on Prox-1 and VEGFR3 in murine models, demonstrating anti-lymphangiogenic capabilities. [214] Additionally, the VEGFR3 human immunoglobulin G subclass 1 monoclonal antibody LY3022856 was studied in a

phase 1 dose-escalation study to measure its anti-tumor effects. [215] Although the study concluded that the antibody had insignificant anti-tumor activity, this phase 1 trial demonstrates that investigators are gradually bringing attention to lymphangiogenesis mechanisms as targets for treating pathologies.

Nanoparticles have additionally emerged as a controlled gene delivery system that suppresses lymphangiogenesis sustainably through targeted delivery and then continuous release of genes to tumorigenic areas. For instance, small interfering RNA (siRNA) targeting VEGF-C suppresses tumor lymphangiogenesis in subcutaneous xenografts particularly when the siRNA is complexed with calcium carbonate (CaCO₃) nanoparticles. [216] Divalent calcium ions of the CaCO₃ nanoparticles stabilize the DNA structure by forming ionic complexes with the nucleic acid backbone. This not only protects the siRNA from degradation but also allows the CaCO₃ nanoparticle–siRNA complex to cross the cancer cell membrane via ion channel-mediated endocytosis. In a similar context, VEGFR3-targeting siRNA molecules are complexed with polyethylenimine (PEI)-alginate nanoparticles with an average diameter of 139 nm. [217] Alternatively, Flt23k, an intrareceptor inhibitor of VEGF, is loaded in biodegradable poly-(L-lactic-co-glycolic acid) (PLGA) nanoparticles. [218] The delivery of these nanoparticles to the murine cornea significantly decreases neovascularization and lymphangiogenesis.

In addition to therapeutic applications, nanoparticles have been applied to imaging lymphatics in cancer. Various magnetic resonance imaging (MRI) contrast agents are injected into the interstitial space for visualization of lymphatic vessels and lymph nodes. For example, gadolinium (Gd)-labeled nanoparticles with a polyamidoamine dendrimer core and an average diameter of 10 nm are used to differentiate intralymphatic- from extralymphatic-involved lymphoma. [219] Alternatively, poly(ethylene glycol) (PEG)-conjugated nanoparticles consisting of an ultra-small superparamagnetic iron oxide (USPIO) core and a gold shell as well as dextran-coated SPIO nanoparticles are used to image lymphangiogenesis within tumors. [220] Notably, dextran-coated SPIO nanoparticles have been approved for diagnostic use in certain European countries. [221]

7 Conclusion

Over the last 15 years, lymphangiogenesis has been implicated in numerous pathologies including metastasis, organ graft rejection, and lymphedema. Consequently, gate keepers of the lymphangiogenic process have emerged as a novel class of therapeutic targets. This constitutes a fertile and untapped opportunity; while there are 26 clinically approved approaches to treat pathological angiogenesis, none exist for lymphangiogenesis. Identification of governors of the lymphangiogenic process, along with the experience obtained in the process of developing anti-angiogenic modalities, has set the stage for rapid development of biomarkers and therapeutics for lymphangiogenesis.

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Nonstandard Abbreviations

ADAM17	A disintegrin and metalloprotease 17
Ang	angiopoietin
APCs	antigen-presenting cells
bFGF	basic fibroblast growth factor
CPT1A	carnitine palmitoyltransferase IA
EGFR	epidermal growth factor receptor
ERBB	homologous to erythroblastoma viral gene product, v-erbB
FGFR	fibroblast growth factor receptor
Gd	gadolinium
GVHD	graft versus host disease
HB-EGF	heparin-binding epidermal growth factor
HER1	human epidermal growth factor receptor 1
HER2	human epidermal growth factor receptor 2
HIF-1alpha	hypoxia-inducible factor 1-alpha
HLA	human leukocyte antigen
HSV-1	herpes simplex virus 1
IBD	inflammatory bowel disease
IL-8	interleukin-8
LECs	lymphatic endothelial cells
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
MAPKs	mitogen-activated protein kinases
MRI	magnetic resonance imaging
MMPs	matrix metalloproteinases
micro-MRL	micromagnetic resonance lymphangiography
mAb	monoclonal antibody
OS	overall survival

PIGF	placental growth factor
PDGFR	platelet-derived growth factor receptor
PEI	polyethylenimine
PFS	progression-free survival
PGFD	Prox1-GFP/Flt1-DsRed
PEG	poly(ethylene glycol)
Prox-1	prospero-related homeobox 1
PAH	pulmonary arterial hypertension
SNPs	single nucleotide polymorphisms
siRNA	small interfering RNA
sVEGFR	soluble vascular endothelial growth factor receptor
TGF-β	transforming growth factor beta
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

Biographies

Michael Yamakawa received his bachelor's degree in Biophysics from Johns Hopkins University. His research there utilized x-ray crystallography to characterize ligand-binding promiscuity and to advance knowledge on the binding mechanisms of multi-drug recognizing transcription factors. He received a grant to evaluate didactic methods in biophysics using an open-source Java viewer of macromolecular structures. He received a second grant to evaluate a set of adjusted eligibility criteria for heart transplantation and study the effectiveness of third-generation ventricular assist devices as a destination treatment for heart failure. He also worked at Northwestern University to determine the structure of PDR3, a transcription factor within the yeast pleiotropic drug resistance network and elucidate its DNA-binding mechanisms. After graduating, he was an intern at a start-up pharmaceutical company in Baltimore where he helped a life-saving pediatric orphan drug achieve FDA approval. He is currently a second-year medical student at the University of Illinois at Chicago (UIC) College of Medicine with a research focus on understanding the development of pathological blood and lymphatic vasculatures in the cornea through the study of vascular endothelial growth factors and their receptors. He also takes part in a selective, specialized program called Innovation Medicine (IMED), which allows him to bridge technology with medical education and practices.

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Mario Montana, M.D. is currently a visiting research scholar at the University of Illinois at Chicago. He received his M.D. degree from Universitas Pelita Harapan in 2012. His current research interest is in the characterization of murine corneal blood and lymphatic vessel growth. More specifically, his research focuses on the effects of limbal deficiency induced by limbal epithelial scraping on Prox1-GFP/Flt1-DsRed (PGFD) mice with or without conditional knockout of lymphatic or angiogenic VEGFR-2. He has also contributed in writing a review paper entitled Angiogenesis and Lymphangiogenesis in Corneal Transplantation, which is currently pending review.

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Andrius Kazlauskas, PhD received his BS (1982) and PhD (1986) degrees in chemistry from Cleveland State University. His graduate studies focused on elucidating the signaling events responsible for growth factor-driven proliferation of cells. He continued in this area of basic research at the Fred Hutchinson Cancer Research Center in Seattle (1986–1991) and then in his own research lab at the National Jewish Center in Denver (1991–1996). He joined the Schepens Eye Research Institute and Department of Ophthalmology at Harvard Medical School in 1996 to investigate the signaling events driving the pathogenesis of blinding eye diseases such as proliferative diabetic retinopathy, age-related macular degeneration, and proliferative vitreoretinopathy. In 2015, Dr. Kazlauskas transitioned to F. Hoffman-La Roche in Basel, Switzerland in order to fully engage in translational research leading to new therapies for patients with sight-threatening conditions. In 2017, Dr. Kazlauskas transitioned

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Figure I. Trend in FDA approval of anti-angiogenic therapies

In 2004, bevacizumab became the first anti-angiogenic therapy approved for the treatment of metastatic colorectal cancer, and the approval of erlotinib and pegaptanib followed shortly after in the same year. Regorafenib, a treatment for hepatocellular carcinoma, is the most recently approved anti-angiogenic therapy. We observe that from 2008 to 2012 there was a steady increase of the number of FDA-approved drugs. A total of 7 anti-angiogenic drugs were approved by the FDA. This steady increase reflects the growing interest in using anti-angiogenic drugs in cancer treatment. There are 26 molecules approved for anti-angiogenic therapy, some with multiple, unique indications. Drugs were counted once per approved indication (e.g., Afatinib is counted for one approval in 2013 and one approval for a separate indication in 2016.)

Figure II. Number of papers found in PubMed searches for "lymphangiogenesis" per year since 1986

The published literature devoted to lymphangiogenesis research has grown dramatically since 1996, when VEGF-C and -D were discovered to be lymphangiogenic factors. The number of published studies steadily increased from 1997 to 2013 from less than 10 to more than 300. The volume of research on lymphangiogenesis increased by more than 50 times after the discovery of VEGF-C, VEGF-D, and the characterization of three lymphatic vessel biomarkers—LYVE-1, Prox-1, and podoplanin. These events proved to be ground-breaking for research into lymphangiogenesis.

Figure III. Clinical trials of anti-angiogenic chemotherapy and anti-lymphangiogenic chemotherapy

An advanced search of NIH *U.S National Library of Medicine* [ClinicalTrials.gov](https://clinicaltrials.gov) shows a steady approval rate of clinical trials for anti-angiogenic therapies. In contrast, clinical trials for therapies targeting lymphangiogenesis are sparse. The only observable approvals of anti-lymphangiogenic therapies occurred in 2005 and 2010, with less than 10 approvals in total. While the number of anti-angiogenic chemotherapies approved in 2008 was well beyond 40, the number of anti-lymphangiogenic therapies was far fewer.

Figure IV. Active Phase III clinical trials for anti-angiogenic therapies (as of August, 2017)

Lenalidomide and bevacizumab have been tested in the highest numbers of clinical trials among all anti-angiogenic therapies. Sorafenib has been tested in the most clinical trials for receptor tyrosine kinase inhibitors. Sunitinib and other receptor tyrosine kinase inhibitors account for around 40% of all related clinical trials, while monoclonal antibody (mAb) drugs account for around 42%, of which more than 25% trials tested bevacizumab. The rest of the trials were for immunomodulatory drugs like thalidomide and lenalidomide.

Figure V. Schematic of VEGF pathway target sites of FDA-approved anti-angiogenesis drugs and potential VEGF pathway target sites for future anti-lymphangiogenesis drugs

Axitinib, Bevacizumab, Cabozantinib, Nintedanib, Pazopanib, Pegaptanib, Ramuricumab, Ranibizumab, Regorafenib, Sorafenib, Sunitinib, and Vandetanib are approved anti-angiogenic therapies that target VEGF-mediated angiogenesis. Inhibition of a VEGF ligand, a VEGFR binding site, or VEGFR tyrosine kinase activity leads to a reduction in angiogenesis. This strategy can potentially be translated for lymphangiogenesis-targeted drug design. Development of anti-lymphangiogenic therapies may follow the design of anti-angiogenic therapies by interrupting the lymphangiogenic factor VEGF-C, its receptors VEGFR2 and VEGFR3, or the tyrosine kinase activity of VEGFR2 and VEGFR3. HA, hemangiogenic; LA, lymphangiogenic.

Figure VI. Schematic of EGF pathway target sites of FDA-approved anti-angiogenesis drugs EGFR (also known as ERBB1 and HER1) and ERBB2 (also known as HER2/neu) are the main therapeutic targets of currently available anti-angiogenic drugs. ERBB2 lacks the ability to bind growth factor ligands and forms heterodimers with EGFR, ERBB3, or ERBB4. Monoclonal antibodies targeting the extracellular domain such as trastuzumab and pertuzumab prevent heterodimer formation, while others such as cetuximab, necitumumab, and panitumumab competitively inhibit EGFR ligand binding. Receptor tyrosine kinase inhibitors target the intracellular kinase domains and prevent signaling downstream of ligand-binding and dimer formation.

Table I

FDA-approved anti-angiogenic therapeutics.

Anti-angiogenic agent	Targeted Pathway	Clinical Indications (year approved)
Afatinib	Receptor tyrosine kinase inhibitor (epidermal growth factor receptor [EGFR], human epidermal growth factor receptor 2 [HER2])	Non-small cell lung cancer (2013); squamous cell lung carcinoma (2016)
Aflibercept	VEGFR1/VEGFR2/IgG chimeric protein (VEGF-A, VEGF-B, and placental growth factor [PlGF])	Wet age-related macular degeneration (2011); metastatic colorectal cancer (2012)
Axitinib	Receptor tyrosine kinase inhibitor (VEGFR, platelet-derived growth factor receptor [PDGFR], c-KIT)	Renal cell carcinoma (2012)
Bevacizumab	Humanized monoclonal antibody (VEGF-A)	Metastatic colorectal cancer (2004); lung cancer (2006)
Cabozantinib	Receptor tyrosine kinase inhibitor (c-MET, VEGFR2, AXL, RET)	Metastatic medullary thyroid cancer (2012)
Cetuximab	Chimeric monoclonal antibody (EGFR)	Metastatic colorectal cancer (2012); recurrent or metastatic head and neck cancer (2011)
Erlotinib	Receptor tyrosine kinase inhibitor (EGFR)	Lung cancer (2004)
Gefitinib	Receptor tyrosine kinase inhibitor (EGFR)	Metastatic non-small cell lung cancer (2015)
Lapatinib	Receptor tyrosine kinase inhibitor (EGFR, HER2)	HER2+ breast cancer (2007)
Lenalidomide	Small molecule that binds to cerublon and thereby reduces the level of certain transcription factors	Myelodysplastic syndrome (2005)
Necitumumab	human IgG1 monoclonal antibody (EGFR)	Metastatic squamous non-small cell lung cancer, in combination with gemcitabine and cisplatin (2015)
Nintedanib	Receptor tyrosine kinase inhibitor (VEGFR, fibroblast growth factor receptor [FGFR], PDGFR)	Idiopathic pulmonary fibrosis (2014)
Panitumumab	Human IgG2 monoclonal antibody (EGFR)	Colorectal cancer (2006)
Pazopanib	Receptor tyrosine kinase inhibitor (c-KIT, FGFR, PDGFR, VEGFR)	Renal cell carcinoma (2009); soft tissue sarcoma (2012)
Pegaptanib	RNA aptamer (VEGF-A ₁₆₅ isoform)	Wet age-related macular degeneration (2004)
Pertuzumab	Humanized monoclonal antibody (HER2)	Neoadjuvant treatment of HER2+ breast cancer in combination with Trastuzumab and Docetaxel (2012)
Pomalidomide	Small molecule that binds to cerublon and thereby reduces the level of certain transcription factors	Multiple myeloma (2013)
Ramucirumab	IgG1 monoclonal antibody (VEGFR2)	Metastatic gastric or gastroesophageal junction adenocarcinoma and platinum-resistant metastatic non-small cell lung cancer (2014); metastatic colorectal cancer (2015)
Ranibizumab	Fab fragment of bevacizumab (VEGF-A)	Neovascular age-related macular degeneration (2006)
Regorafenib	Receptor tyrosine kinase inhibitor (VEGFR2, TIE2)	Metastatic colorectal cancer (2012); GIST (2013); and hepatocellular carcinoma (2017)
Sorafenib	Kinase inhibitor (VEGFR, PDGFR, RAF family)	Renal cell carcinoma (2005); hepatocellular carcinoma (2007)
Sunitinib	Receptor tyrosine kinase inhibitor (PDGFR, VEGFR, c-KIT, RET, granulocyte colony-stimulating factor receptor [G-CSFR], FLT3)	Metastatic renal cell carcinoma and gastrointestinal stromal tumor (2006); pancreatic neuroendocrine tumors (2011)
Temsirolimus	Small molecule inhibitor (mammalian target of rapamycin [mTOR])	Multiple myeloma in combination with dexamethasone (2006)
Thalidomide	Small molecule that binds to cerublon and thereby reduces the level of certain transcription factors	Multiple myeloma (2003)
Trastuzumab	Humanized monoclonal antibody (HER2)	HER2+ breast cancer (2006); HER2-overexpressing metastatic gastric or gastroesophageal (GE) junction adenocarcinoma (2010)

Anti-angiogenic agent	Targeted Pathway	Clinical Indications (year approved)
Vandetanib	Receptor tyrosine kinase inhibitor (VEGFR, EGFR, RET)	Unresectable, locally advanced, or metastatic medullary thyroid cancer (2011)

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Table II

Phase II/III clinical trials of anti-angiogenic drugs for patients with metastatic colon cancers (modified from Nandikolla and Rajdev 2016) [35]

Angiogenesis inhibitor	Identification number	Clinical trial	Primary Outcome	Phase
Aflibercept	NCT02331927	PERMAD: Personalized Marker-driven Early Switch to Aflibercept in Patients With Metastatic Colorectal Cancer (PERMAD)	PFS	II
	NCT02384759	Aflibercept +/- LV5FU2 in First Line of Non-resectable Metastatic Colorectal Cancers (FOLFA)	Radiological PFS	II
Bevacizumab	NCT01640405	Study of 5-Fluorouracil/Leucovorin/Oxaliplatin (FOLFOX) + Bevacizumab Versus 5-Fluorouracil/Leucovorin/Oxaliplatin/Irinotecan (FOLFOXIRI) + Bevacizumab as First Line Treatment of Patients With Metastatic Colorectal Cancer Not Previously Treated and With Three or More Circulating Tumor Cells (VISNU-1)	PFS	III
	NCT01718873	Optimization of Bevacizumab Scheduling With Chemotherapy for Metastatic Colorectal Cancer (OBELICS)	No. of objective responses	III
	NCT01249638	Combination of capecitabine and bevacizumab versus the combination of capecitabine, bevacizumab and irinotecan.	Time of failure strategy	III
	NCT02339116	Folfoxiri Plus Bev Followed by Reintroduction of Folfoxiri Plus Bev at Progression Versus Folfox Plus Bev Followed by Folfiri Plus Bev in mCRC (TRIBE2)	PFS	III
	NCT02141295	A Study Comparing the Efficacy and Safety of Vanucizumab and FOLFOX With Bevacizumab and FOLFOX in Participants With Untreated Metastatic Colorectal Cancer (McCAVE)	PFS	II
	NCT01206530	FOLFOX/Bevacizumab/Hydroxychloroquine (HCQ) in Colorectal Cancer	Response rate	II
	NCT00200200	Hepatic Arterial Infusion With Floxuridine and Dexamethasone Combination With Chemotherapy With/Without Bevacizumab for Hepatic Metastases From Colorectal Cancer	TTP	II
	NCT00544700	Bevacizumab in Treating Patients Who Have Undergone First-Line Therapy for Metastatic Colorectal Cancer	TTP	III
	NCT00217737	Oxaliplatin, Leucovorin Calcium, and Fluorouracil With or Without Bevacizumab in Treating Patients Who Have Undergone Surgery for Stage II Colon Cancer	Disease-free survival	III
	NCT00952029	Combination Chemotherapy and Bevacizumab With or Without Bevacizumab Maintenance Therapy in Treating Patients With Metastatic Colorectal Cancer	Disease-control duration	III
	NCT00098787	Bevacizumab and Oxaliplatin Combined With Irinotecan or Leucovorin and Fluorouracil in Treating Patients With Metastatic or Recurrent Colorectal Cancer	Objective response rate	II
	NCT01279681	Combination Chemotherapy Plus Bevacizumab With or Without Oxaliplatin in Treating Older Patients With Metastatic Colorectal Cancer	PFS	III
Famitinib	NCT02390947	Safety and Efficacy Study of Famitinib in Patients with Advanced Colorectal Adenocarcinoma (FACT)	OS	III
Nintedanib	NCT02149108	Nintedanib (BIBF 1120) vs Placebo in Refractory Metastatic Colorectal Cancer (LUME-Colon 1)	OS	III
Panitumumab	NCT01814501	Panitumumab and Chemotherapy in Patients With Advanced Colorectal Cancer After Prior Therapy With Bevacizumab	PFS	II
Ramucirumab	NCT01079780	Irinotecan Hydrochloride and Cetuximab With or Without Ramucirumab in Treating Patients With Advanced Colorectal	PFS	II

Angiogenesis inhibitor	Identification number	Clinical trial	Primary Outcome	Phase
		Cancer With Progressive Disease After Treatment With Bevacizumab-Containing Chemotherapy		
Regorafenib	NCT02368886	Lower or Standard Dose Regorafenib in Treating Patients With Refractory Metastatic Colorectal Cancer	Proportion of patients in each arm who completed 2 courses of treatment	II
Sorafenib	NCT00826540	Sorafenib and Bevacizumab in Treating Patients With Metastatic Colorectal Cancer	PFS	II