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REVIEW PAPER

Anti-angiogenesis for cancer revisited: Is there a role for combinations with immunotherapy?

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Abstract Angiogenesis is defined as the formation of new blood vessels from preexisting vessels and has been characterized as an essential process for tumor cell proliferation and viability. This has led to the development of pharmacological agents for anti-angiogenesis to disrupt the vascular supply and starve tumor of nutrients and oxygen, primarily through blockade of VEGF/VEGFR signaling. This effort has resulted in 11 anti-VEGF drugs approved for certain advanced cancers, alone or in combination with chemotherapy or other targeted therapies. But this success had only limited impact on overall survival of cancer patients and rarely resulted in durable responses. Given the recent success of immunotherapies, combinations of anti-angiogenics with immune checkpoint blockers have become an attractive strategy. However, implementing such combinations will require a better mechanistic understanding of their interaction. Due to overexpression of pro-angiogenic factors in tumors, their vasculature is often tortuous and disorganized, with excessively branched leaky vessels. This enhances vascular permeability, which in turn is associated with high interstitial fluid pressure, and a reduction in blood perfusion and oxygenation. Judicious dosing of anti-angiogenic treatment can transiently normalize the tumor vasculature by decreasing vascular permeability and improving tumor perfusion and blood flow, and synergize with immunotherapy in this time window. However, anti-angiogenics may also excessively prune tumor vessels in a dose and time-dependent manner, which induces hypoxia and immunosuppression, including increased expression of the immune checkpoint programmed death receptor ligand (PD-L1). This review focuses on revisiting the concept of anti-angiogenesis in combination with immunotherapy as a strategy for cancer treatment.

Keywords Antiangiogenesis · Immunotherapy · VEGF · Normalization · Hypoxia · PD-1

Introduction

Angiogenesis, the formation of new blood vessels from preexisting blood vessels via a process called sprouting, is one of the hallmarks of cancer. Angiogenesis may occur under physiological conditions, such as during embryonic development or during wound healing in adults. In cancer progression, pathological angiogenesis is driven by the overexpression of pro-angiogenic factors. This creates a local imbalance between pro-angiogenic and anti-angiogenic factors, which leads to recruitment of a new vascular supply. Unlike wound healing, where angiogenesis undergoes a resolution phase, tumor angiogenesis continues abnormally in growing tumors, as they require vascular supply to provide essential nutrients and oxygen to proliferating cancer cells [1, 2]. Angiogenesis is often triggered by low oxygenation concentrations in tissues (hypoxia), which leads to expression of multiple growth factors via the hypoxia-induced factors (HIFs) by cancer cells and stromal cell recruited to tumors (fibroblasts, macrophages) [3–8].

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The late Dr. Judah Folkman [9] proposed the concept that solid tumors require pathological angiogenesis for their growth. This concept was supported by many reports published over the last half of century [10–13]. There is an important distinction between physiological and pathological angiogenesis in that the latter leads to a vasculature with abnormal structure and function. Solid tumor vessels are often tortuous and disorganized, and excessively leaky. This enhances vascular permeability, which in turn is associated with high interstitial fluid pressure, and a reduction in blood perfusion and oxygenation [14–16]. While tumors have the ability to grow and progress despite the inefficient blood supply and hypoxia, delivery of drugs and their efficacy is reduced [17–20]. During physiological angiogenesis, pericyte recruitment plays an essential role in the maintenance of the structure and function of vasculature. However, pericyte coverage is often lacking or abnormal (loose) in the tumor vasculature. This feature contributes to vascular permeability to fluids and metastatic cancer cells [21–23].

Disruption of the vascular supply by blocking the pro-angiogenic factors or inhibiting activity of their cognate receptors with pharmacological agents has been pursued initially to promote tumor starvation, trigger cell death, increase the vulnerability to the exposure to the standard care of treatment, with the goal of increasing overall survival (OS), progression-free survival (PFS) or regression of tumors [10, 12, 24]. However, pruning of vessels after anti-angiogenic treatments was shown to increase hypoxia, which in turn promoted the rapid tumor progression via multiple mechanisms, including increased migration, inflammation and stem-like cell phenotype [25, 26]. Moreover, hypoxic tumors are more resistant to the standard cytotoxic treatments, including chemotherapeutic agents and or radiation therapy [27, 28].

Dr. Rakesh K. Jain introduced the concept that the appropriate dose of anti-angiogenic treatment can lead to a normalization of the tumor vasculature, by reducing vascular permeability and interstitial fluid pressure and improving blood flow and tumor perfusion. The normalized tumor vasculature can reduce tissue hypoxia and enhance the delivery of cytotoxic agents and of oxygen for radiation therapy, but also anti-tumor immunity [15, 25, 26, 29]. Preclinical and clinical studies supported the hypothesis that anti-angiogenic therapy can normalize the tumor vasculature, at least transiently. In addition, vascular normalization was associated with improved survival in brain, breast, colorectal and lung cancer patients treated with cytotoxics [30–34].

Moving forward, these insights may be useful in designing approaches to more substantially improve OS in cancer patients, for example by combining anti-angiogenic agents with immunotherapy. This review focuses on this potential new avenue in cancer therapy.

Tumor angiogenesis and VEGF

The vascular endothelial growth factor (VEGF) family of growth factors and their receptors—VEGFR-1, VEGFR-2, VEGFR-3, neuropilin (NRP)-1 and NRP-2—play intricate roles in initiating and promoting tumor angiogenesis [35–37]. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factors (PlGF1-4) [38, 39]. They have been linked with tumor angiogenesis (VEGF-A, PlGF), maintenance of new blood vessels (VEGF-B), lymphangiogenesis and angiogenesis (VEGF-C/D), vascular permeability (VEGF-A/C), chemotaxis (VEGF-A, PlGF), migration (VEGF-A, PlGF), differentiation (VEGF-D) and survival (VEGF-A/B/C, PlGF). VEGF-A (referred to as VEGF), initially discovered as the vascular permeability factor (VPF), is a critical mediator of tumor angiogenesis in many solid tumors [11, 16, 40]. There are two major isoforms of VEGF, a soluble (VEGF121) and an insoluble form (VEGF165). VEGF binds to VEGFR-1 with higher affinity than to VEGFR-2, but VEGFR-1 has lower tyrosine receptor kinase activity compared to VEGFR-2. In fact, VEGFR-1 is able to sequester VEGF, thereby preventing the tyrosine kinase activation of VEGFR-2. The role of VEGFR-1 activity is less well characterized compared to VEGFR-2. VEGF165 binds to the VEGFR-2 co-receptor NRP-1 and induce endothelial cell migration. NRP-1 serves a co-receptor also for class 3 semaphorin (SEMA3A) to control vascular permeability and vessel maturation during angiogenesis. VEGFR-3 and NRP-2 receptors are thought to predominantly regulate lymphangiogenesis induced by their ligands VEGF-C and VEGF-D. Another form of VEGFR-1 is truncated, referred to as soluble VEGFR-1 (sVEGFR-1 or sFLT1), and functions as an endogenous inhibitor of angiogenesis by sequestering/blocking VEGF and PlGF [13, 35, 36, 41–43].

Tumor angiogenesis can be initiated also independently of VEGF-related pathways. The angiopoietin (Ang)/Tie-2 (Tek) pathway is another player in tumor angiogenesis. Ang-1, which binds to the Tie-2 receptor, can promote tumor angiogenesis as well as pericyte coverage maturation of the vascular network. Endothelial cells secrete Ang-2, which binds to Tie-2 to increase tumor angiogenic activity by destabilizing the vessels in the presence of VEGF, but it may decrease endothelial cell survival in the absence of VEGF. Thus, the balance between Ang-1 and Ang-2 plays an important role in tumor angiogenesis [44–50].

VEGF-induced angiogenesis is mediated in part through the expression of adhesion molecules such as the $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins, which regulate the attachment of endothelial cells to the extracellular matrix, thereby promoting migration and survival of the tumor vasculature.

Other integrins (for example αvβ3, αvβ5 and α5β1) have also been shown to mediate angiogenesis [51–53]. Additional mediators of tumor angiogenesis include basic fibroblast growth factor (bFGF/FGF-2), platelet-derived growth factor-B (PDGF-B), stromal cell-derived factor (SDF)-1α, stem cell factor (SCF), interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, endothelin-1 (Et-A, Et-B), vashohibin-1/2, thrombospondin (TSP), and transforming growth factor (TGF)-β and their receptors [18, 36]. Other important receptors in angiogenesis include delta-like 4 ligand (DLL4), Notch4 and ephrin B2, which can interact with the VEGF pathway in the modulation of tumor angiogenesis [54, 55]. Intracellular factors such as Src can increase VEGF-A mRNA transcription and synthesis, as well as the secretion of VEGF. Activation of Src enhances activity of its downstream target, focal adhesion kinase (Fak) and paxillin, which in turn induces VEGF-mediated tumor vascular permeability and migration. Because Src and Fak increase vascular permeability and migration, they can also play critical roles in the metastatic cascade [56–59]. Other pro-angiogenic factors include midkine transcript 2 (MDK), endoglin (ENG), follistatin (FST), angiogenic factor G path and FHA domains 1 (AGGF1). So far, 28 pro-angiogenic factors/genes have been discovered to regulate VEGF or independently mediate tumor angiogenesis [17, 18].

Anti-angiogenic therapy for solid tumors: anti-VEGF agents

VEGF is overexpressed in a vast majority of solid tumors and is widely considered to be a key player in mediating tumor angiogenesis [35, 36]. For this reason, over the past decades, the development of anti-angiogenic treatment was predominately focused on the development of VEGF inhibitors. Preclinical evidence provided that monotherapy of blocking VEGF reduced microvascular density inhibited tumor growth in subcutaneous human xenografts of many cancer types. Anti-angiogenic treatment even resulted into a marked decrease in metastasis and in particular in colorectal cancer preclinical models [60, 61]. The group of Dr. Napoleone Ferrara (Genentech) designed and developed the first anti-angiogenic inhibitor (bevacizumab), a recombinant humanized monoclonal antibody that blocks VEGF-A [10, 62]. Intravenous administration of bevacizumab depletes VEGF in the bloodstream, which inhibits the interaction between VEGF and VEGF receptors [63]. In an initial phase III clinical trial, bevacizumab did not improve OS when combined with chemotherapy in breast cancer [64]. However, several subsequent trials of bevacizumab with chemotherapy increased OS and/or PFS in metastatic colorectal cancer (first and second line), as well as lung cancer, ovarian cancer, endometrial cancer, mesothelioma and cervical cancers [30, 65–75]. A combination of bevacizumab with interferon alpha (IFN-α) immunotherapy prolonged PFS and is now a standard of care in metastatic renal cell carcinoma [76]. In the majority of cancers (melanoma, breast, pancreatic and prostate cancer), bevacizumab failed to increase survival when combined with chemotherapy [77, 79]. A VEGF receptor chimera, VEGF-Trap or aflibercept, was developed as a blocker of multiple VEGF family members, VEGF, VEGF-B and PlGF, and more broadly inhibit VEGFR-2 kinase activity. Aflibercept showed increased OS and PFS in a randomized phase III clinical trial when used in combination with chemotherapy as second-line treatment in metastatic colorectal cancer patients [80] (Table 1).

Anti-angiogenic therapy for solid tumors: anti-VEGFR agents

Others pursued the development of angiogenesis inhibitors that target the VEGF receptors' activity. Tyrosine kinase inhibitors (TKI) and monoclonal antibodies were developed to inhibit VEGF receptors and their downstream targets, in order to suppress endothelial proliferation and disrupt the vascular supply of nutrients and oxygen. Multiple TKIs have been approved as monotherapy for cancer based on improvement of OS or PFS in phase III trials. These include (1) sorafenib in advanced renal cell carcinoma, metastatic differentiated thyroid cancer and unresectable hepatocellular carcinoma, (2) sunitinib in advanced renal cell carcinoma, gastrointestinal stromal tumors and pancreatic neuroendocrine tumors, (3) axitinib in advanced renal cell carcinoma, (4) regorafenib in chemo-refractory metastatic colorectal cancer, unresectable hepatocellular carcinoma and GIST, (5) pazopanib in metastatic soft tissue carcinoma and advanced renal cell carcinoma, (6) vandetanib in unresectable locally advanced or metastatic medullary thyroid carcinoma, (7) cabozantinib in refractory advanced renal carcinoma, metastatic medullary thyroid cancer, pancreatic neuroendocrine tumors, and (8) lenvatinib in locally recurrent or metastatic, progressive, radioactive iodine-refractory differentiated thyroid carcinoma, unresectable hepatocellular carcinoma and renal cell carcinoma (with everolimus) [81–94]. Ramucirumab, a monoclonal antibody against VEGFR-2, was shown to increase OS when combined with chemotherapy in gastroesophageal junction adenocarcinoma or gastric adenocarcinoma, metastatic non-small cell lung carcinoma and colorectal cancers [95–97]. Strikingly, monotherapy increased OS in metastatic gastroesophageal junction adenocarcinoma or gastric adenocarcinoma. The reasons for this activity remain unknown but may be highly

Table 1 FDA-approved anti-angiogenic compounds for solid tumors

Molecular target	Therapy	Malignancy	Improvement in (months)	
			PFS	OS
VEGF-A	Bevacizumab	Glioblastoma	N/A	0.5
VEGF-A	Bevacizumab + fluoropyrimidine-based chemotherapy	Metastatic colorectal cancer	1.7	1.4
VEGF-A	Bevacizumab + FOLFOX4	Metastatic colorectal cancer	2.6	2.1
VEGF-A	Bevacizumab + irinotecan + fluorouracil + leucovorin	Metastatic colorectal cancer	4.4	4.7
VEGF-A	Bevacizumab + paclitaxel or cisplatin, topotecan	Recurrent metastatic cervical cancer	2.3	3.7
VEGF-A	Bevacizumab + carboplatin + paclitaxel	Recurrent metastatic non-squamous NSCLC	1.7	2
VEGF-A	Bevacizumab + IFN-α	Advanced renal cell carcinoma	4.8	NS
VEGF-A	Bevacizumab + pemetrexed + cisplatin	Pleural mesothelioma		2.7
VEGF-A	Bevacizumab + paclitaxel or Pegylated liposomal doxorubicin or topotecan	Peritoneal cancer or recurrent epithelial ovarian cancer fallopian tube cancer	3.8	5.3
VEGF-A	Bevacizumab + paclitaxel	Metastatic HER2- inflammatory breast cancer	1.7	7.9
VEGF-A, VEGF-B, PlGF	Aflibercept + FOLFIRI	Metastatic colorectal cancer	2.2	1.4
VEGFR-2	Ramucirumab	Gastroesophageal junction adenocarcinoma or gastric adenocarcinoma	0.8	1.4
VEGFR-2	Ramucirumab + paclitaxel	Advanced gastroesophageal junction adenocarcinoma or gastric adenocarcinoma	1.5	2.2
VEGFR-2	Ramucirumab + docetaxel	Metastatic NSCLC	N/A	1.4
VEGFR-2	Ramucirumab + FOLFIRI	Metastatic colorectal carcinoma	1.2	1.6
VEGFR-1-2, PDGFR-α/β	Sunitinib	Pancreatic neuroendocrine tumors	4.8	N/A
VEGFR-1-2, PDGFR-α/β	Sunitinib	Gastrointestinal stromal tumors	4.5	NS
VEGFR-1-2, PDGFR-α/β	Sunitinib + IFN-α	Advanced clear cell carcinoma renal cancer	25.3	N/A
VEGFR-1-3, PDGFR-α, FGFR1-4	Lenvatinib	Recurrent or metastatic radioactive iodine-refractory differentiated thyroid carcinoma	14.7	N/A
VEGFR-1-3, PDGFR-α, FGFR1-4	Lenvatinib	Advanced hepatocellular carcinoma	*	*
VEGFR-1-3, PDGFR-α, FGFR1-4	Lenvatinib + everolimus	Advanced renal cell carcinoma	9.1	NS
VEGFR-2	Vandetanib	Advanced differentiated thyroid carcinoma	6.2	N/A
VEGFR-2, PDGFRβ	Sorafenib	Advanced hepatocellular carcinoma	NS	2.8
VEGFR-2, PDGFRβ	Sorafenib	Advanced renal cell carcinoma	2.7	NS
VEGFR-2, PDGFRβ	Sorafenib	Recurrent or metastatic differentiated thyroid cancer	5.0	N/A
VEGFR-1-3, PDGFRβ, FGFR-1-2	Pazopanib hydrochloride	Advanced soft tissue carcinoma	6.2	N/A
VEGFR-1-3, PDGFRβ, FGFR-1-2	Pazopanib hydrochloride	Advanced renal cell carcinoma	5.0	N/A
VEGFR-1-3, PDGFRβ	Axitinib	Advanced renal cell carcinoma	6.2	N/A

Table 1 continued

Molecular target	Therapy	Malignancy	Improvement in (months)	
			PFS	OS
VEGFR-1-3, PDGFRβ, FGFR-1-2	Regorafenib	Advanced gastrointestinal stromal tumors	2.2	1.4
VEGFR-1-3, PDGFRβ, FGFR-1-2	Regorafenib	Chemo-refractory metastatic colorectal cancer	0.2	1.4
VEGFR-1-3, PDGFRβ, FGFR-1-2	Regorafenib*	Refractory hepatocellular carcinoma	7.2	NS
VEGFR-1-3	Cediranib + carboplatin or cisplatin	Ovarian cancer	1.1	**
VEGFR-2, Tie2	Cabozantinib	Refractory advanced renal carcinoma	3.6	4.9
VEGFR-2, Tie2	Cabozantinib	Metastatic medullary thyroid cancer	7.2	NS
VEGFR-2, Tie2	Cabozantinib	Pancreatic neuroendocrine tumors	**	**

* Not licensed yet

Current list of FDA-approved anti-angiogenic drugs for solid tumors (* significantly improved progression-free survival and overall survival, but not yet FDA approved, ** clinical trial is still recruiting patients and data need to be fully analyzed, but the trial met the endpoint and therefore the drug is likely to be FDA approved)

relevant for combinations with immunotherapy in this disease.

Cediranib (a pan-VEGFR inhibitor) prolonged PFS when combined with platinum-based chemotherapy in relapsed platinum-sensitive ovarian cancer; the OS endpoint is currently being analyzed for this clinical trial [98] (Table 1). Similar to other anti-VEGF drugs, this agent failed as monotherapy or with chemotherapy in multiple other solid tumors [79, 99–101].

In summary, TKIs and antibodies targeting VEGFR-2 demonstrated efficacy (increased PFS and OS) when used alone or with chemotherapy or other molecularly targeted agents in certain cancers. While several other phase III clinical trials are still ongoing (Table 2), the urgent question emerging from these studies is what mediates resistance to this treatment modality, which typically prolongs survival by 2–3 months on average. The concept that resistance to anti-VEGF therapy is mediated by pro-angiogenic factors other than VEGF has been proposed based on preclinical studies, but is yet to be translated into a survival benefit in the clinic.

Clinical development of other anti-angiogenic agents in phase III trials

Most preclinical and clinical research was dedicated to the drug development for VEGF and VEGFR inhibition. However, other pro-angiogenic factors are also involved in regulating tumor angiogenesis. Anti-angiogenic agents are currently in clinical development for these targets, and some have reached phase III clinical trial testing. Preclinical studies revealed that cilengitide, an inhibitor for αvβ3,

Table 2 Selected ongoing phase III clinical trails involving anti-angiogenic inhibitors

Anti-angiogenic target	Therapy	Malignancy	Trails
VEGFR-2 PDGFRβ	Sorafenib + stereotactic body therapy	Liver cancer	NCT01730937
VEGFR-2	Ramucirumab + erlotinib	Advanced NSCLC	NCT02411448
VEGFR-1-3 PDGFRβ	Tivozanib	Refractory advanced renal carcinoma	NCT02627962
VEGFR-1-3, PDGFRβ	Cediranib maleate + olaparinib + standard chemotherapy	Refractory ovarian, fallopian tube and peritoneal cancer	NCT02502266

Current list of phase III angiogenesis inhibitors tested in solid tumors

αvβ5 and α5β1 integrins, reduced vascular density and vascular permeability and increased survival in a model of orthotopically implanted glioblastoma in rats [102]. Cilengitide induced tumor cell apoptosis, promoted endothelial cell detachment, disassembly of the cytoskeleton and inhibited SRC/FAK/AKT pathway [103]. However, a phase III clinical trial showed that the combination of temozolomide, radiotherapy and cilengitide failed to extend PFS or OS in newly diagnosed patients with methylated O_6-methylguanine-DNA methyltransferase (MGMT, a biomarker of response to temozolomide) glioblastoma [104].

Targeting of Ang-1/2 and Tie2 receptor axis with trebananib, a peptide-Fc fusion protein, reduced endothelial cell proliferation, angiogenesis and growth of ovarian xenografts tumors in preclinical studies [105, 106]. Although trebananib increased PFS (median 7.2 vs. 5.4 months), it failed to improve the OS in a phase III clinical trial in recurrent ovarian cancer patients [107].

Inhibition of FGFR-1-4, PDGFRβ and VEGFR-1-3 with dovitinib demonstrated anti-tumor activity in xenografts models of renal cell carcinoma, but dovitinib failed in a phase III trail to prolong OS and PFS in renal cell carcinoma [108, 109]. A preclinical study showed that treatment with brivanib a FGFR-1, VEGFR-1-2 inhibitor reduced proliferation, microvascular density and growth of tumors in hepatocellular carcinoma models in mice. However, brivanib failed to demonstrate efficacy in phases III trials in advanced hepatocellular carcinoma patients [110–113].

Mechanisms of resistance to anti-angiogenics

Activation of alternative pro-angiogenic signaling pathways

Anti-VEGF/VEGFR therapy is currently a standard of care in multiple solid tumors. However, even in these patients, tumors are inherently resistant or develop adaptive resistance to VEGF/VEGFR inhibition. This can be mediated through many different mechanisms, but is mainly thought to be governed by activation of alternative angiogenic pathways that promote tumor angiogenesis in a VEGF-independent manner, as seen in preclinical models [114, 115]. Some clinical correlative studies support this hypothesis. For example, treatment with anti-VEGF agents has been shown to increase VEGF-C, VEGF-D, PlGF, SDF1-α, and other factors in the blood circulation of cancer patients [61, 116]. The roles of these and other pro-angiogenic factors (PDGF, Ang-2, bFGF, endoglin, TGF-β, etc.) remain to be established in cancer patients [101, 117].

Another approach is to target the hypoxia inducible factor (HIF)-1α, which is activated/stabilized in many cancers and is upstream of multiple pro-angiogenic genes (VEGF family members, PDGF, Ang-2, etc.) [118, 119]. Since HIF-1α is induced by hypoxia, which may be aggravated after anti-angiogenic therapy, this factor may be involved in treatment resistance. Indeed, HIF1-α was upregulated in bevacizumab resistance metastatic colorectal cancer and might be a potential biomarker of anti-VEGF therapy resistance [120].

Anti-angiogenic resistance mediated by stromal cells

Anti-angiogenic adaptive resistance might be also mediated by the recruitment of local and distal (bone marrow-derived cells or BMDCs) stromal cells [3, 8, 114, 121, 122]. Anti-angiogenic therapies can upregulate SDF1-α, CXCR4, G-CSF and PlGF expression, leading to increased recruitment of pro-angiogenic bone marrow cells and cancer-associated fibroblasts (CAF) [123, 124].

Excessive pruning of vessels after anti-VEGF treatment increases intratumoral hypoxia in tumors, which in turn upregulates SDF1-α/CXCR4 axis and HIF-1-α that regulates both VEGF-dependent and VEGF-independent angiogenesis. These factors promote the recruitment of BMDCs such as M2 (pro-tumor) tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs). All these immune populations can promote angiogenesis, tumor growth, epithelial-to-mesenchymal transition (EMT) and metastasis, but also mediate immune suppression [78, 124–128].

Adaptive (or aquired) resistance to anti-angiogenic therapy may also be mediated by the increased infiltration and activation of CAFs, which produce PDGF, SDF1-α, IGF, TGF-β, MMPs, IL-6, Ang-2 and other growth factors that promote tumor invasion, angiogenesis, immunosuppression, growth and metastasis [117]. The presence of CAFs in solid tumors correlates with worse outcome in patients. The role of CAFs in anti-angiogenic resistance is poorly defined and needs a better characterization [117]. Recently, we have shown that blocking the SDF1-α/CXCR4 pathway with the FDA-approved drug, AMD3100 in hepatocellular carcinoma may overcome sorafenib resistance mediated in part by CAFs (activated hepatic stellate cells) [124].

Anti-angiogenesis resistance due to alternative modes of tumor vascularization

Angiogenesis is known to be an essential hallmark for solid tumors, but other modes of tumor new vessel formation exist and have been linked with intrinsic or adaptive resistance to anti-angiogenic treatment [126]. Numerous reports in preclinical studies demonstrated that cancer cells are able to use

co-option of the existing vasculature for nutritional and oxygen supply [129]. Vascular co-option was first observed in glioblastoma, but also was later reported in other types of solid tumors [130]. This phenomenon has been also linked with hypoxia. Hypoxic cancer cells can co-opt the normal blood vessels and migrate within the host solid tumor organ. Hypoxia is considered to play a crucial factor in vascular co-option by promoting an aggressive behavior of the cancer cells, by promoting EMT, tumor invasiveness and metastasis. Vascular co-option has been also reported in (primary and metastatic) liver and lung tumor, and it has been proposed as a resistance mechanism to anti-angiogenic treatment with bevacizumab in colorectal cancer and sorafenib in hepatocellular carcinoma [131–133]. However, the specific molecular mechanisms leading to vascular co-option that distinguish it from angiogenesis remain partly elusive. Other types of neovascularization involve the direct participation of malignant cells, such as vasculogenic mimicry (when cancer cells line microvascular channels) or vasculogenesis from cancer stem cell differentiation to endothelial cells (reported in glioblastoma [134–138]. Vessel intussusception (splitting angiogenesis) is another mode of new vessel formation by splitting of one vessel into two or more vessels and has been observed in solid tumors [139–141]. Finally, vasculogenesis based on bone marrow-derived precursors of endothelial cells has been studied extensively, but its involvement in tumor angiogenesis and treatment resistance remains controversial [142, 143].

Revisiting anti-angiogenesis

There is a very large body of the literature demonstrating the efficacy of anti-angiogenic treatments alone or with other therapies in preclinical models of primary and metastatic tumors. In the clinic, anti-angiogenesis using multiple drugs has become a standard and widely used treatment modality for cancers such as renal, lung, liver or colorectal cancer. However, addressing the problem of treatment resistance remains an unmet need, as anti-angiogenics have not made a similar impact in other cancers, despite clear evidence (based on pharmacodynamic markers) that treatment had a biologic effect (for example decrease in vascular permeability in brain tumors). Clearly, new strategies involving the use of anti-angiogenic treatment with novel interventions that could have durable benefits, such as immunotherapy, have become particularly attractive.

Reprogramming the tumor vasculature by normalization to enhance anti-tumor immunity

As discussed above, anti-VEGF therapies could lead to hypoxia and acidosis in cancer, in a time and dose-dependent manner due to excessive pruning of tumor vessels [144, 145] (Fig. 1). In addition to affecting the cancer cells, and the delivery and efficacy of anticancer agents, hypoxia and acidosis severely compromise the functionality of immune effector cells, while leading to preferential recruitment of pro-tumor immune cells (Tregs, MDSCs, M2-type TAMs) [15, 29, 146, 147]. Preclinical studies and clinical evidence indicate that judicious dosing of anti-angiogenic treatment can normalize tumor vasculature, at least transiently [30–33, 148, 149]. Anti-VEGF therapies can normalize the tumor vasculature by increasing the pericyte coverage that improves vessel stabilization and by reducing vascular permeability/leakiness [21, 150]. These changes also inhibit intravasation of metastatic seeking cancer cells. Functionally, vascular normalization reduces hypoxia and increases the delivery and efficacy of cytotoxic agents [25, 33, 151]. But normalization of the tumor vasculature can also reduce immunosuppression exerted by Tregs and regulatory B cells and promote anti-tumor immunity by enhancing the uptake of antigen presentation in dendritic cells, M1-associated macrophages and activation of cytotoxic $CD8^+$ T cells [15]. This has been demonstrated in a preclinical model of breast cancer using various doses of anti-mouse VEGFR-2 antibody (DC101 ImClone/Eli Lilly). When used at lower doses, DC101 decreased hypoxia and cancer cell proliferation [152]. Clinical studies have not been usually designed to compare various doses of anti-angiogenics, but when they did they showed a difference or an advantage for the lower dose of the anti-angiogenic agent (such as the use of 5 mg versus 10 mg of bevacizumab plus fluorouracil with leucovorin in metastatic colorectal) [30]. The effect of dose titration of anti-angiogenics on the immune microenvironment of human cancer remains unknown. However, based on preclinical evidence, clinical trials of anti-angiogenics with immunotherapy should take into account the appropriate dosing of anti-angiogenic agents and their timing/scheduling with immunotherapy that would ensure synergy (Fig. 1).

Cancer immunotherapy

The immune system is highly effective in mediating immune response against foreign antigens, but malignant cancer can evade immune surveillance via multiple mechanisms of immunosuppression. Tregs are potent suppressors of immune effector cells. Indeed, whereas Foxp3+ Tregs are often increased, only low numbers of cytotoxic $CD8^+$ T cells are infiltrating in solid tumors. High numbers of Tregs in tumors contribute to the evasion of tumor antigen recognition by APC and conventional T cells and therefore prevent cytotoxic $CD8^+$ T cell activity [153, 154]. Conventional T cells are characterized by

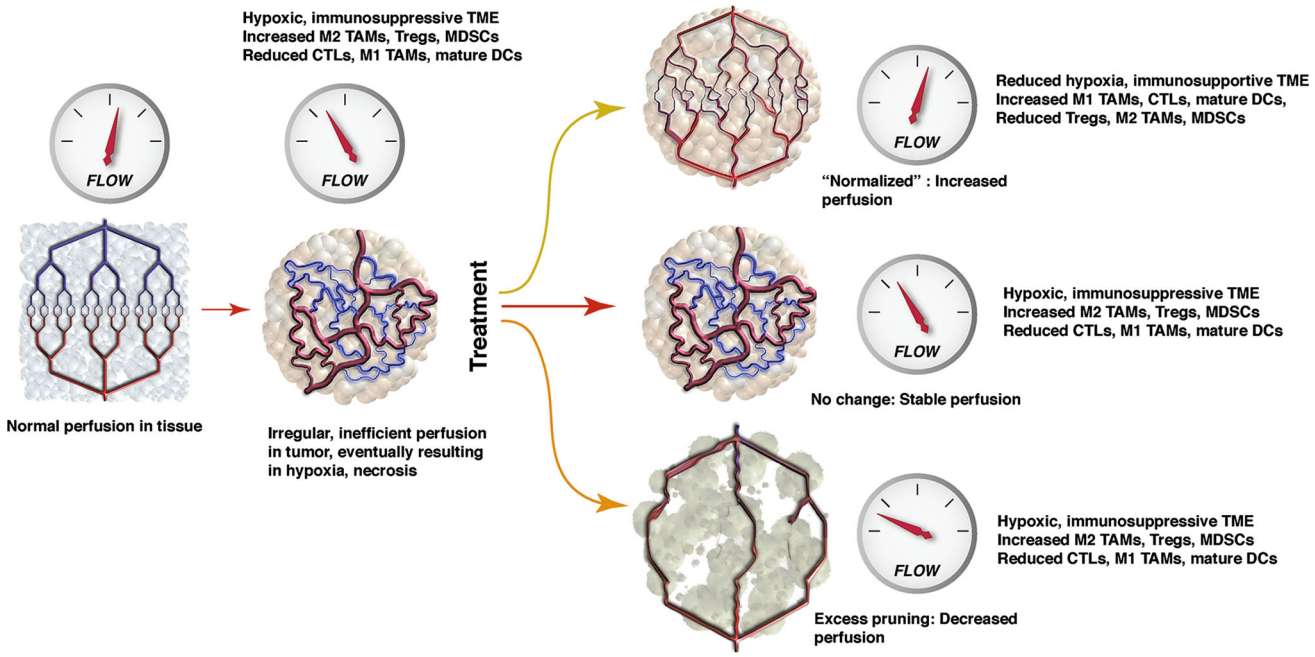

Fig. 1 Potential effects of anti-angiogenic therapy on tumor vascular function and impact on anti-tumor immunity. Blood vasculature is normal tissues maintained by a balanced expression of pro- and anti-angiogenic molecules, which ensures a normal structure and function (blood flow, *left*). The normal architecture and function is lost in cancer, due to abnormal expression of pro-angiogenic factors by malignant and stromal cells. This leads to inefficient blood perfusion, hypoxia and an immunosuppressive tumor microenvironment (TME). This is characterized by increased infiltration by T regulatory cells (Tregs), myeloid suppressor cells (MDSCs) and pro-tumor (M2-type) tumor-associated macrophages (TAMs), and reduced infiltration by cytotoxic T cells (CTLs) and mature dendritic cells (DCs)/antigen-presenting cells. Anti-angiogenic therapy may have different effects on tumor vasculature, depending on the dose, timing and type of anti-angiogenic agent. Treatment may result in: (1) a transiently normalized vasculature, which leads to reductions in hypoxia and reprogramming of the immune TME toward an immunosupportive one, with predominance of anti-tumor (M1-type) TAMs, CTLs and mature DCs, (2) no change or (3) excessive pruning of the vasculature, with further decreases in blood flow and increases in hypoxia. These effects may be critically important for combination of anti-angiogenics with immunotherapies (see Ref. [200]). Illustration courtesy of Dr. Lance L. Munn (MGH Boston)

expression of the αβ T cell receptor and a co-receptor (CD4 or CD8). Tregs regulate immunosuppression through dendritic cells, NK cells, macrophages, B cells and $CD4^+$ and $CD\text{-}8^+$ T cells via cell–cell contact and humoral actions. The balance between Treg and $CD8^+$ T cells is thought to control the balance between active anti-tumor responses and immunosuppression.

Over the last decade, cancer immunotherapy has emerged as a breakthrough for various types of cancer, including NSCLC, urothelial carcinoma, melanoma and head and neck carcinoma [75, 155–163] (Table 3). Multiple preclinical and clinical studies revealed that several solid tumors overexpress immunoregulatory proteins (called immune checkpoint molecules), which correlates with worse prognosis in survival of patients. The immune checkpoint programmed death-1 (PD-1) is a checkpoint molecule that is expressed on the extracellular surface of natural killer T cells (NKT), B cells, dendritic cells, monocyte/macrophages, $CD4^+$ and $CD8^+$ T cells [164, 165]. PD-1 interacts with its ligand-programmed death-ligand 1 (PD-L1)—present on immune cells as well as on certain types of cancer and stromal cells—to inhibit activation, proliferation and survival of T cells. The PD-1 receptor also interacts with PD-L2, a ligand selectively expressed on certain types of macrophages and tolerogenic dendritic cells, whose functionality is poorly defined. Under physiological conditions, PD-1, PD-L1 and PD-L2 play important roles in the activation of T cells during peripheral tolerance, which involves macrophages and tolerogenic dendritic cells that inhibit auto-reactive T cells to cause immune tissue damage and preventing autoimmunity. The activation of PD-1/PD-L1 pathway may result in the induction of T cells-mediated anergy, apoptosis and "exhaustion" to initiate T cell suppression [165, 166]. Solid tumors are capable of "hijacking" the PD-1/PD-L1 axis to induce immune suppression and to escape T cell receptor recognition. The activation of PD-1/PD-L1 pathway regulates $CD4^+$ T cells differentiation into Treg expressing Foxp3, thereby initiating immune suppression. The expression of PD-L1 by cancer cells may limit the attack of conventional cytotoxic $CD8^+$ T cells and prevent tumor lysis. The regulation of PD-1 and PD-L1 expression

Table 3 FDA-approved cancer immunotherapies for solid tumors

Anti-tumor immunity target	Therapy	Malignancy	Improvement in (months)	
			PFS	OS
PD-1	Nivolumab	Advanced squamous-cell NSCLC	0.7	3.3
PD-1	Nivolumab	Advanced non-squamous NSCLC	NS	2.9
PD-1	Nivolumab	Recurrent or metastatic HNSCC	N/A	2.4
PD-1	Nivolumab	Advanced renal cell carcinoma	NS	5.4
PD-1	Nivolumab	Metastatic urothelial carcinoma	N/A	8.7
PD-1	Nivolumab	Metastatic melanoma	2.9	>60
PD-1	Pembrolizumab	Metastatic non-squamous NSCLC	4.3*	Ongoing*
PD-1	Pembrolizumab	Recurrent or metastatic HNSCC	Ongoing*	Ongoing*
PD-1	Pembrolizumab	Unresectable or metastatic melanoma	1.4*	Ongoing*
PD-L1	Atezolizumab	Metastatic NSCLC	N/A	4.2 2.9
PD-L1	Atezolizumab	Metastatic urothelial carcinoma	NS	3.5
CTLA-4	Ipilimumab	Metastatic melanoma	NS	3.6 2.1
PD-1 + CTLA-4	Nivolumab + ipilimumab	Metastatic melanoma	8.6	N/A
Anti-tumor vaccine	Talimogene	Recurrent unresectable melanoma	NS	4.4
Oncolytic virus	Laherparepvec (T-Vec)			
Dendritic cell-based anti-tumor vaccine	Sipuleucel-T (Provenge)	Hormone refractory prostate cancer	5.1	4.5

Current list of FDA-approved cancer immunotherapies for solid tumors (* clinical trial is still recruiting patients and data need to be fully analyzed, but the results are promising when compared to standard care of treatment)

in tumor-infiltrating immune cells and cancer cells is incompletely understood, but it has been linked with oncogenic mutations, hypoxia and sustaining immune suppression. Blocking PD-1/PD-L1 axis has been shown to lead to a reversion of an immunosuppression phenotype and activation of the adaptive immune system. Inhibition of the PD-1/PD-L1 pathway triggers tumor antigen recognition, proliferation, infiltration and activation of cytotoxic $CD8^+$ T cells [154, 167, 168]. Most importantly, blockade with monoclonal antibodies against PD-1 (nivolumab, pembrolizumab) or PD-L1 (atezolizumab) in phase III clinical trails has proven successful in inducing durable responses and increasing survival various types of cancer, which led to their approval by the US FDA [155–160, 163, 169] (Table 3).

Tregs constitutively express other immunosuppressive molecules, including cytotoxic T lymphocyte-associated protein 4 (CTLA-4), which is an exclusive extracellular surface protein with strong co-inhibitory capabilities to control immune suppression. The T cell receptor can be stimulated to mediate immunosuppression by CTLA-4 or be activated through co-stimulatory signaling of CD28 in conventional T cells. It has been proposed that CTLA-4 binds with high affinity or can downregulate co-stimulatory molecules CD80/CD86 ligands on APC by outcompeting them, to prevent the co-stimulation of conventional T cells. In order to sustain Treg-mediated immunosuppression, the expression of CTLA-4 and co-immune modulating stimulatory signals, including LFA-1, is required. Tumors frequently contain CTLA-4-expressing T cells, which inhibit tumor antigen T cell receptor recognition and reduce the proliferation, infiltration, activation and tumor cell eradication by $CD8^+$ T cells. CTLA-4 blockade with a humanized monoclonal antibody as monotherapy or combined with anti-PD-1 antibodies increased survival in advanced melanoma [170–174] (Table 3). It is thought that blockade of CTLA-4 releases "the break" of the immunosuppressive function and reverses into a strongly enhanced tumor antigen recognition paired with anti-tumor immunity controlled by $CD8^+$ T cells [175]. However, the molecular pathways for the exact regulation of T cell immune suppression by CTLA-4 signaling remain incompletely characterized.

Foreign antigens are normally recognized by APCs and presented via major histocompatibility complex II (MHC II), which initiates antigen processing to smaller peptides that is presented on the surface with an MHC II molecule for antigen presentation and for T cell receptor recognition, which triggers T cell [176] proliferation and activation [176, 177]. However, APCs are unable to recognize tumor antigens lacking MHC II molecules and to recruit T cells for activation of the adaptive immune system [178]. Sipuleucel-T is a cancer therapeutic vaccine that targets patient-specific tumor epitopes. The patients APCs are exposed to antigen prostatic acid phosphatase (95% expressed in prostate cancer), fusion protein and

granulocyte–macrophage colony stimulation factor (GM-CSF) for the maturation of the APCs [179–181]. The patient's matured and exposed APCs with tumor antigen recognition are then reinfused in the patients. It is postulated that these APCs carrying tumor antigens are directly recognized by T cells, which is followed up with an expansion and activation of $CD4^+$ helper and $CD8^+$ T cells. But the exact mechanism of action of sipuleucel-T is elusive [182, 183]. Sipuleucel-T is FDA approved based on an increased OS by 3 months in hormone refractory prostate cancer patients [162, 184] (Table 3).

Overall, cancer immunotherapy has evolved as a success in several types of advanced cancer and in particular blockade of CTLA-4, PD-1/PD-L1 or their combination resulted in durable responses in many patients [174]. This has lead to a tremendous interest in the field, with hundreds of clinical trials of immune checkpoint blockers and/or vaccination strategies.

Combining immunotherapy with anti-angiogenic treatment for cancer

Despite these advances, immunotherapy is effective in a subset of cancer patients (up to approximately 20% in most cancers). It is thought that these are the cases where there is a preexisting immune response against the tumors. In all the other cases, tumors rapidly develop resistance to treatment. Less is known about the resistance to treatment of cancer immunotherapies, but there are hints provided by preclinical studies that upregulation of immune suppressive molecules, including indoleamine 2-3-dioxygenase (IDO), TIM3, glucocorticoid-induced TNF receptor (GITR), lymphocyte-activation gene 3 (LAG3), IL-10, IL-35 and TGF-β, might contribute to the resistance to treatment [185].

There are intricate relationships between angiogenesis and immunity in tumors. Vascular endothelium plays a barrier function and has an important role in activation of immunity by increasing the expression of endothelial cell adhesion molecules that directly interact with macrophages, NK cells, granulocytes, B and T cells for antigen recognition, rolling, adhesion and extravasation during immune responses. In tumors, vascular endothelial often has abnormal expression of adhesion molecules, including CD34, intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Down-regulation of these adhesion molecules is in part mediated by angiogenic factors, including VEGF. VEGF also inhibits the maturation of dendritic cells, which suppresses immune activation [147, 186–188].

As discussed above, potent anti-angiogenic treatment can promote tumor vessel pruning and hypoxia. Hypoxia increases the expression of SDF1-α and CCL28, which initiates a state of tolerance by recruiting Tregs, MDSCs and M2-type TAMs to induce an immunosuppressive tumor microenvironment [6, 189, 190]. Intratumoral hypoxia also compromises APC functionality and the stimulation of T cell responses. For example, sunitinib elevated intratumoral infiltration of $CD4^+$ and $CD8^+$ T cells, but also increased intratumoral infiltration of Foxp3+ Tregs.

Testing of combinations of anti-angiogenic treatment with immunotherapy has reached phase III development and includes trials in hepatocellular carcinoma, advanced renal cell carcinoma, NSCLC and ovarian cancer patients (Table 4).

Interactions between anti-angiogenic treatment and immunotherapy

Preclinical and clinical studies have shown that CTLA-4 blockade plus sunitinib reduced Tregs, increased CD8+ T cells and inhibited the growth of renal cell carcinoma. Interestingly, anti-angiogenic treatment with sorafenib, sunitinib or bevacizumab increased PD-L1 expression [124, 147, 191]. PD-L1 is considered to be a target of HIF1-α, and the elevation in its expression may be a consequence of anti-angiogenic treatment-induced hypoxia [192]. But other studies have shown that anti-angiogenic treatment could elevate the expression of PD-L1 independently of hypoxia or HIF1-α. In addition, VEGF could increase PD-1 expression on T cells and mediate "exhaustion" of $CD8^+$ T cells in tumors [154, 164, 193]. Our previous studies have shown that standard (high doses) of sorafenib modestly delayed liver tumor growth, but also induced hypoxia. As a result, there was an activation of the SDF1-α/CXCR4 pathway after sorafenib treatment, increased tumor infiltration by Tregs, MDSCs and M2-type TAMs, and increased expression of EMT genes and PD-L1 in the cancer cells. This mediated treatment resistance, as inhibition of SDF1-α/CXCR4 axis plus sorafenib, prevented these immunosuppressive effects and significantly delayed primary and metastatic tumor growth. However, the reduced immunosuppression did not result in increased intratumoral infiltration by $CD8^+$ T cells. It was only when blockade of anti-PD-1 was added to sorafenib and CXCR inhibition that we found increased intratumoral infiltration and activation of $CD8^+$ T cells [124]. In another study, we demonstrated that a high dose of VEGFR-2 blocker led to hypoxia and immunosuppression mediated by Tregs and M2-type TAMs in breast cancer. In contrast, a lower dose of the same VEGFR-2 blocker increased perfusion, infiltration of $CD8^+$ T cells and polarized M2-type TAMs toward the M1-type TAMs. In addition, combination of low-dose VEGFR-2 blockade with a cancer vaccine increased IFN-γ + $CD8^+$ T cells and decreased Tregs and

Table 4 Selected ongoing phase III clinical trails involving anti-angiogenic inhibitors combined with cancer immunotherapy

Anti-angiogenic target	Anti-tumor immunity target	Combination drugs	Malignancy	Trails
VEGF-A	PD-L1	Bevacizumab + atezolizumab	Advanced renal cell carcinoma	NCT02420821
VEGFR-1-3, PDGFRβ,	PD-L1	Axitinib + avelumab	Advanced renal cell carcinoma	NCT02684006
VEGF-A	PD-L1	Bevacizumab + MPDL3280A + carboplatin + paclitaxel	Stage IV NSCLC	NCT02366143
VEGF-A	PD-L1	Bevacizumab + atezolizumab + pegylated liposomal doxorubicin hydrochloride	Recurrent ovarian, fallopian tube peritoneal cancer	NCT02839707
VEGFR-2, PDGFRβ,	GM-CSF (virus-based vaccine)	Sorafenib + Pexa Vec	Hepatocellular carcinoma	NCT02562755

List of phase III clinical trails of combining anti-angiogenic treatment with cancer immunotherapies in solid tumors

led to a prolongation of overall survival in spontaneous MMTV-PyVT breast cancer in mice [15, 152]. In murine models of colorectal and melanoma, combining VEGF inhibition with a GM-CSF-secreting cancer vaccine reduced Tregs, elevated $CD8^+$ T cells and increased survival [194]. Finally, a preclinical study of anti-angiogenic treatment with endostatin, pigment epithelium-derived factor, IL-12 and GM-CSF decreased PD-1 and CTLA-4 expression and delayed tumor growth in a hepatoma model [186, 187, 195].

Initial clinical data also support a potentially synergistic interaction between these treatment modalities. A phase I clinical trial of dual blockade of CTLA-4 (ipilimumab) and VEGF (bevacizumab) showed increased tumor antigen recognition, tumor-associated endothelial cell activation and infiltration of T cells in melanomas [196]. Another clinical study revealed that atezolizumab (an anti-PD-L1 antibody) combined with bevacizumab increased the number of intratumoral $CD8^+$ T cells and antigen-specific T cell migration [197]. Over one hundred phase I/II clinical trials are currently testing combinations of anti-angiogenics with immunotherapy.

Conclusions

Clinical development of anti-angiogenic therapy was a success and translated into increased OS and PFS in many cancers. This is in line with the concept that tumor angiogenesis is a hallmark of cancer. However, the benefits are limited and there is no method of selection of patients likely to respond to this therapy [198, 199]. Moreover, treatment resistance to anti-angiogenic drugs may be mediated by increased hypoxia, which can also aggravate immunosuppression. This is critically important for optimally combining anti-angiogenic drugs with immunotherapy, which is the most promising strategy currently [24, 29, 51, 53, 200–203]. Understanding the interaction between these two therapeutic interventions, especially as it relates to proper reprogramming of the tumor immune microenvironment to enhance anti-tumor immunity, will be critical for the success of this combination strategy for cancer treatment.

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Compliance with ethical standards

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