Multifaceted approaches to tumor microenvironment modulation and immune checkpoint targeting

Melo Gallegos, Vinicio Alejandro

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CHAPTER 5

Towards immunotherapy-induced normalization of the tumor microenvironment

Vinicio Melo[1], Edwin Bremer[1], John D. Martin[2]

[1]University of Groningen, University Medical Center Groningen, Department of Hematology, Groningen, Netherlands

[2]Materia Therapeutics, Las Vegas, NV, USA.

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ABSTRACT

Immunotherapies modulate the function of immune cells to eradicate cancer cells through various mechanisms. These therapies are successful across a spectrum of cancers, but they are curative only in a subset of patients. Indeed, a major obstacle to the success of immunotherapies is the immunosuppressive nature of the tumor microenvironment (TME), comprising the stromal component and immune infiltrate of tumors. Importantly, the TME in most solid cancers is characterized by sparsely perfused blood vessels resulting from so-called pathological angiogenesis. In brief, dysregulated development of new vessels results in leaky tumor blood vessels that inefficiently deliver oxygen and other nutrients. Moreover, the occurrence of dysregulated fibrosis around the lesion, known as pathological desmoplasia, further compresses tumor blood vessels and impairs blood flow. TME normalization is a clinically tested treatment strategy to reverse these tumor blood vessel abnormalities resulting in stimulated antitumor immunity and enhanced immunotherapy efficacy. TME normalization includes vascular normalization to reduce vessel leakiness and reprogramming of cancer-associated fibroblast to decompress vessels. How immunotherapies themselves normalize the TME is poorly understood.

In this review, we summarize current concepts and progress in TME normalization. Then, we review observations of immunotherapy-induced TME normalization and discuss the considerations for combining vascular normalizing and immunotherapies. If TME could be more completely normalized, immunotherapies could be more effective in more patients.

INTRODUCTION

Cancer cells coopt the surrounding tissue resulting in an organ-like structure with abnormal physiology. Specifically, they can promote unrestrained angiogenesis (i.e., the formation of new vessels) and desmoplasia (i.e., the formation of new and excessive connective tissue). The extent of each process depends on the type of tumor. For example, hepatocellular carcinoma (HCC) is highly angiogenic, whereas pancreatic ductal adenocarcinoma is highly desmoplastic. Dysregulated angiogenesis produces leaky blood vessels while desmoplasia compresses them [1]. Thus, both processes reduce the capacity of blood vessels to deliver oxygen to tumors through independent mechanisms [1-3]. Sub-physiological oxygen tension is referred to as hypoxia [4, 5]. Besides promoting disease progression [6] and resistance to radiation [7] and some chemotherapies [8], hypoxia causes immunosuppression in the tumor microenvironment (TME) by altering immune cell phenotype, infiltration, migration, and function [9]. Simultaneously, newly formed immature blood vessels cannot traffic and distribute infiltrating immune cells efficiently [10], whereas excessive fibrosis poses a physical barrier to immune cell migration into the tumor [11]. Accordingly, hypoxia is associated with poor survival across tumor types [12] and alleviating hypoxia through 'normalization' of the TME increases the efficacy of immunotherapies in preclinical cancer models [13-21]. Circulating, tissue and imaging biomarker studies in patients with glioblastoma [22] and breast cancer [23, 24] support the notion that normalizing blood vessels with antiangiogenic therapies (AATs) correlates with increased antitumor immune cell infiltration and better treatment outcomes. Similarly, imaging studies in patients with lung cancer and glioblastoma indicate that increased blood flow [25, 26] and reduced hypoxia [27] during AAT treatment correlated with response rates and overall survival. Furthermore, regimens of AATs combined with immune checkpoint inhibitors (ICIs) are approved by the USA Food and Drug Administration in patients with HCC, renal cell carcinoma, and non-small-cell lung cancer [28]. Whereas no benefit has been demonstrated in clinical trials directly comparing AAT and ICI versus ICI alone, the combination of bevacizumab with atezolizumab increased overall survival in patients with unresectable HCC compared to first-line treatment, sorafenib, which is a multikinase inhibitor with antiangiogenic properties [29]. In contrast, ICI monotherapy did not increase overall survival compared to sorafenib [30, 31]. Thus, there is preclinical and clinical evidence supporting the notion that alleviating hypoxia through TME normalization increases immunotherapy efficacy. If we could understand how to better increase oxygen delivery when normalizing the TME for immunotherapy, then we might be able to improve outcomes for patients.

The impact of the abnormal tumor microenvironment on vessel function

Cancer cells induce nearby non-malignant cells to produce a microenvironment that promotes disease progression [6, 32, 33] and immunosuppression [34, 35]. As described above, the TME affects blood vessel formation through either disturbed angiogenesis

or excessive fibrosis. The former process results from cancer cells sending signals to vascular, mesenchymal, and immune cells that impair physiological processes and blood vessel formation [36, 37]. In brief, pathological angiogenesis is characterized by cancer cells stimulating endothelial and perivascular cells through angiogenesis and hypoxia signaling to produce new blood vessels through various mechanisms [38-40]. The main angiogenic signaling player in this context is vascular endothelial growth factor (VEGF), as it is released in response to hypoxia and triggers vascular cells to dissociate, migrate and remodel the surrounding tissue. These newly-forming tumor blood vessels do not mature because of constant pro-angiogenic VEGF signaling [41, 42], which limits expression of integrins and cell adhesion molecules [43]. The latter two are required for fortification of the mural cell (*i.e.*, pericytes and vascular smooth muscle cells) of tumor blood vessels [44] (**Figure 1A**). Consequently, in the tumor the blood vessels are abnormal in shape and spatial distribution [42]. Endothelial and mural cells become migratory, lose their interactions with each other [45-49], and cannot adhere infiltrating immune cells [10]. As a result, blood vessels become leaky and are ineffective in maintaining blood flow, resulting in plasma [50] and protein [51, 52] accumulation in the interstitial (i.*e.* extravascular) space (**Figure 1A**).

The second process of excessive fibrotic tissue formation is triggered by cancer cell-mediated activation of fibroblasts, which increases fibroblast contractility and production of fibrosis (**Figure 1B**). These cancer-associated fibroblasts (CAFs) have numerous phenotypes and also play a key role in immunosuppression [53]. Cancer cells and CAFs generate physical forces that compress tumor vessels [54]. Also, CAFs produce and maintain elevated levels of extracellular matrix (ECM) components (*i.e.,* structural components of the tissue including collagen I and hyaluronan) that transmit forces towards compressing blood vessels [54-56] (**Figure 1B**). The expansion of a growing tumor is resisted by the surrounding host tissue thereby increasing the magnitude of compression exerted on tumor tissue [57, 58]. Thus, leaky and compressed tumor blood vessels induce hypoxia.

Besides promoting hypoxia leading to immunosuppression, cancer cells suppress the activation, priming, trafficking, infiltration, migration, and function of antitumor immune cells to reduce antitumor immunity. In fact, all steps of the cancer-immunity cycle, which describes the processes that must be perpetuated for antitumor immunity, are subjected to negative regulation in the TME including through hypoxia signaling [59, 60]. For instance, activated $CD8^+$ T cells primed against cancer antigens must traffic to and infiltrate into tumors [59]. However, this process is inhibited by solid tumors [10], with aberrant VEGF signaling downregulating cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), thereby preventing activated $CD8^+$ T cells to bind and cross the vessel wall [43, 61] (**Figure 1C**). Additionally, a majority of immune cells in the TME are shifted to an immunosuppressive phenotype, such as M2-like rather than M1-like tumor-associated macrophages (TAMs) and regulatory rather than $CD8^+$ T cells [36] (**Figure 1C**). TAMs are shifted to M2-like

phenotypes [9, 62] and regulatory T cells are recruited through angiogenic and hypoxia-induced signaling [63-66]. The TME can exist in different immune phenotypes that reflect various states of immunosuppression [34]. Angiogenesis, desmoplasia and immunosuppression are dysregulated in tumors, and hypoxia is a central downstream effect that leads to disease progression through various mechanisms [1, 9].

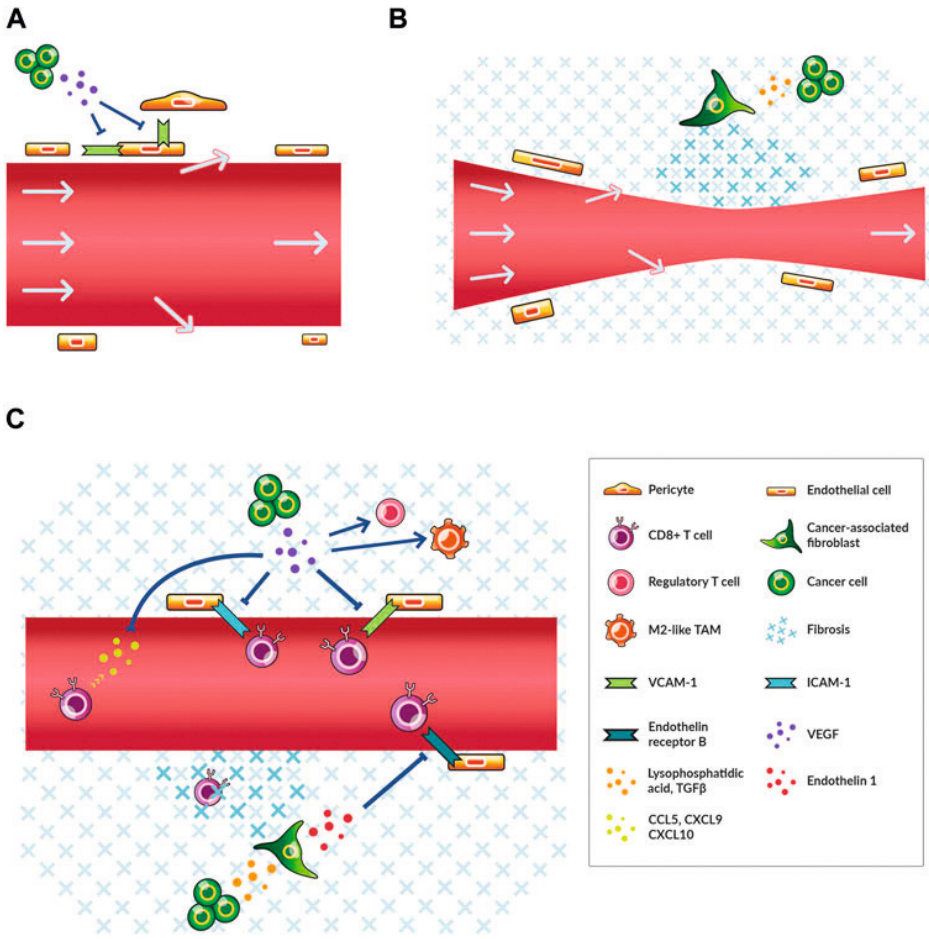

Figure 1 – Cancer cells contribute to angiogenesis, desmoplasia, and immunosuppression thereby reducing blood flow and immune cell infiltration. (**A**) A simplified schematic of leaky tumor blood vessels and relevant cells. Cancer cells secrete angiogenic factors, including vascular endothelial growth factor (VEGF), that reduce the expression of molecules, such as intercellular adhesion molecule 1 (ICAM-1), which facilitate the interaction of endothelial cells with themselves and perivascular cells in normally functioning vessels. Plasma and blood-borne molecules (arrows) flow out of pores in the vessel wall thereby reducing flow. (**B**) A simplified schematic of compressed tumor blood vessels and relevant cells. Cancer cells activate cancer-associated fibroblasts through various signals including transforming growth factor (TGF) b. As a result, more fibrosis is produced and maintained thereby transferring compressive physical force onto blood vessels. Blood flow is reduced. (**C**) A simplified schematic of immunosuppression and relevant cells. As in (**A**) and (**B**), cancer cells secrete VEGF and TGFb among other factors that

affect angiogenesis and fibrosis. VEGF recruits regulatory T cells and shifts tumor-associated macrophages (TAMs) towards M2-like immunosuppressive phenotypes. VEGF also blocks the recruitment and transmigration of CD8+ T cells. TGFb signaling leads to increased fibrosis that physically impedes the migration of CD8+ T cells to cancer cells and blocks the vascular transmigration of these cells through endothelin 1 signaling through the endothelin receptor type B.

Normalization of the tumor microenvironment

Given the central role of hypoxia in poor outcome in patients with cancer and the dependence of oxygen delivery on blood vessels, the normalization hypothesis calls for increasing the function of vessels by modulating stromal cells towards a normal phenotype to enhance the efficacy of chemo-, radio-, and immunotherapies [1, 12, 67]. Though there are numerous physiological mechanisms that can be altered, the two critical abnormalities to be reversed to normalize blood vessels are leakiness and compression [1, 12].

There are two types of TME normalization strategies. One alleviates blood vessel leakiness (vascular normalization, **Figure 2A, B**) and the other reverses compression (CAF reprogramming, **Figure 2A, C**). TME normalization usually refers to a therapeutic strategy to 'normalize' the balance of pro- and anti- factors of angiogenesis and/or desmoplasia signaling [1]. In regards to angiogenesis, Jain introduced the hypothesis of vascular normalization to explain the paradox that, despite the requirement of angiogenesis for tumor growth, starving tumors of their blood supply by therapeutically inducing vascular regression did not improve patient outcome [67]. Instead, preclinical studies demonstrated that balancing elevated pro-angiogenic signaling levels found in tumors with AATs will make the blood vessels phenotypically normal with increased fortification by perivascular cells and ECM (**Figure 2A, B**) [68, 69]. As a result, the blood vessels function normally with decreased vessel leakiness, hypoxia and treatment resistance [1, 12]. Blood vessel normalization has been extensively evaluated preclinically and in patients with cancer for combination with chemo-, radio-, and immunotherapies [1, 12, 28, 70, 71], and vascular normalization could improve responses to ICIs through various mechanisms (**Figure 2A, B**) [72-75]. Indeed, in patients with HCC, the combination of AAT and ICI, but not ICI monotherapy [30, 31], outperforms AAT monotherapy [29].

The second type of normalization involves reversing vessel compression with CAF reprogramming therapies. Hereby, CAFs are turned quiescent such that they produce a smaller magnitude of forces and less amount of ECM such that there is a lesser magnitude of force generated and transmitted within tumors (**Figure 2A, C**) [55, 76-80]. Some CAF reprogramming therapies have been studied in preclinical, retrospective clinical, and prospective clinical studies. One such drug is losartan, which is an anti-hypertensive drug with decades of use in patients with high blood pressure. Losartan and other angiotensin system inhibitors reprogram CAFs to a quiescent phenotype through antagonism of the angiotensin II type I receptor [55]. Dozens of retrospective

studies indicated that patients with certain cancer types receiving angiotensin system inhibitors lived longer [60, 81-83]. Moreover, losartan improved the outcome of patients with pancreatic ductal adenocarcinoma undergoing chemoradiation in a prospective clinical trial [84].

CAF reprogramming also appears to be combinable with ICI [14, 15, 18-20, 85]. For instance, angiotensin system inhibitors prolonged survival of patients with certain tumor types undergoing ICI in a retrospective analysis [86]. Moreover, losartan had direct immunomodulatory effects on immune cells *in vitro* [87] and in clinical studies induced antitumor immunity [81] (**Figure 2A, C**). Additionally, losartan and other angiotensin signaling inhibiting drugs may ameliorate side effects of immunotherapy by enabling ICI dose reduction and inhibiting cytokine storm [88]. With this rationale, ICIs are now being tested prospectively with this combination of losartan and chemoradiation (NCT03563248). Like losartan, metformin is another drug with many effects in cancer and other diseases that has been repurposed to reprogram CAFs [78]. There is evidence from the clinic that it modulates the TME towards antitumor immunity [89]. Additionally, some drugs induce both vascular normalization and CAF reprogramming (**Figure 2**), such as the glucocorticoid steroid dexamethasone [90], but the immunosuppressive properties of this drug are detrimental in many patients taking ICIs [91]. While vascular normalization and CAF reprogramming therapies have advanced to the clinic (**Supplementary Tables 1 and 2**), they have yet to be tested in combination. Nonetheless, mathematical models and preclinical studies demonstrate the value of combining the normalization strategies for ICI (**Figure 2D**) [18, 19, 92].

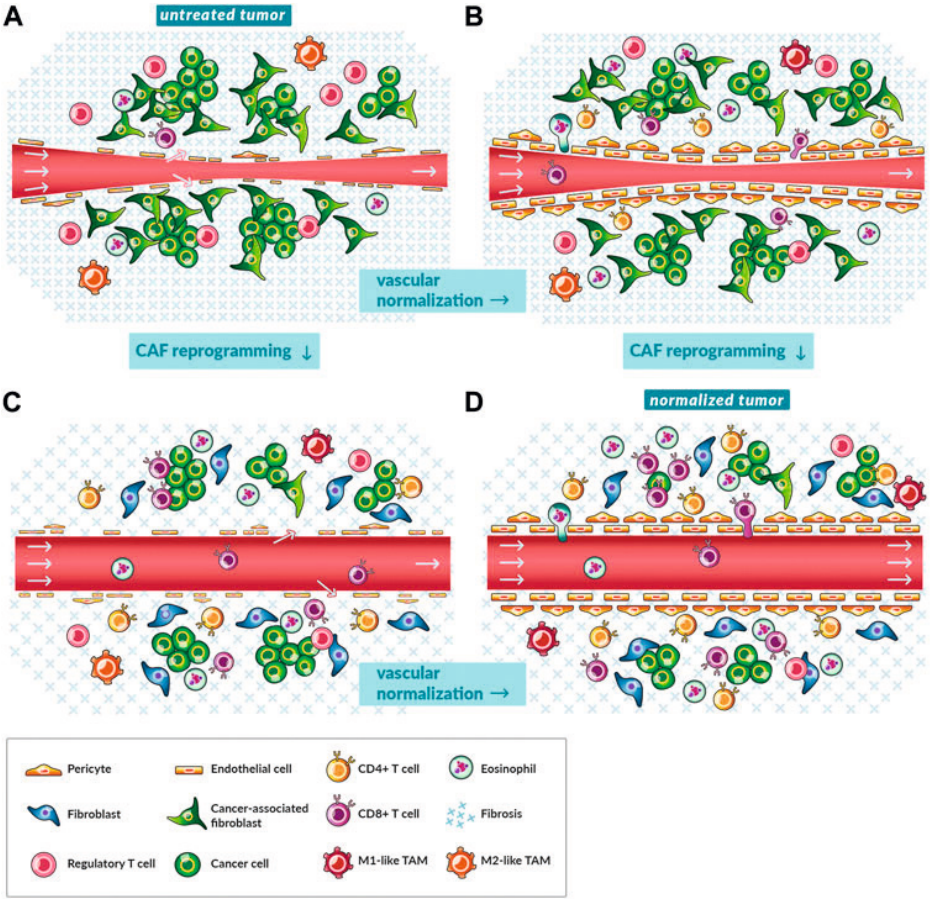

Figure 2 – Vascular normalization and reprogramming of cancer-associated fibroblasts shift the microenvironment towards antitumor immunity. (**A**) A schematic of a magnified, cross-sectional view of a single blood vessel in an untreated tumor. A pinch point in the blood vessel (red tube) and the lack of a consistent endothelial cell layer fortified with pericytes restricts blood flow (gray arrows). The tumor is replete with immunosuppressive cancer-associated fibroblasts (CAFs), fibrosis, and regulatory T cells (CD4+CD25+FOXP3+) while lacking CD8+ and other subsets of CD4+ T cells. (**B**) A schematic of a magnified, cross-sectional view of a single blood vessel in a tumor treated with vascular normalizing therapy. As the balance of pro- and anti-angiogenic factors shifts towards the latter, endothelial cells are aligned, and blood vessels are fortified with pericytes yet remain compressed by mechanical stress. Perfusion increases especially in tumors with low levels of mechanical stress. Immune cells such as CD8+ T cells more efficiently traffic to tumors and transmigrate across the vessel wall. There are fewer immunosuppressive cells because of reduced angiogenic and hypoxia signaling. Vascular normalization by immune checkpoint inhibitors could rely on the accumulation of activated eosinophils. (**C**) A schematic of a magnified, cross-sectional view of a single blood vessel in tumor treated with CAF reprogramming therapy. As CAFs shift to quiescent fibroblasts, they produce and maintain lower levels of fibrosis. Mechanical stress is alleviated and vessels are decompressed. Perfusion increases. Immune cells such as CD8+ T cells flow through tumors and migrate the interstitial space because of less immunosuppressive CAF and hypoxia signaling. Also, there is less physical restriction of migration by components of fibrosis, such as collagen. (**D**) A schematic of a magnified, cross-

sectional view of a single blood vessel in tumor treated with both vascular normalizing and CAF reprogramming therapy. Given the reduced signaling and physical barriers, immune cells such as CD8+ T cells efficiently traffic to tumors, negotiate transport through the vessel wall, and penetrate to clusters of cancer cells.

There are three important aspects to TME normalization. First, stromal cells in the tumor should be reprogrammed towards a non-diseased phenotype. Second, hypoxia should be alleviated, because TME normalization therapies can exacerbate hypoxia at high doses. Specifically, AAT administered at higher doses and for longer times prunes an excessive amount of blood vessels thereby reducing blow flow to the tumor. Besides inducing hypoxia, rapid depletion of stromal components including blood vessels [93], pericytes [94], CAFs [95], hyaluronan [96], collagen [97], and regulatory T cells [98] through either genetic or pharmacological methods results in disease progression. Thus, the third aspect is that stromal components should be reprogrammed rather than destroyed [77].

Normalization by immunotherapies

ICIs are approved for dozens of cancer types, but they only benefit a fraction of patients with cancer [99]. To overcome primary resistance mediated by immunosuppressive hypoxia, angiogenesis and fibrosis signaling, researchers and oncologists are developing ICI combination therapy strategies including with vascular normalizing therapies [72], CAF reprogramming and nanomedicine [7, 60]. In addition to AAT and CAF reprogramming therapies, the contribution of ICI to TME normalization is under investigation currently [92]. Thus, understanding the mechanisms through which immunotherapies normalize the TME could lead to more effective TME normalization and immunotherapy regimens.

There is both preclinical and clinical evidence that ICI monotherapy normalizes blood vessels in tumors that respond to ICI [100]. In murine tumors that respond to ICI with slowed tumor growth, ICI efficiently prunes vessels resulting in enhanced perfusion [100]. However, depletion of CD8+ T cells or inhibition of IFNg production reversed the enhanced perfusion that ICI induced [100]. These results demonstrate that effective ICI therapy, which necessarily promotes $CD8^+$ T cell accumulation and IFNg production, also increases perfusion. Furthermore, these results generate the hypothesis that enhanced perfusion is a biomarker of response to ICI treatment [100].

Both IFNg and CD8+ T cells are necessary for antitumor immunity [101]. At high levels, IFNg induces apoptosis of cancer cells [102]. At low levels, IFNg induces cancer cell stemness resulting in increased metastasis [102]. Also, IFNg has non-immune-mediated antiangiogenic properties [103], and the extent of these effects could depend on the levels of IFNg [73]. AAT at high doses induce vascular regression while low doses induce vascular normalization [1, 3]. Similarly, at high levels of IFNg in inducible models producing ~10 ng per ml [73], IFNg acts on stromal cells independently of cancer cells to induce vascular regression and eliminate blood flow to tumors [104]. At lower levels

generated by adoptive cell transfer or ICI, IFNg might induce vascular normalization, as it induces upregulation of ICAM-1 [105], which promotes adhesion between endothelial cells and leukocytes, and VCAM-1 [106], which promotes adhesion between pairs of endothelial cells and endothelial cells and leukocytes or mural cells. Separately, through IFNg, $CD8^+$ T cells can polarize TAMs to an M1-like phenotype, which is antiangiogenic [36]. ICI also depends on IFNg to induce antitumor immune responses [101]. ICI, TAM polarization, adoptive cell transfer and experimental models of inducible IFNg all introduce different concentrations of IFNg in the TME, thereby, inducing different magnitudes of antiangiogenic effects [73]. Thus, vascular normalization resulting from immunotherapies depends on the context and requires further study.

In patients with glioblastoma, tumors responding to ICI had reduced vascular permeability indicative of vascular normalization [107]. However, this reduction occurred six months after ICI treatment initiation and after a brief period of increased vascular permeability. Therefore, the conflicting kinetics of these processes between murine breast tumors and glioblastoma tumors in patients must be resolved by observing vascular permeability in tumors from patients and related murine models at times before and shortly after ICI treatment initiation [108]. Additionally, vascular regression associated with ischemic tumor necrosis after vaccination and/or ICI was observed in responding melanoma and ovarian tumors from patients [109]. Thus, the kinetics and tumor type dependence of the antiangiogenic effects of immunotherapies in tumors in patients must be clarified.

The cells responsible for producing the IFNg that modulates the vasculature differ between studies. In experiments resembling the clinical 'preventative setting' in which interventions occur before tumors could be diagnosed, knockout models revealed that $CD8^+$ T cells and $CD4^+$ T cells had opposite angiogenic effects. $CD8^+$ T cells induced endothelial cell proliferation, yielding a pro-angiogenic effect. In contrast, the $CD4^+$ T cells induced pericyte recruitment, a critical process for vascular normalization and alleviation of hypoxia [110]. Thus, in the 'preventative setting', $CD4^+$ T cells through IFNg seem to be responsible for vessel fortification through pericyte recruitment [108, 110] consistent with previous studies of angiogenesis in early tumor development [111].

Interventions with ICI in the time window when treatment would typically occur in patients normalized the tumor vasculature in an IFNg-dependent manner, with lack of effect using IFNg receptor knock out mice and upon IFNg-neutralization studies [100]. In T cell depletion studies in wild type mice, ICI-induced normalization and enhanced perfusion proved to be dependent on $CD8^+$ but not $CD4^+$ T cells [100]. Taken together with the results from the preventative setting, these studies collectively indicate that $CD4^+$ T cells play a role in vessel maturation early in tumor development while ICIs act through $CD8^+$ T cells to normalize blood vessels in the treatment setting. The notion that immunotherapy-induced vascular normalization is mediated by $CD8^+$ T cells in

tumors in the treatment setting is supported by observations that adoptive transfer therapy of T cells contributes to normalized vascular morphology [112]. Interestingly, such ICI-induced normalization also depended on eosinophil accumulation in tumors in addition to $CD8^+$ T cell accumulation and IFNg [113]. These findings are consistent with previous studies describing crosstalk between eosinophils and CD8+ T cells that leads to a positive feedback loop of vascular normalization and antitumor immunity [114]. Furthermore, activated eosinophils in the TME, through IFNg, skew TAMs to an antiangiogenic M1-like phenotype. As a result, there is some vascular normalization with elevated expression of VCAM-1, which induces adhesion of eosinophils and T lymphocytes (among other cells) to the endothelium thereby promoting transmigration and infiltration. In turn, these T cells can promote more vascular normalization and skewing of TAMs. Additional tumor-specific effector T cells continue to be trafficked to the tumor by interferon-induced chemoattractants produced by activated eosinophils [114]. In patients, increases in eosinophil and lymphocyte counts after ICI correlated with increased survival [115]. Thus, during ICI treatment there is a positive feedback loop of $CD8^+$ T cells and activated eosinophils stimulating antiangiogenic effects directly through IFNg production and indirectly through TAM polarization, which in turn increases eosinophil and lymphocyte adhesion to the endothelium and tumor accumulation (**Figure 3A**).

Another observation of long-term vascular normalization and antitumor immunity by activated $CD8^+$ T cells, though not an example of immunotherapy causing vascular normalization, adds further support to this positive feedback cycle hypothesis [116]. Specifically, researchers studied the ligand Delta-like canonical Notch ligand 1 (DLL1) [117]. The levels of this ligand are reduced with increased circulating VEGF, which is characteristic of tumors with high levels of angiogenesis [117]. When DLL1-Notch signaling is reduced in bone marrow precursors by circulating VEGF, T cell activation and antitumor immunity is reduced [117]. However, interfering with circulating VEGF-induced DLL1-Notch signaling inhibition by overexpressing DLL1 in cancer cells induced long-term vascular normalization [116]. This normalization was dependent on IFNg production and $CD8^+$ T cell accumulation [116]. In turn, this long-term vascular normalization was necessary for ICI efficacy in the resistant tumor model assayed [116]. Thus, long-term vascular normalization can be induced by immune cells through interference of angiogenic and immunosuppressive signaling, and this normalization can increase the antitumor effect of ICI (**Figure 3A**).

Besides ICI, other immunotherapies have normalizing effects. In melanoma, low-dose local administration of a STING agonist increased expression of antiangiogenic factors resulting in increased endothelial cell pericyte coverage and expression of VCAM-1 [118]. In this case, vascular normalization depended on STING activation of dendritic cells rather than effects on cancer cells, which also express STING receptor [118]. These normalized vessels, along with newly formed tertiary lymphoid structures, increased T

cell infiltration [118]. These processes were studied in more detail in breast, lung, and colorectal cancer models [119]. As in the study in melanoma, STING agonism induced expression of vascular stabilization genes, but there were several additional findings important to understanding STING agonist-induced vascular normalization. First, while STING agonism of hematopoietic stromal cells like dendritic cells were necessary for immune response, agonism of nonhematopoietic stromal cells, particularly endothelial cells, mediated the normalization process [119]. Second, STING agonist-induced vascular normalization depended on $CD8^+$ T cells and IFNg but not TAMs [119]. Taken together, these results demonstrate that STING agonists, like ICI, can induce vascular normalization through $CD8^+$ T cells and IFNg leading to enhanced T cell vascular adhesion, infiltration, and therapeutic effects (**Figure 3B**).

Other immunostimulatory agents, such as oligodeoxynucleotides (ODN) with cytosine-guanine-rich (CpG) motifs (CpG-ODN) normalize vessels as evidenced by the upregulation of ICAM-1 and VCAM-1 in endothelial cells by TAMs directly stimulated by CpG-ODN [120]. With such normalized vessels, adoptively transferred immune cells could better extravasate and infiltrate tumors (**Figure 3C**). Despite clinical testing, CpG-ODN has not succeeded, perhaps in part of the necessity of local administration. Even if CpG-ODN is effective in the tumor in which it is administered and the patient develops systemic antitumor immunity through an abscopal effect, the TME of metastatic lesions could still impair infiltration [60]. Similarly to the effects of CpG-ODN, in some contexts depletion of regulatory T cells also increases the upregulation of ICAM-1 and VCAM-1 adhesion molecules [121]. Depletion of regulatory T cells also lead to other indicators of vascular normalization including reduced vessel diameter and increased perfusion [121]. While depletion of regulatory T cells generates antitumor immunity in most contexts, researchers reported that depletion of regulatory T cells depletes pancreatic ductal adenocarcinoma tumors of fibroblasts, which paradoxically unleashes tumor growth and immunosuppression [98]. Thus, the effect of regulatory T cell depletion could depend on the tumor type. Finally, oncolytic viruses can also reduce vascular density transiently while increasing VCAM-1 gene expression [122], but through what mechanisms and whether vessels are normalized versus regressed is unclear, as is whether hypoxia is alleviated (**Figure 3D**). Thus, whereas STING agonists seem to act through T cells and IFNg as with ICI treatment, oligonucleotide therapies might act through TAMs to increase expression of adhesion molecules. Further research is necessary to determine whether and how these immunotherapies are combinable for enhanced vascular normalization.

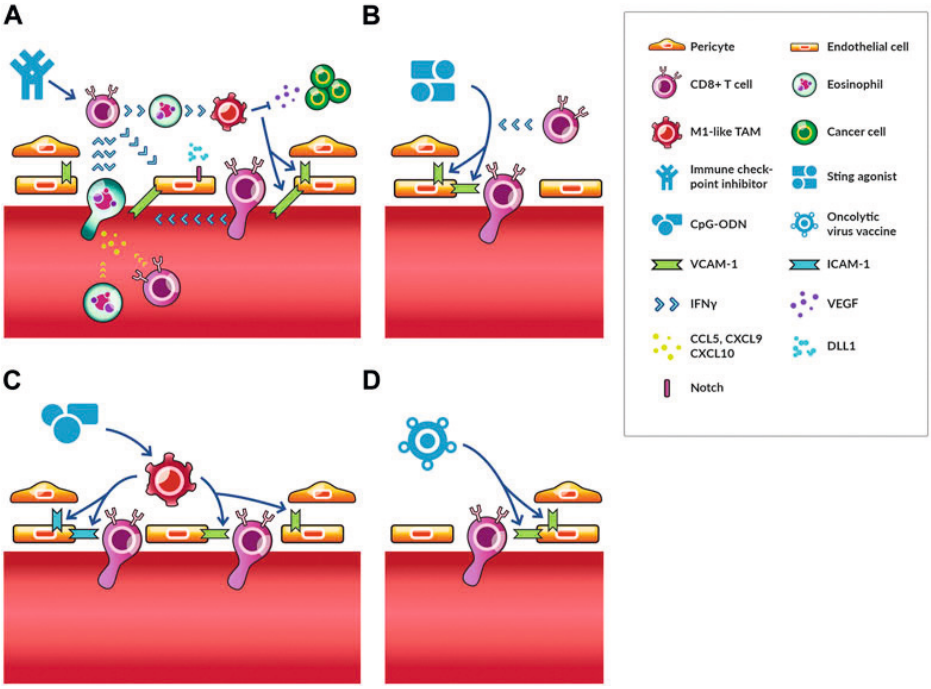

Figure 3 – Vascular normalization through immunotherapies. Immunotherapies stimulate various immune cells to act on endothelial and mural cells resulting in vascular normalization. Several immunotherapies normalize vessels through distinct mechanisms. (**A**) The effects of immune checkpoint inhibitors (ICIs) are the most well-studied. ICIs activate CD8+ T cells, which secrete IFNg. These cells can interact with activated eosinophils through IFNg to induce M1-like TAM phenotypes, which reduces VEGF signaling and induces VCAM-1 expression. As a result, more CD8+ T cells and activated eosinophils adhere to and transmigrate across the endothelium. The later secretes chemokines (i.e., CCL5, CXCL9 and CXCL10), which increase trafficking of CD8+ T cells and eosinophils to tumors. For this reason, activated eosinophil accumulation precedes and is required for increased CD8+ T cell homing to tumors. This process is a potential feedback loop of vascular normalization and antitumor immunity. DLL1-Notch signaling promotes CD8+ T cell activation and IFNg production thereby reinforcing this positive feedback loop for long-term vascular normalization. (**B**) STING agonists cause an increase in antiangiogenic factors, which results in increased pericyte coverage through VCAM-1 expression, which also facilitates the infiltration of T cells. (**C**) CpG-ODN directly act on TAMs promoting an M1-like phenotype, which induces the upregulation of ICAM-1 and VCAM-1 expression. In some contexts, depletion of regulatory T cells has similar effects on ICAM-1 and VCAM-1 expression. (**D**) Oncolytic vaccines reduce vascular density and increase VCAM-1 expression through unelucidated mechanisms.

Combination therapies for tumor microenvironment normalization

Several rationales have been developed for combining AATs and ICI with disparate effects on vascular regression and normalization. One mechanism of synergy involves AATs inducing vascular changes resulting in more recruitment of $CD8^+$ T cells. Although this mechanism is resisted by IFNg acting on endothelial cells to upregulate immune checkpoint expression, this resistance can be neutralized through ICI [123]. This mechanism was investigated in the context of combined VEGF and angiopoietin 2

inhibition, which induced vascular regression leading to tumor necrosis [123]. The remaining vessels were normalized as evidenced by increased pericyte coverage, but the density of vessels was low. This 'passive vessel normalization', in which immature blood vessels are destroyed while the mature vessels remained, was sufficient to facilitate recruitment of $CD8^+$ T cells. Thus, there are mechanisms of synergy between AATs and ICI that are effective when active vascular normalization through pericyte recruitment to immature vessels does not occur while vascular regression does occur. A separate study demonstrated that VEGF inhibition and ICI were complimentary through formation of high endothelial venules, which are blood vessels critical for the recruitment of T cells to antigen-presenting cells within tertiary lymphoid structures or lymph nodes, leading to increased lymphocyte infiltration [124]. As in the other study, researchers observed regression of half of the tumor vasculature [124]. A unique aspect was the investigation of the independent contributions of AAT and ICI to vessel pruning and fortification. While anti-VEGF therapy induced vascular regression and fortification, the addition of ICI did not contribute to vascular regression yet further increased pericyte coverage [124]. Thus, ICI induced active pericyte recruitment, which occurred through an increase in the angiostatic properties of myeloid cells in the TME [124]. After tumor relapse from AAT monotherapy, only the combination of AAT and ICI could reduce vessel density, suggesting the antiangiogenic properties of ICI were non-redundant with VEGF inhibition [124]. Thus, there are mechanisms of synergy between AAT and ICI that induce vascular regression and are independent of vessel normalization, but ICI seems to have stronger effects on vessel fortification rather than regression.

A series of reports in HCC provided an illustrative case of combined AAT and ICI vascular normalization. Sorafenib, which was the only approved AAT for HCC at the time, excessively pruned vessels, thereby, causing hypoxia and stimulating the SDF1a/CXCR4 pathway [125]. Although blocking CXCR4 reduces fibrosis and increases T cell infiltration in some tumor types [15], in HCC after relapse from AAT, blocking CXCR4 alleviated immunosuppressive cell recruitment and angiogenesis resistance mechanisms to some extent [125]. However, adding ICI to AAT and CXCR4 inhibition alleviated these resistance mechanisms further by increasing the amount of IFNg and $CD8^+$ T cells in the tumor center, indicating that vessels could be further normalized [125]. Indeed, a follow-up study demonstrated that ICI induced vessel normalization to a larger extent through $CD4^+$ T cells when combined with an anti-VEGFR2 antibody compared to the AAT antibody alone [16]. Anti-VEGFR2 antibody increased the endothelial cell expression of PD-L1 in an IFNg-dependent manner and PD-1 expression in $CD4^+$ T cells providing further rationale for combination therapy with ICI [16]. Importantly, ICI-induced fortification of vessels (**Figure 4A**) prevented vascular regression caused by higher doses of anti-VEGFR2 antibody thereby increasing the therapeutic index of this AAT [16]. This finding is important given the sensitivity of vascular normalization to anti-VEGF therapy dose [1]. Interestingly, when the AAT sorafenib follows ICI in HCC, the density of blood vessels associated with pericytes increases in a CD8+ T cell dependent

manner (**Figure 4B**), while sorafenib not preceded by ICI causes vascular regression [126]. Thus, ICI fortifies vessels thereby preventing vascular regression induced by subsequent AAT. Along these lines, administering AAT to normalize vessels avoided oncolytic vaccine therapy-induced vascular regression improved therapeutic efficacy [127]. Thus, combinations of AAT and ICI can be administered in various schedules to induce a greater extent of vascular normalization while avoiding regression.

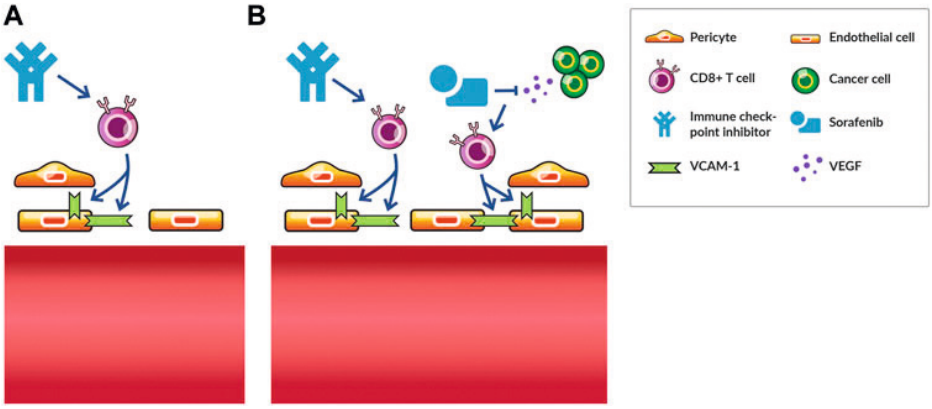

Figure 4 – Vascular fortification by immune checkpoint inhibition promotes vascular normalization by subsequent antiangiogenic therapy in hepatocellular carcinoma. (**A**) Immune checkpoint inhibition monotherapy normalizes tumor blood vessels in various murine models of cancer by increasing the interaction between endothelial cells and with perivascular cells in a CD8+ T cell dependent manner. (**B**) Following immune checkpoint inhibition with antiangiogenic therapy, such as the small-molecule tyrosine kinase inhibitor sorafenib, increases the density of tumor blood vessels fortified by pericytes and antitumor efficacy in a CD8+ T cell dependent manner. In contrast, sorafenib monotherapy induces vascular regression and does not induce an antitumor effect. Thus, vascular-fortifying ICI can shift the effect of subsequent antiangiogenic therapy from vessel destruction to normalization.

While in certain contexts the combination of ICI and AAT prevents vascular regression caused by high levels of IFNg or high doses of AAT, respectively, in other contexts vascular regression is dependent on the dose of AAT. Specifically, high doses of the small-molecule tyrosine kinase VEGF inhibitor regorafenib combined with ICI in HCC induced vascular regression [17]. Besides dose-dependent normalization, the authors demonstrated that regorafenib induced CXCL10 expression in HCC cells. This chemokine binds to its receptor CXCR3 expressed on circulating lymphocytes to increase their trafficking to tumors [17]. This study provides an additional hypothesis for a feedback loop between T cells and vascular normalization. Specifically, regorafenib and ICI normalize the vasculature and increase CXCL10 expression resulting in increased infiltration of T cells, which induce additional vascular normalization. One AAT resistance mechanism associated with gene expression changes, albeit in stromal cells in this case, involves upregulation of the EGFR pathway in endothelial and perivascular cells of lung adenocarcinoma [128]. Accordingly, blocking EGFR is effective for treating

VEGF-resistant tumors and combining EGFR inhibition with immunotherapies might result in TME normalization. EGFR inhibition using fusion proteins or bispecific antibodies also targeting death receptors [129], immune checkpoints [130], or CD47 [131] signaling have also been developed and their vascular normalization properties should be evaluated.

There is also preclinical evidence that other immunotherapies besides ICI, when combined with AATs, reinforce vascular normalization and induce CAF reprogramming. Specifically, an anti-CD40 antibody, which promotes dendritic cell maturation, antigen presentation and priming of T cells, demonstrated normalization properties. In genetically engineered colorectal cancer models, researchers demonstrated that adding anti-CD40 antibody to combined anti-VEGF and anti-angiopoietin 2 therapy fortified blood vessels even in non-angiogenic tumors [132]. Interestingly, in this study AAT alone induced CAF reprogramming effects, and this effect was increased with anti-CD40 antibody [132]. Of note, TME normalization was T cell independent, even though the antitumor efficacy depended on T cells [132]. Nonetheless, the antitumor activity was also dependent on angiogenic (*i.e.*, angiopoietin 2) signaling [132]. An interesting aspect of this study is that while the tumors were well-perfused at baseline, the increase in antitumor activity appears to be due to increased trafficking and infiltration of T cells to tumors because of increased vessel maturity and increased T cell migration resulting due to reduced fibrosis [132]. Unlike other studies, this study examined well-perfused tumors and normalization did not depend on T cells.Thus combining anti-CD40 and ICI therapies, such as through bispecific antibody fusion proteins [133], might normalize vessels through non-redundant mechanisms. In addition, bispecific antibodies can deliver restricted CD40 signaling to specific cell populations, which can reduce toxicity and normalize the TME [134]. Overall, these studies demonstrate that immunotherapies enhance vascular normalization and CAF reprogramming when combined with AAT, and the resulting increase in antitumor efficacy can occur through various mechanisms that can be independent of increased perfusion and alleviated hypoxia.

DISCUSSION

TME normalization, which reduces hypoxia, is a potentially promising approach to enhance responses to immunotherapy based on the preclinical body of evidence, but clinical data remains to be generated. Immune cells modulate tumor vasculature. Accordingly, various immunotherapies including ICIs, oncolytic viral vaccines, and immunostimulatory therapies such as STING agonists induce antiangiogenesis, often through IFNg and $CD8^+$ T cells, but also through promoting angiostatic properties in TAMs. The tumor type and amount of IFNg produced seem to determine the extent of antiangiogenic effect, with large amounts of IFNg leading to vascular regression and hypoxia rather than normalization and normoxia. In preclinical cancer models, efficacious ICI stimulates $CD8^+$ T cells and IFNg production thereby fortifying vessels with pericytes leading to increased perfusion. This process could stimulate a positive feedback loop of increased T cell recruitment and normalization. Further work could clarify how different immune cells differentially regulate aspects of vascular normalization, including vessel pruning and pericyte recruitment. Additionally, the tumor type dependence and kinetics of vascular normalization in patients must be studied further.

AAT and ICI combinations are efficacious through various mechanisms and can induce vascular regression or normalization (**Supplementary Table 3**). In either case, ICI contributes to the fortification of blood vessels with pericytes and combination with AAT increases immune cell recruitment. Fortifying vessels with immunotherapies can help avoid excessive vessel pruning by high doses of AAT. Alternatively, normalizing vessels with AAT can inhibit vascular regression caused by subsequent immunotherapy. These effects seem to depend on the tumor type, treatment type, and kinetics of response. By understanding these interactions, long-lasting normalization might be more effectively achieved by combination immunotherapies with AATs. To what extent CAF reprogramming therapy through alleviation of hypoxia and immunosuppressive signaling can increase the efficacy of combined AAT and ICI remains unclear. Relatedly, further research should unravel whether immunotherapies reprogram CAFs. The more effectively the positive feedback loop of activated immune cells inducing vascular normalization can be harnessed, the more effectively immunotherapies can induce antitumor immunity.

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Supplementary Table 1. FDA-approved vascular normalization agents in clinical trials with immunotherapy. Colors indicate FDA-approval, phase III clinical trial active or recruiting or not yet recruiting and no phase III clinical trial.

Drug	Target	Approved indications	Clinical trials in combination with ICBs	Brief Description
Axitinib	TKI (VEGFR-1–3, PDG-FR-β)	RCC	Approved for: first line treatment of patients with advanced RCC with either avelumab or pembroli-zumab NCT02684006 RCC NCT02853331 RCC	Tested as a first line therapy in combination with ave-lumab in patients with RCC.
Bevacizumab	Anti-VEGF-A antibody	CRC, NSCLC, breast, RCC	Approved for: first line treatment of pa-tients with metastatic non-squamous, NSCLC in combination with atezolizumab and che-motherapy. Approved for treat-ment of patients with persistent, recurrent or metastatic cervical cancer whose tumors express PD-L1 (CPS ≥1) in combination with pembrolizumab plus chemotherapy. Approved for treatment of patients with HCC in combination with atezolizumab NCT03038100 Ovarian cancer NCT04194203 NSCLC NCT04102098 HCC NCT04487067 HCC NCT03737643 Advanced ovarian cancer NCT02891824 Epithelial ovarian cancer NCT03991403 NSCL NCT03353831 Recurrent ovarian car-cinoma NCT03847428 HCC NCT04712643 HCC NCT04803994 HCC NCT04732286 HCC	Tested as first line therapy in combination with atezoli-zumab and chemotherapy in patients with NSCLC, HCC, malignant pleural meso-thelioma, metastatic CRC, cervical cancer, and head and neck cancer. Tested as adjuvant or first line therapy in combination with atezolizumab in patients with high risk of recurrence of HCC, unresectable HCC, unresectable HER2$^-$ breast cancer, and metastatic HCC. Tested as first line therapy in combination with durvalum-ab and chemotherapy in patients with advanced ovarian cancer. Tested in combination with durvalumab as first line therapy in patients with advanced HCC. Tested as a first line ther-apy in combination with transarterial chemoemboli-zation and atezolizumab or durvalumab in patients with HCC unsuitable for curative therapy. Tested as first line therapy in combination with pembroli-zumab and chemotherapy in patients with persistent, recurrent, and metastatic cervical cancer. Tested in combination with pembrolizumab as a first line therapy for recurrent ovarian cancer and NSCLC. Tested as first line therapy in combination with nivolumab and chemotherapy in meta-static CRC.

Supplementary Table 1. Continued

Drug	Target	Approved indications	Clinical trials in combination with ICBs	Brief Description
			NCT05116189 Recurrent ovarian cancer NCT04732598 Breast cancer NCT03762018 Malignant pleural mesothelioma NCT03778957 HCC NCT03635567 Cervical cancer NCT03740165 Ovarian cancer NCT02997228 Metastatic colorectal adenocarcinoma NCT02839707 Ovarian, fallopian tube, or primary peritoneal cancer NCT03414983 CRC NCT03556839 Cervical cancer NCT03434379 HCC NCT05063552 Head and neck cancer NCT03178552 NSCLC	
Cabozantinib	TKI (VEGFR-2, Tie2)	Medullary thyroid, RCC, HCC	Approved for: combination with nivolumab as first-line treatment for patients with advanced RCC NCT04338269 RCC NCT03755791 HCC NCT04471428 NSCLC NCT03937219 RCC NCT04446117 Prostate cancer NCT05092958 Urothelial cancer NCT03793166 Advanced kidney cancer	Tested as a second line therapy in combination with atezolizumab patients with inoperable RCC, metastatic castration-resistant prostate cancer and metastatic NSCLC. Tested as a first line therapy in combination with atezolizumab in patients with advanced HCC. Tested as a first line therapy in combination with nivolumab and ipilimumab in patients with metastatic urothelial cancer. Tested in combination with nivolumab following treatment with ipilimumab and nivolumab in patients with advanced kidney cancer.
Everolimus	mTOR inhibitor	RCC, PNET, GI, lung	None	
Thalidomide	Chemotherapy pleiotropic	Multiple myeloma	NCT02726581 Multiple myeloma	Tested as a first line therapy in combination with nivolumab in patients with multiple myeloma.

Supplementary Table 1. Continued

Drug	Target	Approved indications	Clinical trials in combination with ICBs	Brief Description
Lenvatinib	TKI (VEGFR-1–3, PDGFR-α, FGFR1–4, KIT, RET)	DTC, RCC, HCC, endometrial carcinoma	Approved for: first line treatment of patients with renal cell carcinoma and endometrial carcinoma with pembrolizumab NCT03713593 HCC NCT04889118 Melanoma NCT03820986 Melanoma NCT03976375 NSCLC NCT04776148 CRC NCT03517449 Endometrial carcinoma NCT03884101 Endometrial carcinoma NCT04676412 NSCLC NCT03829332 NSCLC NCT04716933 NSCLC NCT03829319 NSCLC NCT02811861 RCC NCT04770896 HCC	Tested as a first line therapy in combination with pembrolizumab in patients with HCC and melanoma. Tested in combination with pembrolizumab in patients with CRC, endometrial carcinoma, and metastatic NSCLC. Tested as a first line therapy in combination with chemotherapy and pembrolizumab in patients with NSCLC. Tested as a first-line therapy in combination with pembrolizumab in patients with advanced RCC. Tested as a second line therapy in combination with atezolizumab in patients with locally advanced or metastatic HCC.
Nintedanib	TKI (VEGFR-2, PDGFR-α/β, FGFR-1)	NSCLC	None	
Pazopanib	TKI (VEGFR-1–3, PDGFR-β, FGFR-1–2)	RCC, soft tissue sarcoma	None	
Ramucirumab	Anti-VEGFR2 antibody	Gastric, HCC, NSCLC, CRC	None	
Regorafenib	TKI (VEGFR-1–3, PDGFR-β, FGFR-1–2)	CRC, GIST, HCC	NCT04879368 Gastro-oesophageal cancer	Tested as a second line therapy in combination with nivolumab in patients with gastro-oesophageal cancer.
Sorafenib	TKI (VEGFR-2 & 3, KIT, Raf, PDGFR-β)	RCC, HCC, thyroid cancer	NCT04770896 HCC	Tested as a second line therapy in combination with atezolizumab in patients with locally advanced or metastatic HCC.
Sunitinib	TKI (VEGFR-1–2, PDGFR-α/β, KIT, RET, CSFR-1, FLT3)	RCC, GIST, PNET	None	

Supplementary Table 1. Continued

Drug	Target	Approved indications	Clinical trials in combination with ICBs	Brief Description
Vandetanib	TKI (VEGFR-2)	Medullary thyroid cancer	None	
Aflibercept	Protein blocking VEGF (VEGF-A, VEGF-B, PlGF)	Metastic CRC	None	
Pralsetinib	TKI (RET, DDR1, TRKC, FLT3, JAK1-2, TRKA, VEGFR2, PDGFR-β, FGFR1-2)	NSCLC, advanced or metastatic RET-mutant and RET fusion-positive thyroid cancers, medullary thyroid cancer	None	
Infigratinib	FGFR 1-4	Cholangiocarcinoma	None	
Tivozanib	TKI (VEGFR-1–3, PDGFR-β, KIT)	RCC	NCT04987203 RCC	Tested in combination with nivolumab in patients with advanced RCC who have had 1 or 2 prior lines of therapy, one of which was an ICI.
Erdafitinib	FGFR 1-4	Metastatic bladder cancer	None	
Pemigatinib	FGFR 1-3	Cholangiocarcinoma	None	
Selpercatinib	TKI (RET)	Lung and thyroid cancers	None	
Ripretinib	**PDGFR-α/β**	GIST	None	
Pexidartinib	CSF1R, CD117, KIT, FLT3, PDGFR-β	TGCT	None	
Sirolimus	mTOR inhibitor	Perivascular epithelioid cell tumor	None	
Temsirolimus	mTOR inhibitor	RCC	None	

On April 22, 2022 we searched clinicaltrials.gov for the [name of anti-angiogenic therapy] AND pembrolizumab OR atezolizumab OR nivolumab OR cemiplimab OR ipilimumab OR durvalumab OR avelumab OR ipilimumab AND Cancer [DISEASE] with filters for Phase III and active or recruiting.

Supplementary Table 2. Investigational CAF reprogramming and extracellular matrix modifying agents in clinical trials with immunotherapy. Colors indicate approval or clinical trial active or recruiting or not yet recruiting and no approval or no clinical trial. Updated from the senior author's previous work [60].

Drug name/class, target	FDA approved for cancer/other	Clinical stage with immunotherapy
Losartan/angiotensin system inhibitors, TGF-b	No/yes	NCT03563248 Phase II ongoing in combination with nivolumab, radiation and FOLFIRINOX in PDAC
Paricalcitol/vitamin D receptor agonist, vitamin D receptor	No/yes	NCT02930902 Phase II ongoing in combination with pembrolizumab and chemotherapy in resectable (NCT02930902, NCT03519308) or metastatic (NCT02754726) PDAC)
Plerixafor/ immunostimulant, CXCL12/CXCR4	Yes/yes	Phase II ongoing – in combination with cemiplimab in metastatic pancreatic cancer
Metformin/treatment for diabetes, TGF-b	No/yes	Phase II ongoing in combination with nivolumab in NSCLC (NCT03048500) and CRC (NCT03800602), pembrolizumab in melanoma (NCT03311308), and in metastatic head and neck (NCT04414540 and NCT04114136), advanced melanoma, RCC, NSCLC, HCC (Child Pugh Class A only), MSI-High solid tumors, urothelial cancer, GE junction/gastric adenocarcinoma, durvalumab in head and neck cancer (NCT03618654), and in breast cancer (NCT01042379)
All-trans retinoic acid	Yes/yes	Phase II ongoing in melanoma in combination with pembrolizumab (NCT03200847) and ipilimumab (NCT02403778)
PEGPH20/ hyaluronidase, hyaluronan*	No/no	Early Phase I with avelumab in metastatic PDAC (NCT03193190), with pembrolizumab in gastric cancer (NCT03281369)
Pentoxifylline/ treatment for occlusive artery disease	No/yes	None
Pirfenidone/treatment for idiopathic pulmonary fibrosis, TGF-b	No/yes	Phase I with atezolizumab as a second line therapy in NSCL (NCT04467723)
Tranilast/anti-histamine, TGF-b	No/no (approved as an anti-histamine in Japan and Korea)	None
Hydralazine/vasodilator	No/yes	None
Fasudil/vasodilator, Rho-kinase	No/yes	None
Relaxin/hormone, collagen**	No/No	None
Halofuginone/ antiprotozoal, collagen	No/no (veterinary)	None

* PEGPH20 does not reprogram CAFs, but rather depletes desmoplasia, which has been shown in preclinical studies to induce tumor progression.
**Some studies have demonstrated a correlation between relaxin levels and tumor progression.
For trials in combination with ICB, on April 22, 2022 we searched clinicaltrials.gov for the [name of stromal normalizing therapy] AND pembrolizumab OR atezolizumab OR nivolumab OR cemiplimab OR ipilimumab OR durvalumab OR avelumab OR ipilimumab AND Cancer [DISEASE] with filters for and active or recruiting.

Supplementary Table 3. Vascular normalizing characteristics of antiangiogenic therapies and immune checkpoint inhibitors. The efficacy, pharmacodynamic, and adverse effects of the therapies are summarized.

	Antiangiogenic therapy	Immune checkpoint inhibition therapy	Combination therapy
Anti-tumor efficacy	Low except in highly angiogenic tumor types such as HCC.	High in some patients	High in patients with tumors that are angiogenic and expressing immune checkpoints. High in patients with tumors that exclude immune cells and cause immunosuppression through angiogenic signaling.
Vascular pruning	High. Dependent on dose.	Low in most studies. This could depend on the level of IFNg generated.	Less than additive. Additional fortification of vessels by the combination could reduce the amount of vessels pruned.
Vascular fortification	High	High	Could be additive or greater.
Toxicity	Low	Low	Low

List of abbreviations in Supplementary Tables

CRC	Colorectal cancer
CSF1R	Colony stimulating factor 1 receptor
CXCL12	C-X-C motif chemokine ligand 12
CXCR4	C-X-C chemokine receptor type 4
DDR1	Discoidin domain receptor 1
DTC	Differentiated thyroid cancers
FGFR	Fibroblast growth factor receptor
FLT3	Fms-related tyrosine kinase 3
GI	Gastrointestinal cancer
GIST	Gastrointestinal stromal tumor
HCC	Hepatocellular carcinoma
ICI	Immune checkpoint inhibitor
JAK	Janus kinase
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
MSI	Microsatellite instability
NSCLC	Non-small-cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
PDGFR	Platelet-derived growth factor receptor
PEGPH20	Pegvorhyaluronidase alfa
PNET	Primitive Neuro-Ectodermal Tumors
RCC	Renal cell carcinoma
RET	Rearranged during transfection proto-oncogene
TGCT	Tenosynovial Giant Cell Tumor
TGF- β	Transforming growth factor β
TKI	Tyrosine kinase inhibitor
TRKA	Tyrosine kinase receptor A
TRKC	Tropomyosin receptor kinase C
VEGFR	Vascular endothelial growth factor receptor


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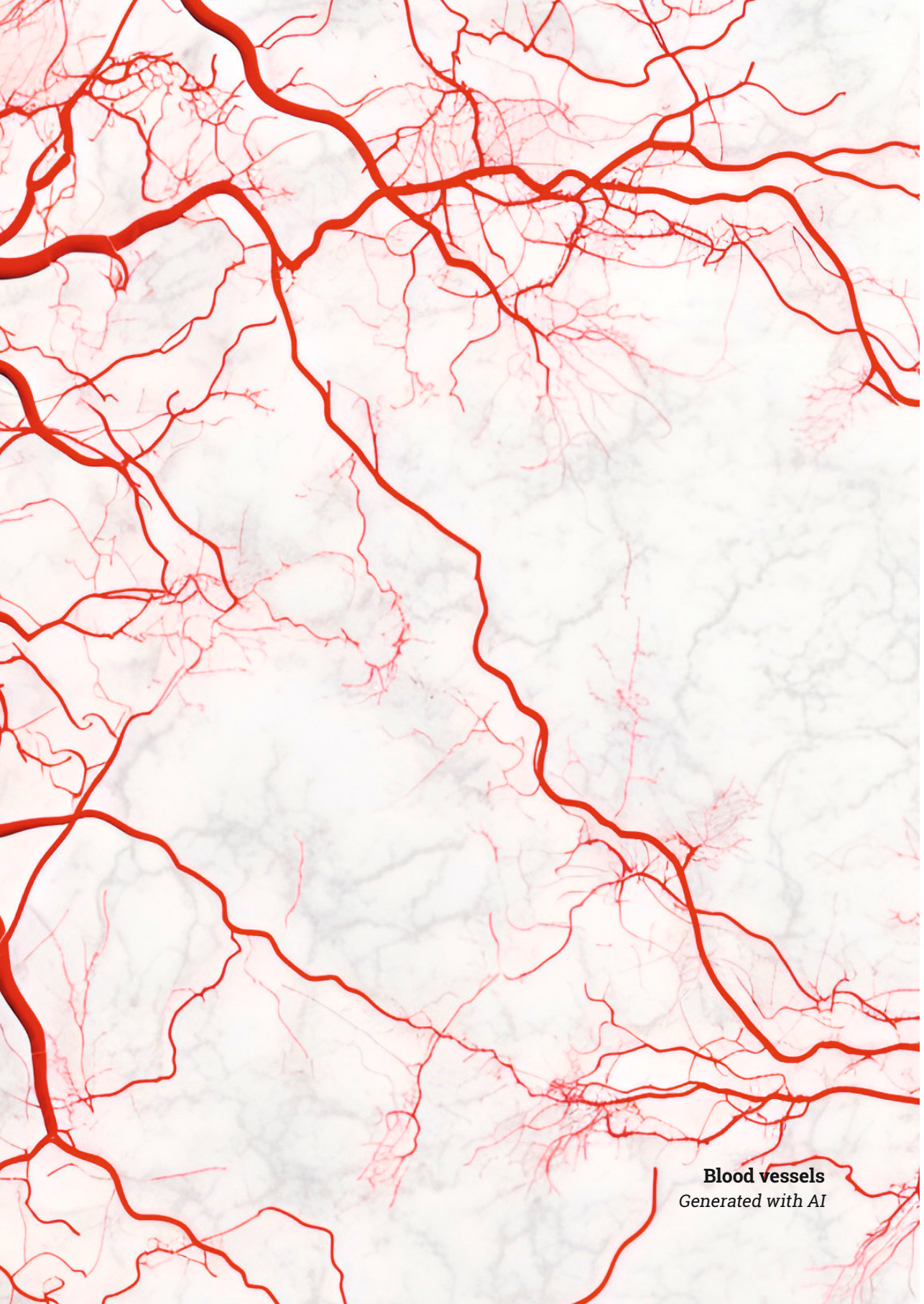

Blood vessels
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