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Tumor vasculatures: A new target for cancer immunotherapy

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Abstract

Immune cells rely on a functional vascular network to enter tissues. In solid tumors, blood vessels are abnormal and dysfunctional, so immune effector cell infiltration is impaired. Although normalizing the tumor vasculature has been shown to improve the efficacy of cancer immunotherapies, recent studies suggest that enhanced immune stimulation also, in turn, improves tumor vascular normalization. This new paradigm of immune-vessel mutual reprogramming thus opens up the possibility of identifying new cancer treatment strategies that combine vascular targeting and immunotherapies. In this Opinion, we highlight the current evidence supporting immune-vascular cross-talk and outline how this relationship can provide new rationales for developing more effective combination immunotherapy strategies for treating human cancers.

Keywords

Cancer immunotherapy; vascular normalization; immune checkpoint blockade; tumor vasculature; tumor microenvironment

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Cancer immunotherapies, including immune checkpoint blockers (ICBs), have significantly improved clinical outcomes for patients with cancer.[1–8] However, despite the prolonged overall survival in some patients treated with ICBs, their response rates remain suboptimal, so efforts are now being increased to explore additional strategies that can overcome immunosuppression mediated by tumors.[9–13] In addition to the intrinsic inhibitory signals generated by cancer cells, a growing body of evidence indicates that both structural and functional abnormalities within the tumor microenvironment also contribute greatly to its dysfunctional immune landscape.[9,10,13–16]

In this Opinion article, we highlight research showing that the morphologically and functionally abnormal tumor vasculatures produced from a dysregulated balance between pro- and anti-angiogenic signals contribute to the immune suppressive microenvironment within solid tumors. We also review recent evidence showing that, although tumor blood vessel normalization using anti-angiogenic therapy (AT) improves effector T cell infiltration and reduces immune inhibitory processes to augment the effect of cancer immunotherapies, cancer immunotherapies such as ICBs may also remodel the tumor vasculature, which in turn improves tissue perfusion. Therefore, this reciprocal interaction between tumor vascular normalization and immune activation provides opportunities for developing new combination strategies to improve the efficacy of cancer immunotherapies. Finally, we propose potential translational applications that would take advantage of ICB-induced vascular normalization effects to treat human cancers.

Tumor vasculature normalization

Tumor angiogenesis is a hallmark of cancer.[17,18] New blood vessel formation provides tumor cells with nutrition and facilitates tumor progression and metastasis.[19] It is logical, therefore, that AT would be an ideal strategy to disrupt the tumor vasculature with the goal of starving tumors to death.[20,21] However, the initial excitement surrounding AT soon met with clinical reality.[22,23] Yet, when anti-angiogenic agents such as the monoclonal antibody targeting the vascular endothelial growth factor (VEGF) were given with systemic chemotherapies, an improvement in overall survival was observed in patients;[24,25] this improvement was also observed with radiation treatment.[26] Since chemotherapies require functional blood vessels to enter the tumor parenchyma, and ionizing radiation relies on the presence of oxygen to generate free radicals to induce cytotoxic tumoricidal effects, it was unclear how destroying the very vascular network necessary for transporting these molecules would improve therapeutic efficacy. In answer to this seemingly paradoxical observation, it was hypothesized that perhaps certain ATs, instead of completely obliterating tumor vessels, induce a normalizing effect.[23,27] As the normalized tumor blood vessels regain a certain degree of their perfusion capacity, they are more efficient in transporting chemotherapies and oxygen into tumors, thus increasing the antitumor efficacy of cytotoxic treatments.[25]

Anti-angiogenic therapy-induced tumor blood vessel normalization possesses several unique characteristics: structural normalization, functional normalization, a normalization window, and dose-dependence.[28,29] Structural normalization due to anti-angiogenic treatments is characterized by morphological changes in the tumor vascular network, wherein tumor vessel diameters, density and tortuosity are drastically reduced. Functionally, vascular

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permeability is decreased with increased coverage of pericytes. This leads to improved vessel perfusion and reduced tissue hypoxia. Both structural and functional normalization of tumor vessels in the setting of AT are transient in nature and exhibit dose-dependent relationships. Administering a higher dose of anti-angiogenic agents often results in a brief normalization window. Conversely, low-dose AT tends to prolong the vascular normalization effect.[25] In preclinical models of colorectal cancer, high-dose anti-VEGF antibody treatment decreased microvessel density and increased tumor hypoxia, findings that are consistent with vascular pruning, resulting in inadequate tissue perfusion.[30] When used in lower dosages, anti-VEGFR2 antibody treatment was found to drastically improve tumor vessel perfusion and reduce tumor tissue hypoxia, compared to IgG control or high-dose anti-VEGFR2 antibody treatment in murine breast cancer models.[31] Furthermore, the more durable normalization window produced by low-dose anti-VEGFR2 antibody treatment augmented cancer immunotherapies by improving intratumoral effector T cell infiltration as well as by relieving immunosuppressive signals within the tumor microenvironment, thus providing direct evidence of a potential synergism between vascular and immune targeted therapies in cancer.[31] Although the precise dose of AT needed to generate the optimal vascular normalization effect is difficult to determine and is likely to vary across different tumor types, stages of tumor development, particular angiogenesis targets, and specific clones of antibodies used, the discovery of the vascular normalization window has resulted in the identification of therapeutic strategies that target tumor blood vessels to enhance conventional cancer treatments.

Strategies to normalize tumor vasculatures

One of the main strategies to remove the excess pro-angiogenic signals within tumors is to disrupt the signaling mediated by the interactions between angiogenic growth factors and their receptors. Genetic disruption of regulator of G-protein signaling 5 (Rgs5) expression in mice resulted in a shift in pericytes from an immature (PDGFR-β^+) to a mature (α-smooth muscle actin, αSMA$^+$; and neural/glial antigen 2, NG2$^+$) phenotype.[32] Despite a lack of changes in the overall vascular coverage by these pericytes, the phenotypic changes resulted in tumor vessel normalization and promoted an influx of immune effector cells into the tumor.[32] However, the cellular component that mediates the Rgs5 knockout (KO)-induced vascular normalization effect remains unclear. Previous studies have found that deleting Rgs5 causes both endothelial cell and macrophage apoptosis.[33] Therefore, it is possible that vascular normalization in the setting of Rgs5 deletion may be due to direct vascular regression through endothelial cell apoptosis, or it may be a secondary response to changes in perivascular cells such as pericytes or monocytes.

Beyond the classical VEGF-VEGFR axis, the angiopoietin (Ang)/Tie2 signaling pathway also plays a critical role in tumor angiogenesis. Ang1, which is primarily expressed by perivascular cells, maintains the survival and quiescence of endothelial cells in mature blood vessels.[34] Ang2, by contrast, competes with Ang1 for Tie2 binding and promotes the sprouting of tumor vessels, leading to vascular destabilization.[35] Simultaneous activation of Tie2 and blockade of Ang2 induced tumor vessel normalization and enhanced the delivery and antitumor effect of chemotherapy.[36] Targeting Tie2 and Ang2 simultaneously produced enhanced antitumor responses than Ang2 blockade alone, which was attributed to decreased

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expression of multiple pro-angiogenic factors that are often upregulated in response to Ang2 blockade-induced tissue hypoxia.[36] The need for a multi-targeted approach to elicit tumor vascular normalization was further demonstrated by dual blockade of VEGFa and Ang2 signaling, which improved tumor vessel normalization compared to VEGFa inhibition alone.[37,38] More importantly, the antitumor responses of this treatment strategy were further enhanced by adding anti-PD1 treatment, as endothelial cells release IFNγ to upregulate PD-L1 in order to promote adaptive resistance to the dual blockade.[38]

In addition to disrupting pro-angiogenic signaling, the local delivery of angiostatic factors can also induce tumor vascular normalization.[39] Intratumoral delivery of low dose TNFα stabilized the tumor vascular network, improved vessel perfusion, and, as a result, substantially enhanced cancer vaccine and adoptive T cell therapies. This was markedly different from intratumoral injection of IFNγ, which caused rapid vessel loss and impeded antitumor immunity.[39,40] The intratumoral delivery of TNF superfamily member LIGHT, using a tumor homing peptide (CGKRK), induced tumor vascular normalization.[41] Furthermore, CGKRK-LIGHT treatment induced the formation of high endothelial venules (HEVs), which are specialized structures that facilitate T cell infiltration into solid tumors, resulting in improved cancer immunotherapy responses.[41,42] These studies confirm that interventions to reduce pro-angiogenic signaling and/or to increase angiostatic factors within the tumor microenvironment can lead to vascular normalization responses that may improve the antitumor efficacies of cytotoxic or immunotherapies.

Immune-mediated tumor vessel normalization

Although immune cells are known to regulate endothelial cell functions and, thus, play an essential role in tumor angiogenesis, it was only recently found that immune cell stimulation can also promote the normalization of tumor vessels.[43,44] In addition to eliciting immune-mediated tumor cell eradication, ICB was also shown to promote tumor vascular normalization in orthotopic breast and ectopic colon tumor models.[43,44] In both studies, blockade of CTLA-4 or PD-1 reduced tumor vascular density, improved vessel perfusion, and decreased tumor tissue hypoxia, all of which are hallmarks of the vascular normalization effect.[43,44] However, because the tumor microenvironment is strongly influenced by local cellular and molecular signals, it is unclear whether the ICB-mediated vessel normalization observed in these tumor models is indicative of a universal effect. Since the therapeutic responses to ICBs themselves differ across tumor types and even across similar tumors established at different organ sites,[45] it is reasonable to suspect that the associated vascular normalization effect is also subject to such variation.

In $CD4^{-/-}$ and $CD8^{-/-}$ mouse models, it was found that blockade of CTLA-4 and PD-1 promoted tumor vessel normalization via the activation of Th1 $CD4^+$ helper T cells.[43] Another study found that $CD4^+$ T cells alone were insufficient to induce tumor vasculature remodeling effects. Instead, the depletion of $CD4^+$ T cells resulted in the accumulation of $CD8^+$ T cells and increased production of IFNγ, as well as a tumor vessel normalization response. The authors thus suggest that the vascular normalizing effect of immune checkpoint therapy is probably mediated by the activation of $CD8^+$ T cells via the IFNγ signaling pathway.[44] At the present, the precise role of $CD4^+$ and $CD8^+$ T cell in promoting

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the tumor vascular normalization effect in the setting of ICB is unclear and likely depends on the tumor type and, perhaps more importantly, on the cross-talk between immune cell subtypes and other cellular components of the tumor microenvironment. On one hand, using monoclonal antibodies to pharmacologically deplete $CD4^+$ or $CD8^+$ T cells may lead to antibody-dependent cell-mediated cytotoxicity (ADCC), which might elicit inflammatory responses that affect the tumor vasculature. On the other hand, genetic knockout of $CD4^+$ or $CD8^+$ T cells completely removes the presence of lymphocytes from the outset, thus raising the question of whether the tumorigenesis or even the angiogenesis process would be affected in such a lympho-depleted environment. The latter effect may be especially important given that lymphocyte subpopulations are differentially associated with various stages of tumor development. For example, the number of Regulatory T cells (Treg) ($CD4^+CD25^+$) was found to correlate positively with increases in tumor size. In contrast, $CD8^+$ effector T cell numbers decrease as tumors grow.[46] Depleting Tregs using a monoclonal anti-CD25 antibody synergized with anti-PD-1 therapy to inhibit tumor growth and increased IFNγ production within both $CD4^+$ and $CD8^+$ effector T cells.[47] Furthermore, using a Foxp3-LuciDTR-4 mouse model (which simultaneously expressed enhanced green fluorescent protein, luciferase, and diphtheria toxin receptor (DTR)), another study recently demonstrated that selective depletion of Tregs increases the tumor infiltration of $CD11b^+Gr1^{lo}F4/80^+Siglec\text{-}F^+$ eosinophils, which prompts the activation of $CD8^+$ T cells and the normalization of tumor blood vessels.[48] Simultaneous depletion of eosinophils and Tregs abrogates $CD8^+$ T cell activation and vessel normalization, suggesting that $CD8^+$ T cells mediate vessel normalization upon Treg depletion.[48] Therefore, how $CD4^+$ and $CD8^+$ T cells induce vascular normalization responses may depend on context and may rely on coordinating with immune cell types within the tumor microenvironment.

The functional changes within the tumor vascular network from an immune-mediated normalization effect can be monitored by analyzing alterations in tumor blood vessel perfusion through noninvasive imaging techniques. Improved vessel perfusion (IVP) was found mainly in tumors sensitive to ICB, but not in resistant ones.[44] More importantly, increased vessel perfusion appears to positively correlate with a tumor's responsiveness to ICBs.[44] This enables quantification of global IVP using noninvasive imaging to predict the potential therapeutic efficacy of immune checkpoint blockers, even before changes in tumor size become evident.[44] Since noninvasive radiologic methods could be used to monitor vascular perfusion in real time, it is conceivable that IVP measurement could be incorporated into current cancer immunotherapy practice to achieve genuinely personalized cancer immunotherapies to benefit patients.[9]

These newly discovered functions of cancer immunotherapies, including ICBs, exert effects beyond immune cells and act on non-immune cells within the tumor microenvironment, thus providing a strong rationale to explore their combination with other treatment modalities that target these cell populations. For example, given that AT's inhibition of vascular growth factors VEGF and ANG2 also induces adaptive resistance in endothelial cells by upregulating PD-L1,[38] it makes logical sense to combine anti-angiogenic therapy with immunotherapy to achieve the optimal antitumor effect.[49,50] In fact, multiple clinical trials have been initiated to investigate whether combining the two treatment strategies can improve clinical responses.

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Combining vascular and cancer immunotherapy

The increasing evidence supporting a reciprocal interaction between immunotherapy and tumor vasculature suggests that combining anti-angiogenic agents with ICBs may improve clinical efficacy by creating positive feedback loops through which the therapies reinforce each other.[9,51,52] When administered in the right doses,[28] AT could reverse the immune suppressive tumor microenvironment by normalizing tumor blood vessels, which would reduce tissue hypoxia and acidosis and increase the infiltration of effector T cells.[9] The increased infiltration and decreased immune suppressive signals would further augment ICBs to enhance effector T cell activation.[53,54] These activated T cells not only kill tumor cells but also further contribute to modulating and normalizing tumor blood vessels, thus creating a positive reinforcement loop that leads to tumor regression.[9] Meanwhile, anti-angiogenic agents may also directly or indirectly reduce immune suppressive cell populations within the tumor microenvironment. For example, low-dose anti-VEGFR2 therapy, but not high-dose therapy, promoted the polarization of tumor-associated macrophages toward the immune-stimulatory M1 phenotype and increased tumor infiltration of $CD4^+$ and $CD8^+$ T cells.[31] In another study, inhibition of angiogenesis in a murine glioblastoma model promoted macrophage polarization and prolonged survival.[37] In addition to polarizing tumor-associated macrophages, AT may also reduce Treg-mediated immune suppression. Treg cells have been found to express multiple receptors for VEGF, including Neurophilin-1, a co-receptor for VEGF with Flt-1 (VEGFR 1) and KDR (VEGFR2).[55–58] Blockading VEGF using a monoclonal anti-VEGFa antibody significantly decreased the number of Treg cells in CT26 colorectal cancer.[59] Therefore, whether through more efficient recruitment and activation of effector T cells or via decreased immune inhibition mediated by suppressor cells, anti-angiogenic treatment appears to promote immunologically favorable effects in the tumor microenvironment.

Recent preclinical studies have also demonstrated a potential synergy between anti-angiogenic agents and ICB.[38,42] Currently, a number of ongoing clinical trials are testing the combination of anti-angiogenic agents and ICBs in solid tumors (Table 1). However, questions still remain regarding the best regimen for administering the two modalities together. Based on lessons learned from anti-angiogenic agents and chemotherapies, the dose and timing of these agents' administration will probably be key to their potential success. Although AT may provide an initial normalization effect and decrease immune suppressive signals within the tumor microenvironment, excessive inhibition may lead to vessel regression, resulting in impeded drug delivery and lymphocyte infiltration into the tumor. As phase I trials are often designed to identify the dose limiting toxicities and maximal tolerated doses of the drug combination, they may not reflect the doses needed to generate optimal antitumor responses. Furthermore, effective dosages of anti-angiogenic agents for normalizing tumor vasculatures may depend on additional factors, such as tumor size, vascularity, and expression levels of pro-angiogenic growth factors. The complexities governing the interplay between anti-angiogenic therapy and immunotherapy thus highlight the importance of identifying potential biomarkers that can predict treatment responses. Serum-based biomarkers reflecting the functional status of tumor blood vessels have been used to monitor responses to anti-angiogenic agents.[60,61] Some of these biomarkers have

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already demonstrated potential to predict cancer immunotherapy responses.[62,63] Therefore, whether they can be combined with immune biomarkers to serve as predictors for combination anti-angiogenic and immunotherapy remains to be seen.

In addition to antitumor responses, combining AT with ICBs may increase the risk of adverse events over either treatment alone. Based on early clinical trial results, ICBs combined with AT appeared to be safe but are associated with higher incidences of acute Grade 3 or above toxicities in patients compared to either monotherapies (Table 2). The most common adverse reactions from the combination treatment include hematological and liver function abnormalities. Larger populations and longer follow-up are needed to better assess the potential long-term side effects associated with combined AT and ICB treatment in patients.

Neoadjuvant cancer immunotherapy: A new path forward?

The tumor vascular normalization effect of ICBs also provides a strong rationale for its use in the neoadjuvant setting, where it can prime the tumor microenvironment to improve the efficacy of subsequent definitive treatment. Encouraging results have already been reported on the use of neoadjuvant immune checkpoint inhibitors for resectable melanoma and non-small cell lung cancers,[68–71] as well as in more traditionally immune-resistant tumors such as glioblastoma.[72,73] However, most of these studies investigated whether immunotherapy can serve as a more effective alternative to chemotherapy in the neoadjuvant setting to improve surgical resection and pathological complete response rate. Through normalization of the tumor vasculature, the improved tissue perfusion is often accompanied by reductions in hypoxia, acidosis, and interstitial fluid pressure within the tumor microenvironment, all of which help to improve oxygenation and drug delivery into tumors (Figure 2).[9] These pathophysiological changes in the setting of tumor microenvironmental normalization thus make neoadjuvant cancer immunotherapy a potential option to complement existing treatment strategies, such as chemotherapy, targeted therapy and radiation, or emerging modalities like photodynamic therapy, which relies on generating reactive oxygen species to mediate its tumoricidal effect.[74] While the prospect of these combination therapies seems exciting, many questions need to be addressed before clinical translation can occur. The first question pertains to the kinetics of the immune-mediated vascular normalization effect and its duration. The immune-mediated response seems to be more durable than AT-induced transient vascular normalization and, thus, may provide more flexibility in terms of administering combination therapy with other agents. Second, whether vasculature normalization occurs in all tumors that respond to ICBs remains unclear. Finally, the optimum dosing of ICBs to induce tumor vascular normalization is unknown. In the setting of AT, a lower but more persistent dosing regimen of anti-VEGFR agent was found to be more potent in promoting the tumor vascular normalization effect.[23] However, whether this holds true for immune-mediated tumor vascular normalization is unclear, since AT induces vascular changes by directly interacting with blood vessel endothelial cells, while the immune-mediated vascular effect is secondary to IFNγ activities in the setting of T cell activation.[43,44] Regardless, since cancer immunotherapies such as ICBs are increasingly being used in combination with existing treatment modalities, both concurrently or as

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neoadjuvants, addressing these questions would undoubtedly help to identify the best combination regimen and delivery strategies to elicit the most potent antitumor responses.

Future outlook and concluding remarks

The aberrant tumor vascular network has long been established as a major contributor to the immune suppressive microenvironment in solid tumors.[9,75] Therefore, it was not surprising to observe that the normalization of tumor blood vessels can improve cancer immunotherapies. However, the fact that cancer immunotherapy, including ICBs, can also normalize tumor vasculatures through IFNγ produced by activated T cells suggests that immune-vascular cross-talk, at least in the context of tumor immunology, plays a critical role in tumor stromal invasion, immune evasion, and perhaps metastatic spread. At this point, it remains incompletely understood how IFNγ induces tumor vascular normalization, although previous evidence suggests that it is probably mediated via interactions with its receptor expressed on endothelial cells.[40] Similarly, whether this immune-induced vascular normalization effect can also be observed in other IFNγ-producing immunotherapies, such as CAR T cells or oncolytic viruses, is unclear. Moreover, simultaneous blockade of two or more immune checkpoint molecules has been tested in the clinic.[76,77] Although in some instances, targeting multiple immune checkpoints may improve antitumor effects, it is unclear whether this enhances tumor vessel normalization. Furthermore, the heightened T cell responses due to blockade of multiple immune checkpoints may in fact cause vessel regression, as seen in high-dose AT.[31] Therefore, how to optimally induce tumor vessel normalization using cancer immunotherapy warrants further research. Nevertheless, the vascular remodeling effect of immunotherapy further broadens its potential oncological applications and provides new rationale for developing combination immunotherapy strategies with conventional cytotoxic treatment for cancer patients.

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Glossary

Angiopoietin (ANG)
A family of vascular growth factors that play critical roles in angiogenesis.

Antigens
Short sequences of peptides produced from digested proteins that are presented on the cell surface by major histocompatibility complex (MHC).

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4)
Also known as CD152, is a protein receptor that functions as an immune checkpoint. It binds to CD80 or CD86 to downregulate T cell responses.

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IFNγ
Also called type II interferon, is a cytokine that is critical for mediating immune responses against a number of pathogens as well as transformed cells.

Interstitial fluid pressure (IFP)
The pressure exerted by the free interstitial fluid, which helps to determine transcapillary flow. IFP is often elevated in tumors.

Immune checkpoints
A collection of inhibitory pathways within the immune system that maintain self-tolerance by modulating the duration and amplitude of physiological immune responses.

Neoadjuvant therapy
The administration of therapeutic agents before a main treatment, which is usually a more definitive local therapy such as surgery, radiation, or chemoradiation.

PD-1 (Programmed cell death protein 1)
Also known as CD279, is a cell surface protein that suppresses T cell inflammatory activity when binding to its ligands, such as PD-L1.

Vascular normalization
Structural and functional changes within abnormal and dysfunctional tumor blood vessels that make them more closely resemble normal vasculatures.

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Highlights:

- Tumor vascular normalization via angiogenic blockade enhances immune effector cell infiltration and decreases immune suppressive signals within the tumor microenvironment, improving the efficacy of cancer immunotherapies.
- Cancer immunotherapies, such as immune checkpoint inhibitors, can also promote tumor vascular normalization via stimulating γ-interferon release from immune effector cells.
- This immune-vascular cross-talk offers unique opportunities to develop new combination strategies to improve the effectiveness of cancer immunotherapies.
- A deeper insight into the underlying mechanisms governing immune-mediated tumor vessel normalization may guide future clinical trials of combined vascular and immunotherapies for patients with cancer.

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Outstanding questions:

- Does the tumor vascular normalization effect in the setting of ICB extend to other cancer immunotherapies such as CAR T cell or tumor vaccines?
- What is the precise mechanism of γ-interferon–mediated tumor vascular normalization? Is this a direct (via endothelial cells) or indirect (via other cells) effect?
- What are the differences in the nature of immunotherapy-mediated and AT-mediated tumor vasculature normalization effects?
- Is there an immune-mediated mechanism in the development of AT resistance in solid tumors?
- Can immunotherapy-mediated vessel normalization serve as a reliable biomarker for cancer immunotherapy response?
- How will the effect of immunotherapy-mediated tumor vascular normalization differ for different tumors or tumor stages?

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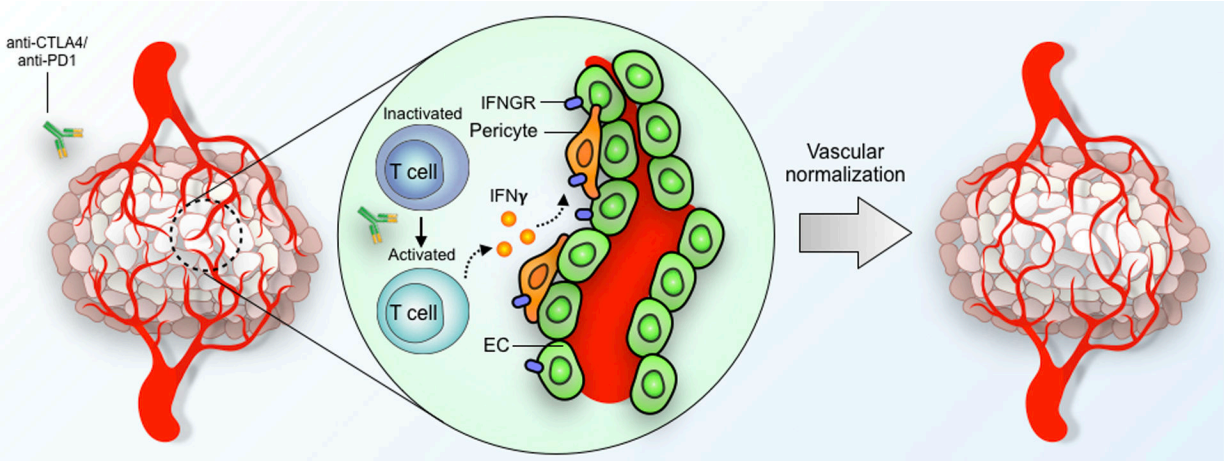

Figure 1. Potential mechanism of immunotherapy-mediated tumor vascular normalization.

Activation of effector T cells in the setting of ICB results in production and secretion of IFNγ, which, through interaction with IFNγ receptors (IFNGR) expressed on vascular cells, results in normalization of the tumor vasculature. EC = endothelial cell.

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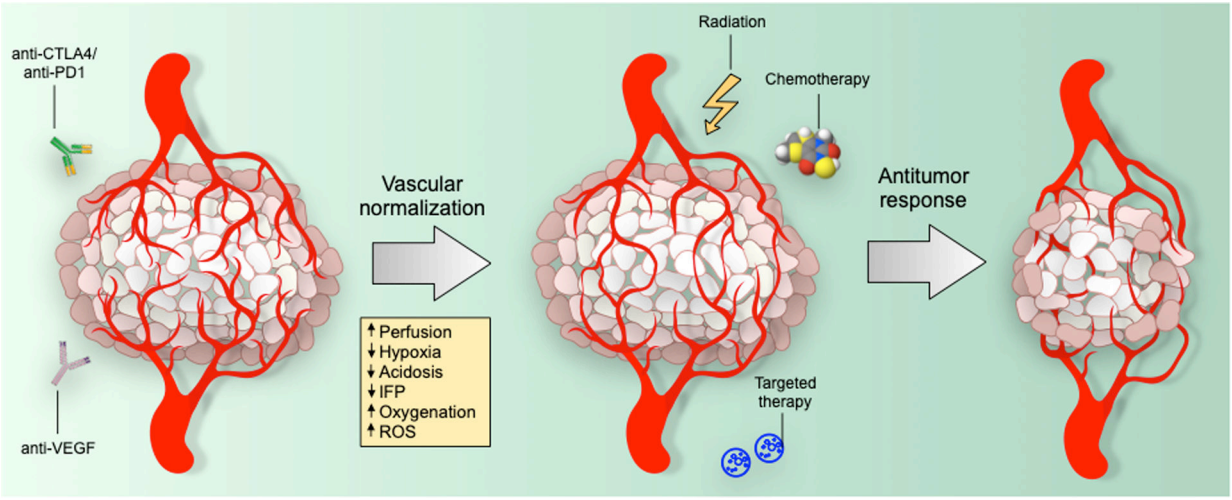

Figure 2: Immune or AT-mediated tumor vascular normalization improves tissue perfusion, leading to a decrease in tumor hypoxia and acidosis.

The reduced vascular permeability and leakiness also decreases interstitial fluid pressure (IFP) and thus helps to facilitate intratumoral delivery of chemo or targeted therapeutic agents. The increased oxygenation also enhances the production of reactive oxygen species (ROS), which further sensitizes solid tumors to definitive local treatments, such as targeted therapy or chemotherapy, as well as to ionizing radiation.

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Table 1:

Ongoing clinical trials investigating ICB in combination with AT for cancer treatments.

Trial ID	Phase	Immunotherapy	Anti-angiogenic therapy	Disease
NCT02982694	II	Atezolizumab	Bevacizumab	Advanced CRC
NCT03074513	II	Atezolizumab	Bevacizumab	Several solid tumors
NCT02724878	II	Atezolizumab	Bevacizumab	Advanced non-clear-cell RCC
NCT02921269	II	Atezolizumab	Bevacizumab	Cervical cancer
NCT02997228	III	Atezolizumab	Bevacizumab	CRC
NCT01984242	II	Atezolizumab	Bevacizumab	Advanced RCC
NCT02420821	III	Atezolizumab	Bevacizumab	Advanced RCC
NCT03133390	II	Atezolizumab	Bevacizumab	Metastatic urothelial carcinoma
NCT02659384	II	Atezolizumab	Bevacizumab	Ovarian cancer
NCT01688206	I	Atezolizumab	Vanucizumab	Epithelial ovarian, fallopian tube or primary peritoneal cancer
NCT03170960	I/II	Atezolizumab	Cabozantinib	Several solid tumors
NCT02493751	I	Avelumab	Axitinib	Advanced RCC
NCT02684006	III	Avelumab	Axitinib	Advanced RCC
NCT02572687	I	Durvalumab	Ramucirumab	Several solid tumors
NCT02336165	II	Durvalumab	Bevacizumab	GBM
NCT02496208	I	Ipilimumab + nivolumab	Cabozantinib	Metastatic genitourinary cancer
NCT00790010	I	Ipilimumab	Bevacizumab	Melanoma
NCT01950390	II	Ipilimumab	Bevacizumab	Melanoma
NCT02210117	I	Nivolumab, ipilimumab	Bevacizumab	RCC
NCT02873962	II	Nivolumab	Bevacizumab	Relapsed ovarian, fallopian tube or peritoneal cancer
NCT02999295	I/II	Nivolumab	Ramucirumab	Gastric cancer
NCT02576509	III	Nivolumab	Sorafenib	Advanced HCC
NCT03172754	I/II	Nivolumab	Axitinib	Advanced RCC
NCT02681549	II	Pembrolizumab	Bevacizumab	Melanoma or NSCLC brain metastasis
NCT02337491	II	Pembrolizumab	Bevacizumab	GBM
NCT02348008	I/II	Pembrolizumab	Bevacizumab	Clear cell RCC
NCT02853318	II	Pembrolizumab	Bevacizumab	Recurrent ovarian, fallopian tube, or primary peritoneal cancer
NCT02856425	I	Pembrolizumab	Nintedanib	Several solid tumors
NCT02133742	I	Pembrolizumab	Axitinib	Metastatic RCC
NCT02853331	III	Pembrolizumab	Axitinib	Metastatic RCC
NCT02636725	II	Pembrolizumab	Axitinib	Sarcoma
NCT02443324	I	Pembrolizumab	Ramucirumab	Several solid tumors
NCT02014636	I/II	Pembrolizumab	Pazopanib	Advanced RCC
NCT02298959	I	Pembrolizumab	Aflibercept	Advanced tumors
NCT02501096	I/II	Pembrolizumab	Lenvatinib	Several solid tumors
NCT03006887	I	Pembrolizumab	Lenvatinib	Several solid tumors
NCT02811861	III	Pembrolizumab	Lenvatinib	Advanced RCC
NCT02722954	I	Pembrolizumab	Demcizumab	Metastatic solid tumors

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Trial ID	Phase	Immunotherapy	Anti-angiogenic therapy	Disease
NCT03603756	II	SHR-1210	Apatinib	Esophageal squamous cell carcinoma
NCT02039674	I/II	Pembrolizumab	Bevacizumab	NSCLC
NCT03424005	I/II	Atezolizumab	Bevacizumab	TNBC
NCT03555149	I/II	Atezolizumab	Bevacizumab	Metastatic CRC
NCT03280563	I/II	Atezolizumab	Bevacizumab	Breast neoplasms
NCT03414983	II/III	Nivolumab	Bevacizumab	Metastatic CRC
NCT03193190	I/II	Atezolizumab	Bevacizumab	Pancreatic adenocarcinoma
NCT03424005	I/II	Atezolizumab	Bevacizumab	TNBC
NCT03175432	II	Atezolizumab	Bevacizumab	Melanoma and other malignant neoplasms of skin

CRC, colorectal cancer; RCC, renal cell carcinoma; GBM, glioblastoma; NSCLC, non-small cell lung cancer; TNBC, triple negative breast cancer.

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Table 2:

Incidence of Treatment-Related Adverse Events when combining AT with immunotherapy.

Trial ID	Phase	Immunotherapy	Anti-angiogenic therapy	Disease	Toxicity	Adverse events[64–67]
NCT02366143	III	Atezolizumab	Bevacizumab	NSCLC	Combined Grade≥3 58.5% Bevacizumab Grade≥3 50%	Hematological 41.8% Hypertension 6.4% Hematological 35.7% Hypertension 6.3%
NCT0308304	Ia/Ib	Anti-PD1 antibody	Apatinib	HCC、EC/ EGJC	Ia Grade 3 26.7% Ib Grade3 60.6%	Biochemistry 6.97% Hypertension 20% Hypertension 15.2% Liver function 15.2%
NCT02853331	III	Pembrolizumab	Axitinib/ sunitinib	Advanced RCC	Avelumab plus Axitinib Grade≥3 75.8% Sunitinib Grade≥3 70.6%	Liver function 20.3% Hypertension 22.1% Diarrhea 9.1% Liver function 5.5% Hypertension 19.3% Fatigue 6.6% Hematological 29.4%
NCT02684006	Ib	Avelumab	Axitinib/ sunitinib	Advanced RCC	Pembrolizumab–Axitinib Grade≥3 71.2% Sunitinib Grade≥3 71.5%	Diarrhea 6.7% Hypertension 19.3% Liver function 9.9% Hypertension 17.1% Liver function 5.7% Hematological 33.1%

NSCLC, non-small cell lung cancer; EC, esophageal cancer; EGJC, esophageal-gastric junction cancer; RCC, renal cell carcinoma.