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Improving immune-vascular crosstalkfor cancer immunotherapy

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Abstract

The vasculature of tumours is highly abnormal and dysfunctional. Consequently, immune effector cells have an impaired ability to penetrate into solid tumours and often exhibit compromised functions. Normalization of the tumour vasculature can enhance tissue perfusion and improve immune effector cell infiltration, leading to immunotherapy potentiation. However, recent studies, have demonstrated that stimulation of immune cell functions can also help to normalize tumour vessels. In this Opinion article, we propose that the reciprocal regulation between tumour vascular normalization and immune reprogramming forms a reinforcing loop that reconditions the tumour immune microenvironment to induce durable antitumour immunity. A deeper understanding of these pathways could pave the way for identifying new biomarkers and developing more effective combination treatment strategies for patients with cancer.

Introduction

The recent clinical successes of cancer immunotherapies such as immune-checkpoint blockade have reaffirmed the importance of the host immune system in preventing and

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Author contributions

Y.H. and W.J. conceived the study. Y.H. and W.J. performed the literature search. Y.H., B.Y.S.K. and W.J. designed and generated the figures. All authors helped to write the manuscript.

Competing interests

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eliminating cancer[1–4]. In contrast to conventional cytotoxic agents, which directly target cancer cells, a major goal of cancer immunotherapy is to alleviate tumour-associated suppression of anticancer immune responses. A significant portion of cancer immunotherapy research over the past decade has focused on heightening the functions of effector T cells, which play a direct role in recognizing tumour-associated antigens and in mediating tumouricidal responses[5]. In this context, therapeutic cancer vaccines that are based on tumour antigens, or engineered chimeric antigen receptor (CAR) T cell therapies both rely on the formation of antigen-specific T lymphocyte clones that can recognize and eradicate tumours harboring a particular antigen or a mutant protein. Both approaches have shown promise in preclinical studies and early-phase clinical trials; in particular, CAR T cell therapy has demonstrated potential as a treatment for hematologic malignancies[6–12] On the other hand, immune checkpoint inhibitors target inhibitory ligand–receptor interactions between T cells and immune suppressor cells within the tumor microenvironment (TME), in particularly those mediated by tumour cells[13]. Ipilimumab, a monoclonal antibody that targets the cytotoxic T lymphocyte associated protein-4 (CTLA4) and antibodies blocking the programmed cell death-1 (PD1)/programmed cell death ligand-1 (PDL1, also known as B7-H1) axis, have led to remarkable clinical responses in several types of metastatic cancer, for example melanoma[14], non-small cell lung cancer (NSCLC)[15,16], Merkel cell carcinoma[17], and renal cell carcinoma[18].

In this Opinion article, we highlight emerging evidence that cancer immunotherapies, such as immune checkpoint blockade, can promote T lymphocyte activation beyond modulating the stimulatory–inhibitory axis and this activation may extend to normalize the immunosuppressive TME. We propose that a reciprocal regulation between immune cells and the tumour vasculature is critical in dictating the antitumour efficacy of cancer immunotherapy and forms the basis of a larger interacting network consisting of tumour vascular remodelling, metabolic homeostasis, and immune reprogramming. Together, these elements establish a positive feedback loop, in which changes in one will reinforce the effect of others. Understanding the mutual regulation among these processes and their collective effects on promoting antitumour immunity is crucial for elucidating the mechanisms of tumour immune evasion and for developing effective combinational cancer immunotherapies.

The aberrant tumour vasculature

The stimulation of local and systemic antitumour immune responses is essential for successful cancer immunotherapy[19]. For activated immune cells to eradicate cancer cells, they first need to penetrate deep into the tumour parenchyma and identify cancer cells as their intended targets. Once inside the tumour, immune cells also need to overcome many of the immune suppressive mechanisms within the TME[20–22].

Effect on immune cell infiltration

Immune cells, like nutrients or oxygen, rely on a functional vascular network to enter tissues[23]. The hypoxic environment within solid tumours induces the continued production of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), transforming

growth factor-β (TGFβ), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF)[24–26]. This results in an imbalance between the levels of pro-angiogenic and anti-angiogenic factors, which are tightly regulated in healthy tissues, and promotes rapid but aberrant tumour blood vessel formation[24,27,28]. Morphologically, tumour blood vessels are tortuous, dilated, and unevenly distributed, with adjacent endothelial cells being loosely attached to one another. Pericytes, which surround the blood vessels and regulate vascular permeability, are usually detached from the endothelial cells, resulting in leaky tumour blood vessels that are characterized by dysfunctional flow characteristics[22,28]. Tumour-associated endothelial cells also express lower levels of cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM1) and intercellular adhesion molecule-1 (ICAM1), which promotes endothelial anergy and reduces the trafficking of immune effector cells into tumours[29–32]. Together, the structural and functional abnormalities of tumour blood vessels decreases the recruitment of immune effector cells, thus limiting the effectiveness of cancer immunotherapies (Fig 1). Strategies to convert a “cold” tumour that is devoid of immune effector cells into a “hot” tumour by increasing tumour infiltration of T lymphocytes is an active area of research, which aims to enhance the effectiveness of cancer immunotherapies[33–35]. It is not surprising that poorly vascularized tumours, for example pancreatic adenocarcinoma, which is densely packed with fibrous stroma with a sparse immune cell presence, are often highly resistant to cancer immunotherapies[36].

Effect on Tumour Immune Microenvironment

In addition to having direct effects on immune cell adhesion and extravasation, the abnormal tumour vasculature also indirectly antagonizes the effectiveness of cancer immunotherapy by promoting TME-mediated immune suppression. The impaired perfusion capacity of tumour blood vessels helps to create a highly hypoxic TME[22,25]. Hypoxia contributes to immunosuppression via several mechanisms (regulation of immunity by hypoxia is Reviewed in ref 37). First, hypoxia promotes the accumulation of myeloid-derived suppressor cells (MDSCs) and facilitates the differentiation and polarization of tumour-associated macrophages (TAMs) into an immunosuppressive M2-like phenotype[38–40]. Second, hypoxia indirectly increases the accumulation of regulatory T cells (T_{regs}) within the TME by upregulating expression of the chemoattractant chemokines (C-C motif) ligand 22 (CCL22) and CCL28 on tumour cells and TAMs[41,42]. Furthermore, hypoxia promotes resident immune suppressor cells within the TME to secrete immunosuppressive factors such as VEGF, TGFβ, and IL-10[22,41–44]. Hypoxia also induces the expression of immune checkpoint molecules such as PDL1 on tumour cells[45] as well as PDL1, T cell immunoglobulin and mucin-domain containing-3 (TIM-3) and CTLA4 on TAMs, MDSCs, and T_{regs}. Moreover, through VEGF, hypoxia can indirectly upregulate PD1 expression on $CD8^+$ T lymphocytes, thus further inhibiting immune effector cell activation and function (Fig 1)[46–51]. Last, hypoxia along with tumour cell necrosis, increase the extracellular concentrations of the immune-inhibitory metabolites adenosine and lactate.[52–55] The accumulation of lactate further leads to metabolic lactic acidosis (a low pH in the blood due to build up of lactic acid) and results in impaired cytotoxic T cell functions by interfering with T cell receptor (TCR)–triggered production of interferon-γ (IFNγ)[56]. These processes act together to inhibit effector immune cell maturation and promote T cell anergy and exhaustion (Fig 1). Therefore, dysfunctional tumour vessels have a major role in

contributing to the immunosuppressive nature of the TME and countering the effect of cancer immunotherapies.

Tumour vascular normalization

Given that the abnormal tumour vasculature promotes immune suppression within the TME, strategies that normalize these aberrant blood vessels may therefore restore immune cell functions and facilitate their antitumour activities. Although initially proposed as a way to improve the delivery of systemic drugs into tumours[27,57,58], the process of vascular normalization was recently shown to potentiate cancer immunotherapy, by promoting immune cell infiltration into tumours and reducing the immune-suppressive elements within the TME.

Genetically induced vascular normalization improves immunotherapy

Genetic disruption of regulator of G-protein signaling 5 (*Rgs5*) expression in mice promoted immature PDGFRβ$^+$ pericytes to become mature αSMA$^+$NG2$^+$ pericytes without affecting their overall coverage of the vasculature. This phenotypic change within tumour vessels consequently reduced tumour tissue hypoxia and vascular leakiness, leading to an influx of immune effector cells into the tumour parenchyma[59]. This study attributed the reduction in intratumoural pressure and hypoxia due to pericyte maturation as the main reasons for the increased T cell entry. However, vasculature normalization also results in an increased and more uniform distribution of adhesion molecules on the luminal surface of endothelial cells lining the tumour blood vessels, thus allowing more efficient immune cell docking and rolling, both of which facilitate immune cell infiltration into tumours[60].

Therapeutically induced vascular normalization improves immunotherapy

Beyond genetic manipulations, the therapeutic blockade of proangiogenic factors can also normalize tumour vasculature and improve cancer immunotherapies. Traditional high-dose antiangiogenic therapy destroys tumour vessels, leading to further hypoxia and inhibition of immune cell recruitment. However, appropriate low-dose antiangiogenic therapy against VEGF/VEGFR was found to induce tumour vascular normalization, reduce hypoxia, facilitate tumour infiltration of CD8$^+$ T lymphocytes, and potentiate cancer immunotherapy[61–65]. The importance of hypoxia in promoting immune suppression within solid tumours was further illustrated by a recent study demonstrating that respiratory hyperoxia induced by breathing high concentration (60%) of oxygen alone could convert the TME from an immunosuppressive to an immunosupportive phenotype, by decreasing intratumoural hypoxia and concentrations of extracellular adenosine[66]. As a result, respiratory hyperoxia resulted in the regression of spontaneously developed tumours in a T cell- and natural killer (NK) cell-dependent manner[66].

Although earlier studies of tumour vascular normalization mostly focused on disruption of the VEGF–VEGFR axis, recent studies have revealed the angiopoietin (Ang) –Tie2 signalling pathway as a promising new target for normalizing tumour vessels[67]. Simultaneous blockade of angiopoietin-2 (Ang2) and activation of Tie2 signalling normalized tumour vessels and induced favorable immunological alterations within the

TME[57]. Moreover, dual targeting of Ang2 and VEGF signaling pathways often results in improved vascular normalization relative to VEGF inhibition alone[68,69] and in various tumour models enhanced the effects of PD1 blockade[70]. The vascular normalization effect of dual Ang2 and VEGF blockade was associated with more efficient lymphocyte priming by antigen-presenting cells, TAM polarization to an M1-like phenotype, and accumulation of activated, IFNγ-expressing $CD8^+$ T cells within the perivascular space[70,71]. Although depletion of $CD8^+$ T cells completely eliminated the antitumor effects of Ang2 and VEGF blockade, it remains to be seen whether the absence of $CD8^+$ T cells will also abrogate the accompanied vascular normalization effect.[69]. Interestingly, the dual blockade of Ang2 and VEGF upregulated PDL1 expression in tumor endothelial cells via IFNγ[70]. This observation raises the possibility that resistance to antiangiogenic therapy may arise, at least in part, due to the development of adaptive immune suppressive processes within tumours[70,72]. Furthermore, IFNγ can induce both PDL1-dependent and PDL1-independent resistance within tumours in the setting of immune checkpoint blockade[73]. Together, these findings further highlight the intricate relationship between immune cells and tumour blood vessels, and provide new rationales for combining antiangiogenic treatments with immunotherapies.

Activated eosinophils promote tumour vascular normalization

In addition to blocking proangiogenic factors, a recent study demonstrated that activated $CD11b^+Gr1^{lo}F4/80^+Siglec\text{-}F^+$ eosinophils can also promote the normalization of tumour vessels, which subsequently help to mediate tumour rejection by $CD8^+$ T cells[74]. The exact mechanism by which the pro-angiogenic eosinophils induce vessel normalization is unclear, but it may be that they act through the polarization of TAMs into the M1-like phenotype via eosinophil-derived IFNγ and tumour necrosis factor (TNF) signalling, resulting in decreased VEGF production. The normalized blood vessels improve T cell infiltration, which results in a positive feedback loop that facilitate further M1-like macrophage polarization, vessel normalization, and VCAM1 expression on endothelial cells to promote more efficient T cell entry.[74] Together, these studies confirm that vascular normalization has an immune supportive role in enhancing antitumour immunity through a multitude of mechanisms, which include: reducing tumour tissue hypoxia, improving the access of tumour-infiltrating T lymphocytes to tumour cells, and polarizing immune suppressive cells toward immune stimulatory phenotypes.

Immune-vascular crosstalk

The interplay between tumour blood vessel remodelling and tumour immune microenvironment reprogramming has led to studies that examined the effect of vascular normalization on immune checkpoint blockade and vice versa[70,72,75]. In $CD4^+$ T cell-deficient mice, pericyte coverage of blood vessels was reduced and tumour tissue hypoxia was increased in breast tumour models, thus indicating that a lack of $CD4^+$ T cells causes vascular abnormalities[75]. Treatment with dual anti-CTLA4 and anti-PD1 therapy, which has been thought to mainly affect T cells[76–81], induced tumour vessel normalization[75]. Although it is well known that immune cell populations possess anti-angiogenic and/or pro-angiogenic activities[82–84], the results of that study suggest that the antitumor effects of immune checkpoint blockade may also stem from its ability to remodel the tumour vasculature[75].

With this new understanding, it is now conceivable that cancer immunotherapies, such as immune checkpoint blockade, may exert effects beyond immune cells and act on non-immune cells within the stromal microenvironment to indirectly enhance their antitumour activities.

A feedback loop of vascular normalization and immune reprogramming

This unexpected action of immune checkpoint inhibitors thus completes a positive feedback loop, in which immune checkpoint inhibitors activate T cells to normalize tumour blood vessels, resulting in the polarization of the immunosuppressive TME into an immune-supportive environment. This in turn facilitates the expansion and improves the functions of intratumoural effector T cells, thus lead to more vascular and TME remodelling, which ultimately produces long-term tumour control (Fig 2). Thus, vascular normalization in the setting of immune stimulation represents a novel mechanism for the antitumor effects of immune checkpoint blockade and provides a new understanding of tumour vascular remodelling and immune reprogramming. Although these findings are exciting, the properties of immunotherapy-induced tumour vascular normalization require further characterization and validation in clinical settings. For example, the conditions required for immunotherapy-induced vascular normalization, the duration of the response, and the differences from antiangiogenic therapy-mediated vascular normalization remain unclear. In addition, the distinct effects of $CD4^+$ and $CD8^+$ T cells populations on tumour blood vessels in the setting of immune checkpoint blockade need further analysis. Thus far, the role of $CD4^+$ T cells on tumour vessels was elucidated through genetic manipulation or antibody-mediated depletion, in which tumour implantation occurred in the absence of $CD4^+$ T cells[75]. It is well known that tissue hypoxia increases as a tumour grows[28], resulting in TME changes that increase T_{reg} population within the tumour[41,42,52]. Several elegant studies have demonstrated that T_{regs} can promote tumor angiogenesis and that T_{reg} depletion activates $CD8^+$ T cells and induces vascular normalization[42,74]. Therefore, the tumour vascular normalization effect of immune checkpoint blockade is likely a dynamic process that involves different immune cell populations at different stages of tumour development.

Molecular mechanisms of the feedback loop

At the molecular level, the interconnection between angiogenesis and immune activation relies on dual-functional signalling of many proangiogenic factors and immune regulator proteins. Previous studies have demonstrated that proangiogenic factors are usually immunosuppressive, whereas immune effector cells and antitumour cytokines possess angiostatic effects. For example, potent angiogenic factors (for example, VEGF, which is secreted by tumour and stromal cells) can interfere with both the maturation of dendritic cells from $CD34^+$ precursors, and the development of T lymphocytes[85–88]. By contrast, major mediators of antitumour immune responses such as IFNγ, the CXC-chemokine ligands (CXCL) 9 and 10, and TNF also inhibit tumour angiogenesis[89–93]. Moreover, IFNγ is a major mediator of antitumor immunity with robust antiangiogenic activity[3,83,84,93]. A recent study induced IFNγ expression in murine fibrosarcoma and adenocarcinoma cells and selectively expressed IFNγ receptor in specific stromal cell types, including myeloid cells, fibroblasts, T cells, and endothelial cells; this study found that IFNγ directly promoted tumour vessel regression and blood flow arrest via its interactions with tumour endothelial

cell IFNγ receptors, leading to intratumoral ischemia and subsequent collapse of the tumour[92]. The apparent discrepancy between this finding and the recent evidence that IFNγ promoted vessel normalization[75] could be explained by potential differences in exposure of endothelial cells to IFNγ[75,92]. The induction of IFNγ expression in tumour cells produced high systemic IFNγ concentrations (~10 ng/mL), which were maintained for a more extended period than the transient IFNγ elevation that can be elicited by adoptively transferred antigen-specific T cells[91]. Therefore, it is likely that the inducible IFNγ system produced more intense and sustained antiangiogenic effects on tumour blood vessels in comparison with IFNγ stimulation from immune checkpoint blockade[75]. Excess antiangiogenic activities mediated by both higher dose and prolonged IFNγ exposure likely promote the transition from vascular normalization to vessel regression, a phenomenon that was also observed in previous studies using anti-VEGFR2 agents[61,63].

In addition to IFNγ, T helper 1 (T_H1) type chemokines (such as CXCL9 and CXCL10) can produce angiostatic effects, in addition to acting as chemoattractants for effector T cells[33]. Human microvascular endothelial cells that express high levels of CXCR3, the receptor for the angiostatic chemokines CXCL4, CXCL9, CXCL10 and CXCL11, were found to exhibit low rates of proliferation and high rates of apoptosis, as well as decreased capability to undergo tube-like vessel formation[89]. Several recent studies revealed that the elevation of histone and DNA methylation within tumours and activation of intrinsic oncogenic signalling suppressed the expression of T_H1 type chemokines and resulted in 'cold' tumour type that is resistant to immunotherapy[33,94,95]. Treatment with epigenetic modulators was found to restore the expression of CXCL9 and CXCL10, promoted $CD8^+$ T cell tumour infiltration, and enhanced the efficacy of immune checkpoint therapy[94]. These studies further underscore the complexity of the interactions between immune cells and tumor blood vessels at the genetic and molecular levels.

Finally, immunosuppressive cell populations such as T_{regs}, MDSCs, and M2-like TAMs not only promote tumour immune evasion, but also foster tumour angiogenesis by secreting VEGF, placental growth factor (PIGF), IL-1β, IL-6, FGF2, stromal cell-derived factor 1 (SDF1), and PDGF, among others[33,93,96,97]. Both TAMs and MDSCs are often intimately associated with tumour blood vessels[98] and secrete membrane-bound or soluble proteases (for example, matrix metalloproteinase (MMP)2, MMP9, and MMP12), which facilitates the growth of tumour blood vessels by degrading the extracellular matrix and improving the availability of proangiogenic growth factors[99,100]. Interestingly, MDSCs that reside within the perivascular space can adopt an endothelial cell-like morphology and express markers such as CD31 and VEGFR2, suggesting that these immature cells may possess the potential to differentiate into cells that become part of the tumour vasculature[96,97]. Therefore, strategies that deplete or polarize immune suppressive myeloid cells within tumours have been investigated to normalize tumour vasculatures. For example, histidine-rich glycoprotein was found to induce TAM polarization away from the immune-suppressive phenotype by downregulating macrophage-derived PIGF. This response resulted in sustained tumour vascular normalization that inhibited tumour growth and metastases[101,102].

New biomarkers for immuno-oncology

The discovery that activated T cells can induce morphologic and functional tumour vessel normalization in the setting of immune checkpoint blockade provides a new opportunity to identify novel biomarkers for cancer immunotherapy[103].

Serum-based biomarkers for immuno-oncology

Serum-based biomarkers reflecting the functional status of tumour blood vessels were previously used to monitor responses to angiogenic therapies[104,105] and may now be explored as predictors of response to cancer immunotherapies. Serum levels of Ang2, a vessel-destabilizing ligand of Tie2 and a critical regulator of blood vessel maturation[106], were found to inversely correlate with both clinical response rate and survival in melanoma patients treated with the anti-CTLA4 antibody, ipilimumab[107]. Similarly, humoral responses against proangiogenic cytokines including Ang2 and VEGFA were found to predict long-term remission and survival in patients with acute myeloid leukemia that had received tumour cell vaccines after allogeneic hematopoietic stem cell transplantation, and also in patients with NSCLC treated with tumour vaccines[108,109]. Together, these findings support the interconnecting relationship between tumour vascular remodeling and the generation of antitumor immune responses, and they further highlight the potential role of using vascular-related biomarkers as a surrogate to predict clinical responses to cancer immunotherapies.

Vessel-based biomarkers for immuno-oncology

Beyond circulating biomarkers, functional measurements of vascular changes with Doppler ultrasonography, perfusion scans, or dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)[110,111], may also provide important information regarding the changes that occur within the TME as a surrogate to tumour responses to immune checkpoint inhibitors (Fig 3). These noninvasive measurements also allow longitudinal monitoring during therapy, a limitation that faces tissue-based biomarkers because of the need for repeat biopsies. Measurements of tumour vascular remodelling more closely reflect changes within the TME in the setting of immune checkpoint blockade therapy, which may not be perfectly represented by systemic biomarkers such as circulatory cytokine levels, immune cell subset counts, or lactate dehydrogenase levels[103]. Finally, tumour vascularity[112] and the degree of perfusion impairment may also serve as a predictor of response to cancer immunotherapy at baseline, before treatment is begun. These new imaging biomarkers can be incorporated into future clinical trial designs, and potentially be used to stratify patients for treatments, as has been done successfully for PDL1 expression in advanced-stage NSCLC[103]. Again, establishing robust vascular biomarkers for cancer immunotherapy requires carefully designed biomarker exploratory studies with independent validation and is still far from clinical translation at this time. Nevertheless, noninvasive measurement techniques provide a unique opportunity to explore TME-based biomarkers based on functional changes induced by immune checkpoint blockade therapy, which extends beyond traditional tumour cell and immune cell analyses.

Combination immunotherapies

The interdependence between vascular normalization and immune reprogramming provides a unique opportunity to identify new and more efficient combination therapy strategies to enhance antitumour immunity. Since the first clinical trial of ipilimumab monotherapy for patients with advanced melanoma nearly a decade ago, research has focused on combining immunotherapies with standard-of-care chemotherapy or radiotherapy to achieve the optimal therapeutic effects[14,113–115]. However, in some cases, it is unclear whether a mechanistic basis exists to support the specific combination regimen used. For example, several institutional and cooperative group trials are ongoing to study the usefulness of combining radiation with cancer immunotherapies. These studies are based largely on the premise that radiation can facilitate the release of tumour-associated antigens, which may help to broaden the antitumour responses in the setting of immune stimulation[116]. However, it is far from clear how the two regimens should be delivered in relation to one another (for example, concurrently versus sequentially) and what type of radiotherapy should be used (for example, conventionally fractionated, where a large number of treatments are delivery with small doses of radiation, versus hypofractionated or stereotactic radiotherapy, where a few large doses of radiation are used to ablate the tumour to yield the best response[117,118]. Ionizing radiation is known to induce changes within the tumour immune microenvironment[119], but the nature of those changes has not always been consistent. For example, studies using a single fraction of low-dose radiation (~2 Gy) have been shown to normalize tumour vasculatures and improve T cell recruitment by inducing inducible nitric oxide synthase (iNOS) expression in TAMs in mouse and human pancreatic tumours[120,121]. However, other studies have found that a single-fraction ablative dose of radiation (as high as 30 Gy) promotes enhanced $CD8^+$ T cell infiltration and decreases MDSCs in murine colorectal tumours[122]. It is unclear whether the differences in these findings mainly reflect variations in the tumour models used or are due to the involvement of distinct molecular processes. Nevertheless, the fact that stimulated T cells in the setting of immune checkpoint blockade also promote tumour vascular normalization, improve tissue perfusion, and alleviate tumour hypoxia provides a strong rationale that a mutual benefit may exist between cancer immunotherapy and radiotherapy. Beyond the traditional belief that radiotherapy serves as an immune adjuvant[119], it is now conceivable that immunotherapy may also have radiosensitizing effects by increasing oxygenation within the tumour, thus paving the way for future studies to examine this reciprocal effect.

The relationship between cancer immunotherapy and conventional systemic therapy also warrants a more detailed examination in light of these new findings. By normalizing the tumour vasculature and improving tissue perfusion, cancer immunotherapy may, at least in theory, improve the delivery of systemic agents into tumours[123]. However, similar to vascular normalization strategies using antiangiogenic agents, the optimal conditions needed to produce notable therapeutic benefits may be technically difficult to accomplish and may vary among different tumours and different patients[61]. Nevertheless, new mechanistic insights into the interplay between vascular normalization and immune reprogramming provide us with a theoretical foundation to explore new combination approaches using cancer immunotherapy and systemic therapies.

Vasculature and beyond

Vascular normalization can also lead to changes in the acellular components of the TME that may facilitate the infiltration and activation of immune effector cells to complement cancer immunotherapies. For example, increased tumour blood vessel permeability and decreased lymphatic drainage lead to an elevated intratumoural interstitial fluid pressure (IFP)[124]. This pathophysiological state in the TME makes it difficult for immune effector cells to enter the tumour parenchyma and they accumulate primarily around the tumour edge[124]. By restoring the transluminal pressure gradient across tumour blood vessels, vascular normalization has been shown to decrease IFP within the TME[125], therefore reducing the restrictions on immune effector cell mobilization and tumouricidal functions.

Another potential benefit of tumour vascular normalization is through its effect on intratumoural lymphatic vessels. Elevated IFP may lead to the compression of intratumoural lymphatic vessels, which may be important for antigen-presenting cells to home to regional lymph nodes and prime T cells[126]. Previous studies of tumour vascular normalization showed that despite restoration of blood flow within previously compressed blood vessels, lymphatic drainage remained sluggish, suggesting that different processes are involved in blood and lymphatic vessel impairment within tumours[124]. Investigations into whether immune cells possess a similar ability to remodel dysfunctional tumour lymphatics, in addition to blood vessels, may further illuminate the antitumour effects of cancer immunotherapies.

Finally, tumour vascular normalization may also help to restore aberrant concentrations of ions within the TME, which are largely caused by increased cell turnover and the inadequate removal of intracellular ions released from cancer cells[127,128]. The ionic imbalance can directly inhibit the functions of effector T cells. A recent study showed that elevations in the extracellular potassium concentration ($[K^+]_e$) in the TME impairs TCR–driven Akt–mTOR phosphorylation via the activities of the serine/threonine phosphatase PP2A[128], thus inhibiting T cell activation. Similarly, T_{regs} that were induced to undergo apoptosis from oxidative stress were found to release and convert a large amount of ATP to adenosine, which had the downstream effect of amplifying immune suppressive signals within the TME[129]. This seminal finding demonstrates that local concentrations of cellular metabolites, such as reactive oxygen species, also have critical roles in regulating the immune TME. The identification of different ionic and metabolic checkpoints further highlights the complexities associated with tumour immune escape, which involves both cellular and acellular elements within the TME. Further investigations into the effect of tumour vascular normalization on the ionic profiles within the TME are therefore needed to fully understand the reciprocal interactions between tumour blood vessel remodeling and immune reprogramming.

Outlook

Although the evaluation of antitumour immune responses has traditionally been focused on assessing the interactions between cancer and immune effector cells, growing evidence now suggests that other components of the TME also have critical roles in determining the

efficacy of cancer immunotherapies[22,56,130,131]. The constituents of the TME form a sophisticated interaction network that ultimately determines the immune landscape within the TME. The complexity and heterogeneity of this immune TME requires a systematic assessment of different molecular (for example, IFNγ, Granzyme B, and various cytokines), cellular (for example, $CD8^+$ T cell activation and myeloid cell polarization), vascular (for example, vessel normalization and tissue perfusion), and acellular responses (for example, metabolic and ionic changes) to cancer immunotherapies. The finding of mutual regulation between tumour vascular normalization and immune reprogramming supports the notion that the antitumour effects of immune checkpoint inhibition may depend not only on the restoration of effector T cell functions within the TME, but also on the normalization of the TME itself. Therefore, the effects of immune checkpoint blockade on the TME, such as the vascular changes it induces, may provide novel variables with which to evaluate and predict cancer immunotherapy responses. The insights gained from studying this reciprocal regulation between immune stimulation and TME normalization has thus opened new avenues to understand how tumours undergo immune evasion and to identify strategies to further boost the antitumour effects of cancer immunotherapies.

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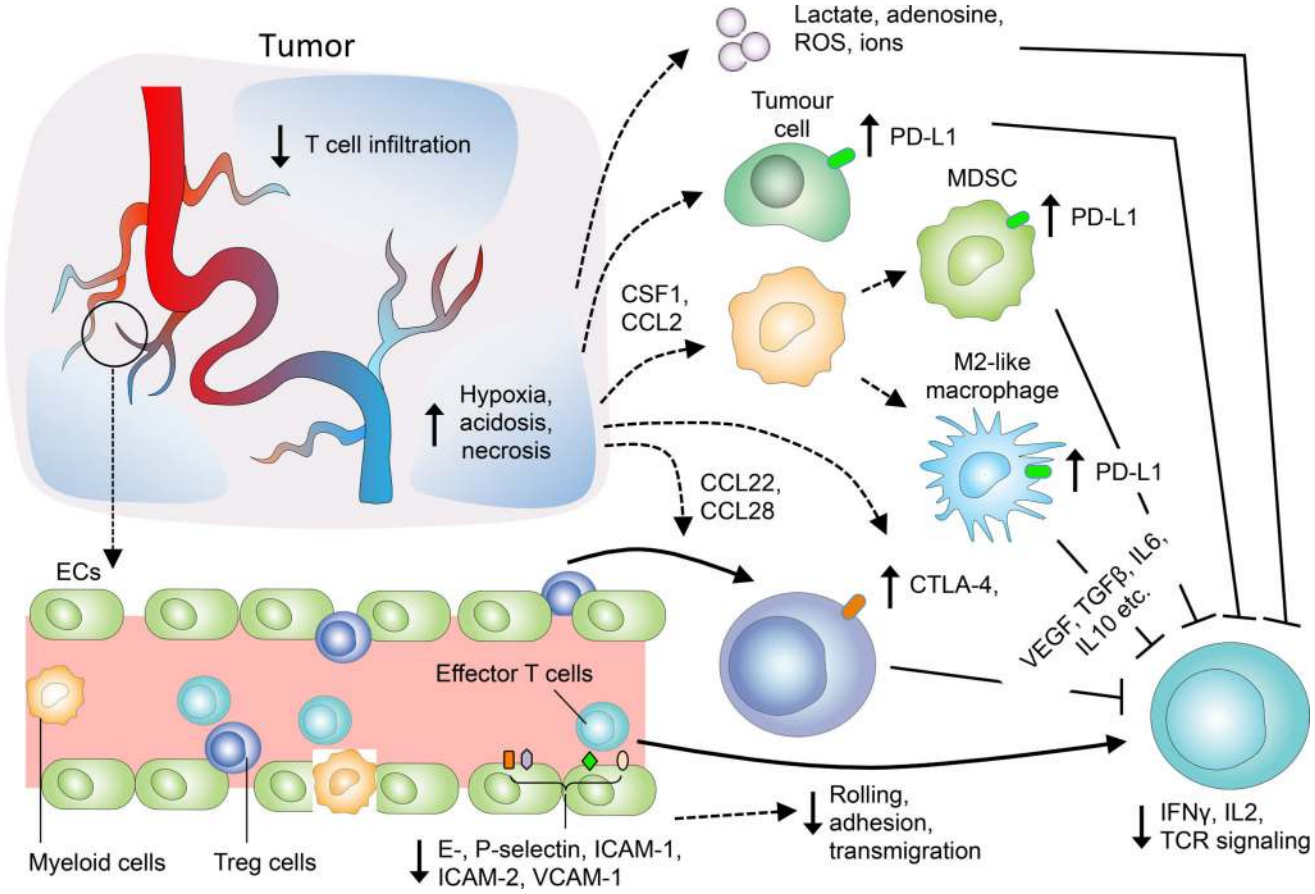

Figure 1. Abnormalities in the tumour vasculature contribute to immune suppression via multiple mechanisms

Impaired vessel perfusion and increased vascular permeability promote tissue hypoxia, acidosis and necrosis, which activate immune suppressive processes to inhibit effector T cell functions. Hypoxia not only induces the secretion of cytokines and chemoattractants to increase the recruitment of immunosuppressor cells, but also upregulates the expression of CTLA4 or LAG3 on T_{reg}, and PDL1 on MDSCs, TAMs[131] and tumour cells.[45] The endothelial cells of tumour vessels also express lower levels of cell adhesion molecules causing endothelial anergy, thereby reducing the ability of effector T cells to infiltrate into tumours. ECs: endothelial cells; ROS, reactive oxygen species; MDSC, myeloid-derived suppressor cell, T_{reg}, regulatory T cells; TCR, T cell receptor; CTLA4, cytotoxic T - lymphocyte associated protein-4, PDL1, programmed cell death ligand-1; TAMs, tumour associated macrophages.

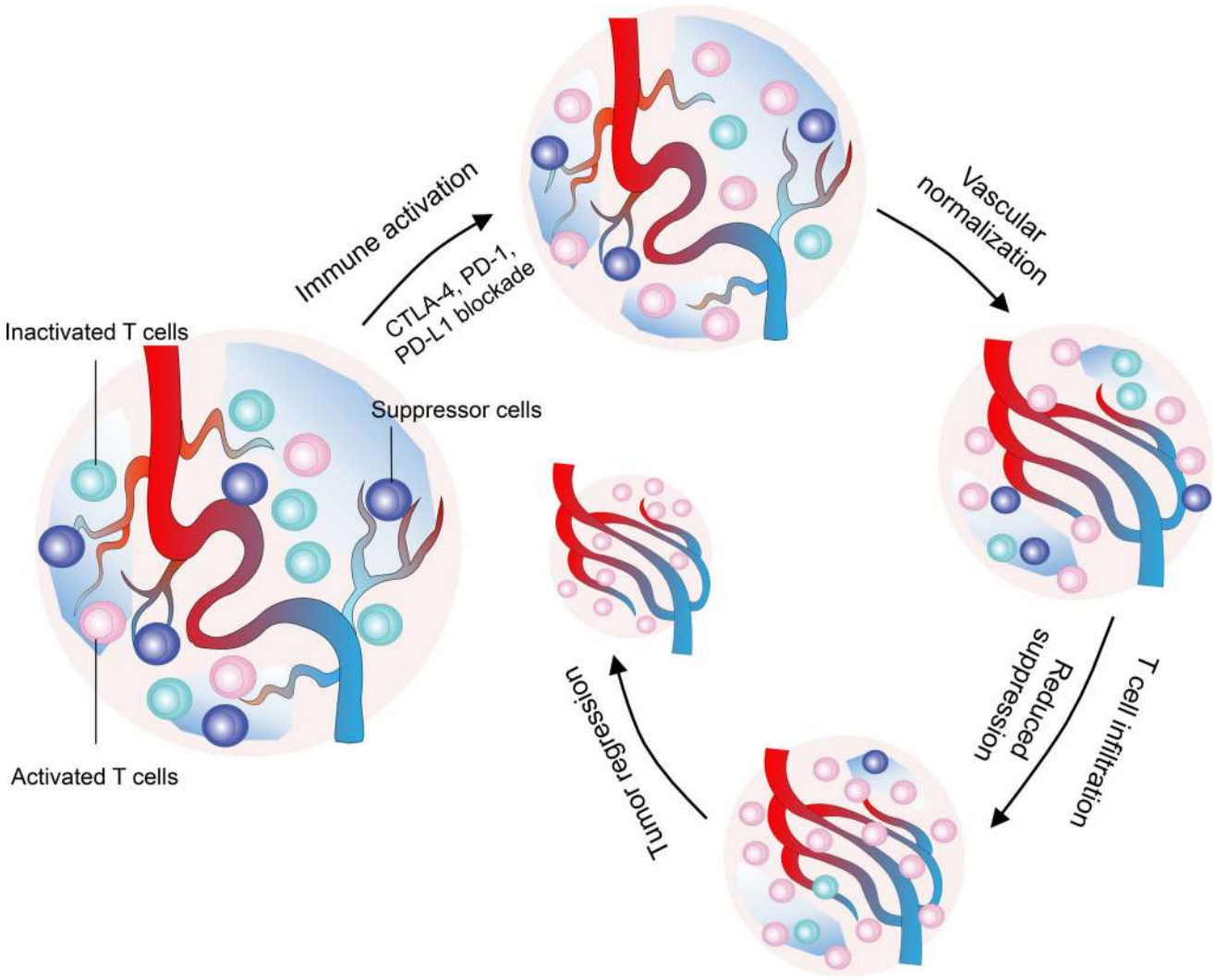

Figure 2. A reinforcing feedback loop of immune reprogramming and tumour vascular normalization

The highly immune suppressive tumour microenvironment is often dominated by the presence of immune suppressor cells and dysfunctional effector T cells. Immune checkpoint blockers activate effector T cells, which in turn promote the normalization of tumour blood vessels. The initial vascular normalization decreases immune suppressive processes within the tumour microenvironment, facilitates the infiltration and enhances the function of effector T cells, leading to further normalization of tumour blood vessels. This feedback loop between immune reprogramming and tumour vascular normalization reinforces each other, ultimately promotes immune-mediated tumour eradication. The disruption or the inability to establish such a positive reinforcement process may lead to transient therapeutic efficacy and decrease long-term tumour control of immune checkpoint inhibitors.

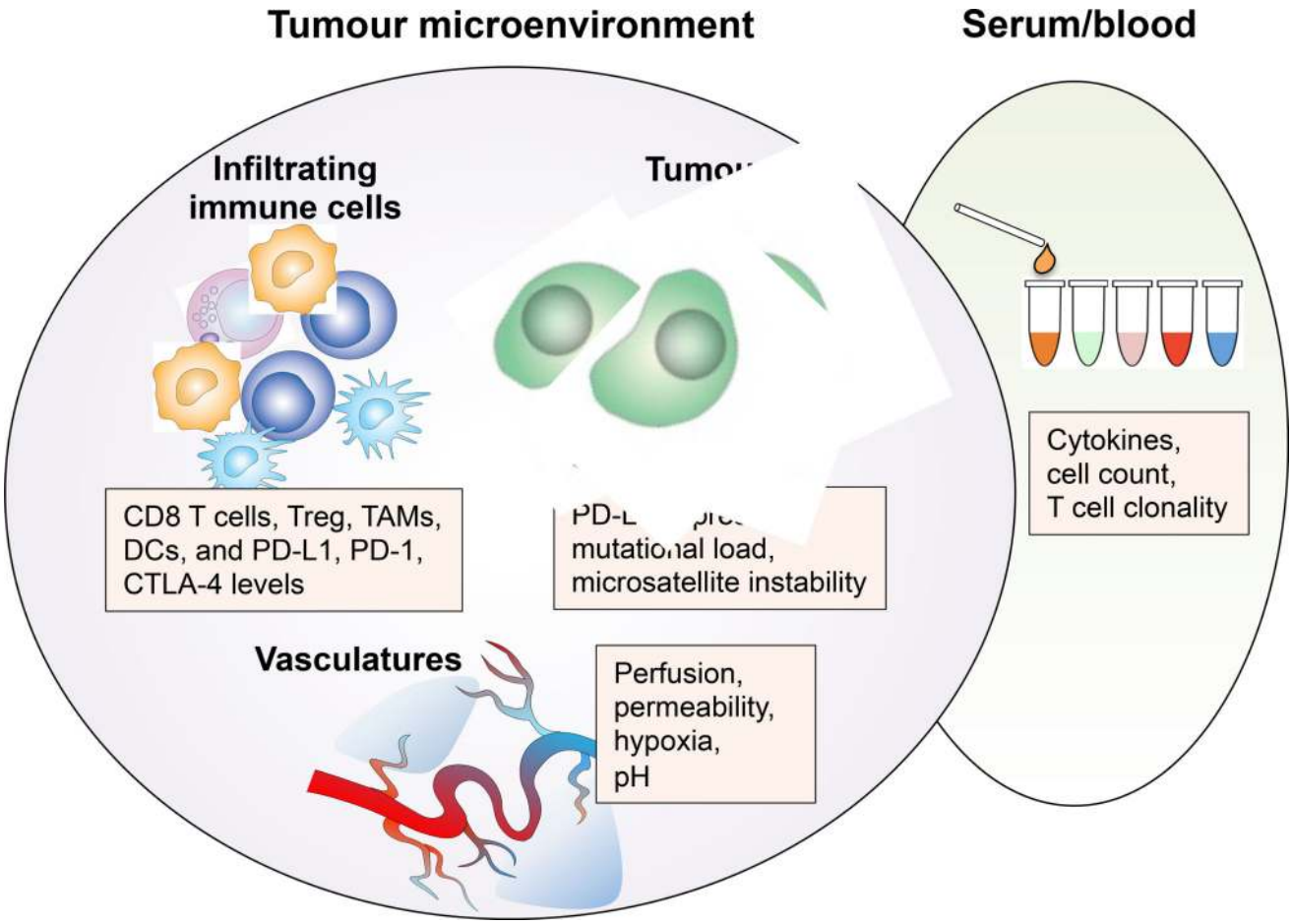

Figure 3. Biomarker discovery for immuno-oncology

The current tissue-based biomarker analysis for immuno-oncology largely focuses on intrinsic tumour cell properties and immune cell profiles within the tumour microenvironment, including PDL1 expression levels, mutational load, as well as the number of infiltrating effector T cells, immune suppressor cells, and their ratios. The vascular remodeling effects of immune checkpoint therapy provide a new rationale for assessing tumour microenvironment-based biomarkers beyond tumour and immune cells. Examination of tumour vascular-related changes such as alterations to tissue perfusion, hypoxia, pH and vascular permeability, in combination with immune cell profiling and tumour cell characterization may provide a more sensitive and dynamic way of monitoring tumour responses to immune checkpoint blockade. Together with serum-based biomarkers, tumour microenvironment-based biomarkers incorporating tumour cell, immune cell, as well as blood vessel analyses will provide a complete picture of cancer immunotherapy induced immunological changes to accurately monitor clinical responses in patients.