Review

Clinical Cancer Research

The Intersection between Tumor Angiogenesis and Immune Suppression

Osama E. Rahma and F. Stephen Hodi

Abstract

Both immune checkpoint inhibitors (ICI) and antiangiogenesis agents have changed the landscape of cancer treatment in the modern era. While antiangiogenesis agents have demonstrated activities in tumors with high vascularization, including renal cell carcinoma and colorectal cancer, the effect of ICIs has been seen mainly in immunologically recognized tumors, with highly immune-infiltrative lymphocytes. The main challenge in the drug development of ICIs is moving their activities to noninflamed tumors and overcoming resistance that is driven, in part, by the immune-suppressive microenvironment. Angiogenesis factors drive immune suppression by directly suppressing the antigen-presenting cells as well as immune effector cells or through augmenting the effect of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM). Those suppressive immune cells can also drive angiogenesis, creating a vicious cycle of impaired immune activation. The combination of bevacizumab and ipilimumab was the first to show the promising effect of antiangiogenesis and ICIs. A plethora of similar combinations has entered the clinic since then, confirming the promising effects of such approach.

Introduction

Angiogenesis and immune tolerance are both normal physiologic mechanisms that are hijacked by tumors. Angiogenesis involves the formation of new vessels from preexisting ones during development and wound healing (1). The modulation of angiogenesis is highly regulated by proangiogenic and antiangiogenic factors, a process that becomes disrupted and dysregulated in cancer (2). Tumor-driven hypoxia increases the expression of proangiogenic factors leading to the formation of new vessels that are vital to the tumor survival and proliferation (3). The VEGF family, consisting of six growth factors (VEGFA-F), plays the most critical role in angiogenesis by binding to their receptors VEGFR1-3 and neuropilin (4). Angiogenesis can also be mediated by the angiopoietin (Ang1-2)/Tie-2 pathway, independent from the VEGF pathway. Angiopoietin-1 is constitutively expressed in many adult tissues and is required for normal vascular homeostasis, whereas Ang-2 is predominantly expressed in tissues undergoing vascular remodeling and in hypoxic tumor microenvironments (5, 6). Ang-2 plays a critical role in regulating blood vessel maturation and is complementary to the VEGF pathway in later stage of vascular formation (7). Elevated levels of VEGF and Ang-2 are associated with a worse prognosis in a number of different tumor types (8, 9). Accordingly, drug development was heavily focused on antiangiogenesis in the past decade as a strategy to deprive tumor's nutrition and inhibit tumor growth. However, despite the modest activities of these agents as single agents or in combination with chemotherapy, tumors can overcome their effects and become resistant (10).

Cancer immunotherapy has emerged as a modality that can effectively treat a variety of cancers with the discovery of immune checkpoints (11). A plethora of investigations with immune checkpoint inhibitors (ICI) has demonstrated a long-lasting clinical activity against many malignancies (12). ICIs block another mechanism hijacked by tumor "immune exhaustion," unleashing the effector immune cells against cancer (13). Primary resistance to ICIs is described in tumors that lack tumor-infiltrating lymphocytes. In addition, tumors that initially respond to ICIs can develop secondary resistance due to defects in antigen-presenting machinery and the overexpression of coinhibitory molecules among other factors (14).

The cancer immunotherapy field is currently heavily focused on discovering factors that drive resistance to ICIs. Angiogenesis plays a major role in immune suppression and can lead to both primary and secondary resistance to ICIs (15). Accordingly, these two phenomena (angiogenesis and immune exhaustion) could unleash the potential of effective combination (16).

The Interaction between the VEGF Family and Immune Suppression

The effect of VEGF-driven angiogenesis on the immune microenvironment

VEGF-driven angiogenesis and immune suppression interact at many different levels (Fig. 1). The VEGF family plays a key role in suppressing tumor immune response by negatively affecting the antigen-presenting cells (APC) and effector T cells while augmenting the effects of immune suppressive cells such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). The binding of VEGF factors to their receptors (mainly VEGFR2) inhibits the differentiation of monocytes into dendritic cells (DC), drives immune evasion by decreasing DC maturation and antigen presentation (an effect that is mediated by the

Center for Immune-Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts.

Corresponding Author: Osama E. Rahma, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-6954; Fax: 617-582-7227; E-mail: Osamae_rahma@DFCI.harvard.edu

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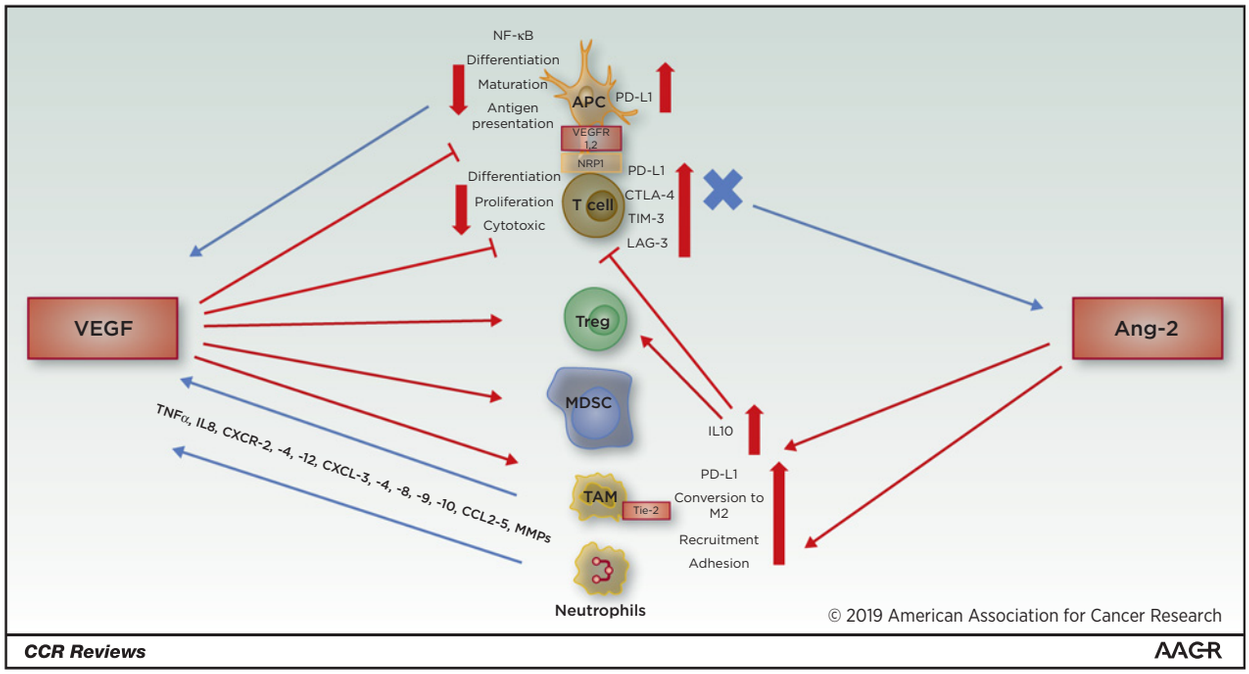

Figure 1.
The interaction between angiogenesis and the tumor immune microenvironment. The VEGF family can suppress the maturation, differentiation, and antigen presentation of APCs, DCs, and T cells, while both VEGF and Ang-2 can augment the suppressive effect of Tregs, TAMs, and MDSCs. MMPs, metalloproteinases.

inhibition of nuclear factor NF-κB; ref. 17), and leads to PD-L1 expression on DCs (18). Likewise, the suppressive effect of VEGF on T cells is driven by the inhibition of the progenitor cells differentiation to $CD4^+$ and $CD8^+$ lymphocytes (19), decreasing T cells' proliferation and cytotoxic effects (20), and increasing T cells' exhaustion by increasing PD-L1, CTLA-4, TIM3, and LAG3 expression on T cells (21). In addition, VEGF-driven angiogenesis creates a barrier for T cells' infiltration by decreasing the endothelial intercellular adhesion molecule-1 expression (22). On the other hand, VEGF-driven angiogenesis leads to the expansion of suppressive immune cells including Tregs and MDSCs (23, 24) and increase the infiltration of tumor-associated macrophages (TAM) to the tumor sites (25). The immune-suppressive effects of angiogenesis have been shown to be reversible using antiangiogenesis, which could suggest that the activity of these agents is immune-driven. For example, the use of bevacizumab (IgG1 antibody that targets VEGF-A) has been shown to normalize the vasculature (26), restore DC maturation, and reduce Tregs in patients with colon cancer (27, 28) and decrease MDSCs in renal cell carcinoma (RCC) mouse model (29).

The effect of the immune microenvironment on VEGF-driven angiogenesis

The modulation effect of angiogenesis on the immune microenvironment is a two-way process as both the innate and adaptive immune cells can lead to endothelial proliferation (Fig. 1; ref. 30). DCs express VEGFR-1 and 2 and can promote angiogenesis when activated (31). Tumor-driven inflammation secretes proangiogenic factors, which facilitates the migration of more inflammatory cells to the tumor site. Monocytes are thought to be the main driver of neovascularization. Both neutrophils and TAMs promote angiogenesis by secreting proangiogenic factors, such as VEGF, TNFα, IL8, and various chemokines including CXCR-2, 4 and 12, CXCL3,4,8,9,10, and CCL2-5 (32, 33). In addition, TAMs secrete metalloproteinases (MMP) that can destabilize the vasculature leading to tumor migration and metastases (34). Although T cells do not directly secrete VEGF, they facilitate its effect by acquiring neuropilin 1 (NRP1) during interaction with DCs, which binds to VEGFA to promote angiogenesis (35).

The Interaction between the Ang-2 Family and Immune Suppression

The effect of Ang-2–driven angiogenesis on the immune microenvironment

Ang-2 is an autocrine cytokine that is produced by endothelial cells and functions as an antagonist of Ang1/Tie2 signaling that is involved in vasculature remodeling, which facilitates VEGF-dependent angiogenesis (7). Tie-2 is expressed on a proangiogenic subpopulation of myeloid cells in circulation and tumors called Tie-2–expressing monocytes/macrophages (TEM; refs. 36, 37). The interaction of Ang2/Tie2 leads to immune suppression by distinct mechanism compared with VEGF. Ang-2 increases the neutrophil recruitment and adhesion of both neutrophils and TEMs to endothelium (38), and increases the conversion to M2-like macrophage phenotype (39). However, unlike VEGF, Ang-2 has no direct effect on T cells but it can stimulate TEMs to secrete IL10, which can promote the expansion of Tregs and the inhibition of effector T cells (38, 39; Fig. 1).

The effect of immune checkpoint blockade on the Ang-2 pathway

The Ang-2 pathway is another potential angiogenic mechanism for ICI resistance. Treatment with ICIs can induce functional Ang-2 antibodies especially in patients who derive clinical benefits from ICIs. In addition, patients treated with CTLA-4 and PD-1 blockade with high pretreatment serum Ang-2 and increased Ang-2 titers posttreatment were found to have worse clinical outcomes compared with patients with lower pretreatment titers (40, 41). Interestingly, the magnitude of Ang-2 titers increase correlated with worse clinical outcomes. In addition, there was a correlation between the increase in Ang-2 expression and tumor infiltration by $CD68^+$ and $CD163^+$ macrophages with Ang-2 promoting the expression of PD-L1 on macrophages. This suggests that the Ang-2 immune resistance mechanism is driven by monocytes and inhibiting Ang-2 and immune checkpoints is potentially synergistic. On the other hand, the combination of ipilimumab and bevacizumab was found to be associated with decreased Ang-2 tumor expression, which could explain the role of VEGF in driving Ang-2 upregulation on tumors, a process that is blocked by bevacizumab (40). Accordingly, this combination could potentially reverse the immune resistance to ipilimumab through TAM suppression. On the basis of these findings, we initiated a clinical trial of anti–Ang-2 inhibitor (trebananib) in combination with PD-1 inhibitor (pembrolizumab). The trial is currently enrolling patients with melanoma and renal cell cancer following PD-1 therapy, and ovarian and colorectal cancer (NCT03239145). The primary objectives of this study are to test the safety of the combination and whether PD-1 resistance driven by angiogenesis and macrophages could be overcome by blocking Ang-2. In summary, Ang-2 is an important component of the angiogenesis process that could drive immune resistance through myelocytes recruitment and targeting the Ang-2 pathway may overcome such resistance.

The Combination of ICIs and Antiangiogenesis in Clinical Trials

Many clinical trials have been conducted to test the efficacy of ICIs and antiangiogenesis combinations to reverse the immune suppression–driven by vasculopathy. Table 1 summarizes some of these clinical trials with available preliminary or final results.

The combination of ICIs and VEGF-targeted therapy

The combination of CTLA-4 antibodies and bevacizumab. Both checkpoint blockade and vaccination strategies have reported severe tumor vasculopathy accompanied by perivascular and intramural lymphoid infiltrates in patients with melanoma (42). This observation led to phase I trial of the combination of bevacizumab and ipilimumab in patients with unresectable stage III or IV melanoma. The results of this trial provided the first experience of combining antiangiogenesis with immune checkpoint blockade (43). The combination revealed promising activity, including a 19.6% best overall response rate (ORR) and a disease control rate (DCR) of 67.4% with an overall survival (OS) of 25 months. Immune-related adverse events included giant cell arteritis, hepatitis, and uveitis. Several notable observations were made in correlative laboratory and pathologic investigations in this trial. Marked infiltration with $CD3^+$, $CD4^+$, and $CD8^+$ T cells as well as $CD163^+$ cells (monocyte/macrophage lineage) were observed after treatment with ipilimumab plus bevacizumab. In contrast, patients treated only with ipilimumab demonstrated less immune cell infiltration, while on therapy. In a subset of pathologic samples, tertiary lymphoid aggregates were noted in posttreatment biopsies, similar to that noted for high endothelial venules in lymph nodes. In addition to morphologic changes of endothelia suggesting activation, biochemical changes in the endothelia were also witnessed including E-selectin, ICAM, and VCAM upregulation as a function of treatment. Flow cytometry analysis on peripheral PBMCs indicated a marked increase in the number of patients exhibiting a $\geq$50% increase in levels of circulating $CD4^+$ and $CD8^+$ memory cells (CD45RO), suggesting that antiangiogenesis may have an effect on circulating immune memory populations. This trial provided a proof of concept for the enhanced immunologic effect provided by adding an antiangiogenic agent to immune checkpoint blockade and provided the basis for future combinational studies. A randomized phase II trial investigating ipilimumab with or without bevacizumab in patients with advanced melanoma finished accrual (ECOG 3612, NCI 01950390).

Table 1. Selected studies with preliminary or final results using the combination of ICIs and antiangiogenesis

Antiangiogenesis drug	Target	ICI drug	Target	Sample size (*N*)	Tumor type	Clinical activity	Correlatives
Bevacizumab	VEGF-A	Ipilimumab	CTLA-4	46	Melanoma	ORR 32% DCR 64% OS 25 months	Increase infiltration of T cells and macrophages ($CD163^+$). Increase circulating memory cells (CD45R0) (43)
Bevacizumab	VEGF-A	Atezolizumab	PD-L1	101	RCC	ORR 32% PFS 11.7 months	Improved outcomes in tumors with immune-suppressed gene signature (44)
Bevacizumab + carboplatin + paclitaxel	VEGF-A	Atezolizumab	PD-L1	356	NSCLC	ORR 63% PFS 8.3 months OS 11.3 months	Subgroups with low PD-L1 and T-effector gene signature benefited (46)
Bevacizumab	VEGF-A	Atezolizumab	PD-L1	101	HCC	ORR 62%	(47)
Axitinib	VEGFR1-3	Pembrolizumab	PD-1	21	RCC	ORR 38% PFS 21 months	(48, 49)
Axitinib	VEGFR1-3	Avelumab	PD-L1	356	RCC	ORR 58%	(50, 51)
Sunitinib	VEGFR1-3	Nivolumab	PD-1	55	RCC	ORR 55% PFS 12.7 months	(53)
Pazopanib	VEGFR1-3	Nivolumab	PD-1	52	RCC	ORR 45% PFS 7.2 months	(53)

Abbreviations: DCR, disease control rate; HCC, hepatocellular carcinoma; NSCLC, non–small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

The combination of PD-1/PDL-1 antibodies and VEGF-targeted therapy. Few trials investigated the combinations of PD-1 or PD-L1 antibodies and bevacizumab showing promising activities. The first study investigated atezolizumab (anti–PD-L1) in combination with bevacizumab in advanced RCC compared with atezolizumab or sunitinib, another antiangiogenesis targeting VEGFR2 (44). The ORRs were 32% including 7% complete response (CR) and 25% partial response (PR) with atezolizumab + bevacizumab, 25% (11% CR, 14% PR) with atezolizumab, and 29% (5% CR, 24% PR) with sunitinib. Median progression-free survival (PFS) was 11.7 months in the intention-to-treat population and 14.7 months in PD-L1$^+$ population in the combination arm compared with 8.4 with sunitinib and 6.1 months with atezolizumab. Although the trial was not designed to compare the efficacy between the three arms but rather to estimate the efficacy of each arm, it shed a light on the important role of targeting angiogenesis to overcome immune suppression. The investigators correlated the clinical activity of these agents with RNA-sequencing gene expression data focusing on three gene signatures (angiogenesis, preexisting immunity or effector immunity, and immunosuppressive myeloid inflammation). While atezolizumab was more effective in tumors with high preexisting immunity and low myeloid inflammation gene signature, the addition of bevacizumab to atezolizumab improved outcomes in tumors with immune-suppressed myeloid signature compared with atezolizumab alone highlighting the role of targeting angiogenesis to overcome the immune-suppressive microenvironment. The combination of bevacizumab and atezolizumab was well tolerated and did not show an increase in immune-related adverse event compared with single-agent atezolizumab. These data confirm prior activity of anti-VEGF and immunotherapy combination in RCC using IFNα. This combination improved PFS compared with IFNα alone leading to its approval in the first-line setting of metastatic RCC (45).

Building on this concept, the same combination of atezolizumab and bevacizumab was tested in the first-line setting of metastatic non–small cell lung cancer (NSCLC) with chemotherapy of carboplatin and paclitaxel. This combination demonstrated an improved PFS and OS compared with the bevacizumab–chemotherapy group, 8.3 months versus 6.8 months ($P < 0.001$) and 11.3 months versus 6.8 months ($P < 0.001$), respectively (46). All subgroups including patients with low or negative PD-L1 expression and most interestingly low T-effector gene signature expression benefited from the atezolizumab–bevacizumab combination providing another evidence for the role of antiangiogenesis in reprograming the immune-suppressive microenvironment. However, the data regarding the group who received atezolizumab and bevacizumab in combination with chemotherapy were not reported in comparison with the group who received atezolizumab in combination with chemotherapy precluding making any conclusion regarding the role of adding bevacizumab to atezolizumab and chemotherapy.

This combination is currently being tested in hepatocellular carcinoma (HCC) with promising preliminary activity (over 60% PR) in treatment-naïve patients (47) and moved to phase III trial compared with sorafenib (NCT03434379). Other indications where this combination is currently being tested are: cervical, endometrial, microsatellite stable colon cancer, glioblastoma, and pancreatic cancer (Table 2).

Axitinib, a tyrosine kinase (TKI) targeting VEGFR1-3, has been recently combined with both PD-1 (pembrolizumab; refs. 48, 49) and PD-L1 inhibitors (avelumab) in RCC (50, 51) demonstrating striking activities in the first-line setting. The primary objectives were PFS and OS in the intention-to-treat population for the pembrolizumab/axitinib trial (49) and in PD-L1$^+$ tumors for the avelumab/axitinib trial (51). Both combinations increased the overall response rate (ORR) by 2-fold compared with single-agent sunitinib (from 25%–35% to 51%–58%) with most responders enjoying durable responses. Most importantly both combinations demonstrated activities in RCC regardless of PD-L1 status or risk group. This observation is unique considering the proven activity of nivolumab and ipilimumab in the intermediate and high-risk RCC groups compared with sunitinib, which was found to be more effective in the good risk group (52).

Although median OS has not been reached, the 12-month OS reported to increase from 78.3% for sunitinib to 89.9% with the pembrolizumab/axitinib combination. If this improvement in OS remains persistent in longer follow-up, it would confirm the survival advantage of combining PD-1 inhibitors and antiangiogenesis in RCC where anti-VEGF–targeted therapies alone were not as effective in prolonging OS. Furthermore, the randomization design of both trials will allow robust correlatives to further understand the immune modulation role of antiangiogenesis and whether tumors with less immune activation at baseline could benefit from the combination as seen in the atezolizumab/bevacizumab studies. The safety profile of these combinations was comparable with each drug safety profile without significant increase in adverse events except for transaminitis in the pembrolizumab/axitinib combination.

The combination of nivolumab and sunitinib or pazopanib (both TKIs targeting VEGFR1-3) in RCC resulted in 82% of patients developing grade 3–4 treatment-related adverse events leading to the discontinuation of further development (53). The most common grade 3–4 side effects were hypertension, transaminitis, which is mainly related to pazopanib or sunitinib, diarrhea, and fatigue. The overall response rate of these combinations was comparable with what was seen with axitinib and pembrolizumab or avelumab combinations including 54.5% and 45 for nivolumab + sunitinib and nivolumab + pazopanib, respectively.

Additional ongoing studies testing the combination of ICIs and antiangiogenesis are summarized in Table 2.

The Dual Blockade of VEGF and Ang-2 in Combination with ICIs

The role of the Ang-2 pathway in the adoptive resistance to VEGF inhibition has been demonstrated in preclinical models. The inhibition of VEGF resulted in the upregulation of Ang-2 and Tie-2 and increased Tie-2-expressing macrophages in the pancreatic neuroendocrine tumors (PNET), while dual Ang-2/VEGFR2 inhibition was effective in delaying PNETs progression and suppressing revascularization (54). Dual blockade of Ang-2 and VEGF has been also shown to be an effective strategy in glioblastoma animal model through TAMs reprograming (55). This concept was tested in clinic using vanucizumab, a bispecific antibody targeting VEGF-A and Ang-2. Vanucizumab was shown to cause treatment-related grade 3

Table 2. Selected ongoing studies using the combination of ICIs and antiangiogenesis

Clinicaltrials.gov ID	Antiangiogenesis drug	ICI drug	Combination with	Competitor arm	Tumor type
NCT03353831	Bevacizumab	Atezolizumab	Chemotherapy	Bevacizumab+ chemotherapy	Ovarian cancer
NCT02997228	Bevacizumab	Atezolizumab	FOLFOX		MSI-high colorectal cancer
NCT03063762	Bevacizumab	Atezolizumab	RO6874281		RCC
NCT03434379	Bevacizumab	Atezolizumab		Sorafenib	HCC
NCT03175432	Bevacizumab	Atezolizumab			Melanoma brain mets
NCT03526432	Bevacizumab	Atezolizumab			Endometrial cancer
NCT03133390	Bevacizumab	Atezolizumab			Cisplatin-ineligible urothelial cancer
NCT03024437	Bevacizumab	Atezolizumab	Entinostat		RCC
NCT03556839	Bevacizumab	Atezolizumab			Cervical cancer
NCT03038100	Bevacizumab	Atezolizumab	Paclitaxel, carboplatin		Ovarian, fallopian tube, or primary peritoneal cancer
NCT03181100	Bevacizumab	Atezolizumab	Chemotherapy		Anaplastic and poorly differentiated thyroid carcinomas
NCT03395899	Bevacizumab	Atezolizumab			Neoadjuvant estrogen receptor–positive breast cancer
NCT03555149	Bevacizumab	Atezolizumab	Regorafenib, imprime PGG, or isatuximab		Microsatellite-stable colorectal cancer
NCT03280563	Bevacizumab	Atezolizumab	Enitinostat, exemestane, fulvestrant, ipatasertib, or tamoxifen		$HR^{+}HER\text{-}2^{+}$ breast cancer
NCT03424005	Bevacizumab	Atezolizumab	Ipatasertib, SGN-LIV1A, bevacizumab, cobimetinib, or chemotherapy		TNBC
NCT03193190	Bevacizumab	Atezolizumab	Chemotherapy		Pancreatic cancer
NCT02336165	Bevacizumab	Durvalumab			Glioblastoma
NCT02337491	Bevacizumab	Pembrolizumab		Pembrolizumab	Glioblastoma
NCT02496208	Cabozantinib	Nivolumab+ ipilimumab		Cabozantinib + nivolumab	Genitourinary tumors
NCT03367741	Cabozantinib	Nivolumab			Endometrial cancer
NCT03635892	Cabozantinib	Nivolumab			Nonclear RCC
NCT03316586	Cabozantinib	Nivolumab			TNBC
NCT03149822	Cabozantinib	Pembrolizumab			RCC
NCT03468218	Cabozantinib	Pembrolizumab			Head and neck cancer
NCT03534804	Cabozantinib	Pembrolizumab			Urothelial carcinoma
NCT01658878	Cabozantinib	Nivolumab ± ipilimumab		Nivolumab, nivolumab + ipilimumab, sorafenib	HCC
NCT03291314	Axitinib	Avelumab			Glioblastoma
NCT03289533	Axitinib	Avelumab			HCC
NCT03472560	Axitinib	Avelumab			NSCLC and urothelial cancer
NCT03172754	Axitinib	Nivolumab			RCC
NCT03736330	Axitinib	Pembrolizumab	D-CIK		RCC
NCT02636725	Axitinib	Pembrolizumab			Soft tissue sarcomas
NCT02853331	Axitinib	Pembrolizumab		Sunitinib	RCC
NCT02684006	Axitinib	Avelumab		Sunitinib	RCC
NCT01472081	Pazopanib Sunitinib	Nivolumab Nivolumab		Nivolumab + ipilimumab	RCC
NCT02014636	Pazopanib	Pembrolizumab			RCC
NCT03277924	Sunitinib	Nivolumab			Soft tissue and bone sarcomas
NCT03211416	Sorafenib	Pembrolizumab			HCC
NCT03439891	Sorafenib	Nivolumab			HCC
NCT03239145	Trebananib (Ang-2 inhibitor)	Pembrolizumab			Melanoma, RCC, ovarian cancer, colorectal cancer
NCT01688206	Vanucizumab (VEGF-A and Ang-2)	Atezolizumab			Solid tumors
NCT02665416	Vanucizumab (VEGF-A and Ang-2)	Selicrelumab (CD40 agonist)			Solid tumors

Abbreviations: HR, hormone receptor; MSI, microsatellite instability; TNBC, triple-negative breast cancer.

toxicities in 41% of patients including one fatal pulmonary hemorrhage initially in a phase I trial with preliminary activity demonstrated in RCC (56). Vanucizumab is currently investigated in combination with atezolizumab (NCT01688206) and CD40 agonist in solid tumors (NCT02665416).

Endothelial Adhesion Proteins and Their Role in Immunity

Endothelial adhesion proteins including integrins and matrix MMPs play a critical role in angiogenesis and influence cancer immunity. Integrins are a family of 24 transmembrane cell-matrix α-β heterodimeric adhesion receptors that bind extracellular matrix proteins to the cell cytoskeleton (57). Many integrins are overexpressed on endothelial cells during the process of angiogenesis making it an attractive target to inhibit angiogenesis for cancer therapeutics (58). However, targeting integrin has not shown promising anticancer activities due, in part, to the integrin switching expression between different subsets (59). Integrins have complex interactions involving the anchoring and transmigration across the endothelia of immune cells from the circulation into tissues (60). In addition, integrins play critical roles in antigen presentation and immune-regulatory cellular interactions (61). Kwan and colleagues demonstrated that integrin-binding peptide combined with albumin/IL2 Fc fusion in combination with PD-1 inhibitor can elicit an innate and adaptive immune response and increase survival in syngeneic mouse models (62). Their significance in immunity is highlighted by the successful targeting of integrins in the treatment of inflammatory conditions including autoimmune diseases such as multiple sclerosis (63).

MMPs are also attractive targets given their role in degrading the extracellular matrix, which is required for vascular basement membrane invasion during angiogenesis and their presence in the epithelial–mesenchymal transition processes (64). These proteinases are expressed on endothelial and inflammatory cells (including DCs, macrophages, and lymphocytes; ref. 65). In addition, there is a cross interaction between MMP-2 and integrin αvβ3 leading to an enhanced mesenchymal cell-invasive activity (66). On another hand, the inflammatory response at the tumor microenvironment driven by neutrophils and macrophages can lead to an inactivation of MMPs (67), making the interaction between endothelial adhesion proteins and the immune microenvironment even more complex. Similar to integrins, targeting MMPs in clinic has not been successful, in part, due to the lack of specificity to their targets (68). More preclinical data are needed to have a better understanding of the role that integrins and MMPs may play in immune modulation and whether combinational approach with ICIs is warranted.

Discussion

In summary, angiogenesis plays a critical role in modulating the tumor immune microenvironment. Both VEGF and Ang-2 families contribute to this process by inhibiting the proliferation and differentiation of activated immune effector cells, while recruiting suppressive tumor-associated immune cells. As detailed in this review, the interaction between angiogenesis and immune regulation is very dynamic and a two-way process.

There is mounting evidence to support the strategy of combining antiangiogenesis and ICIs with promising clinical activities. Such activities remain to be confirmed in a number of

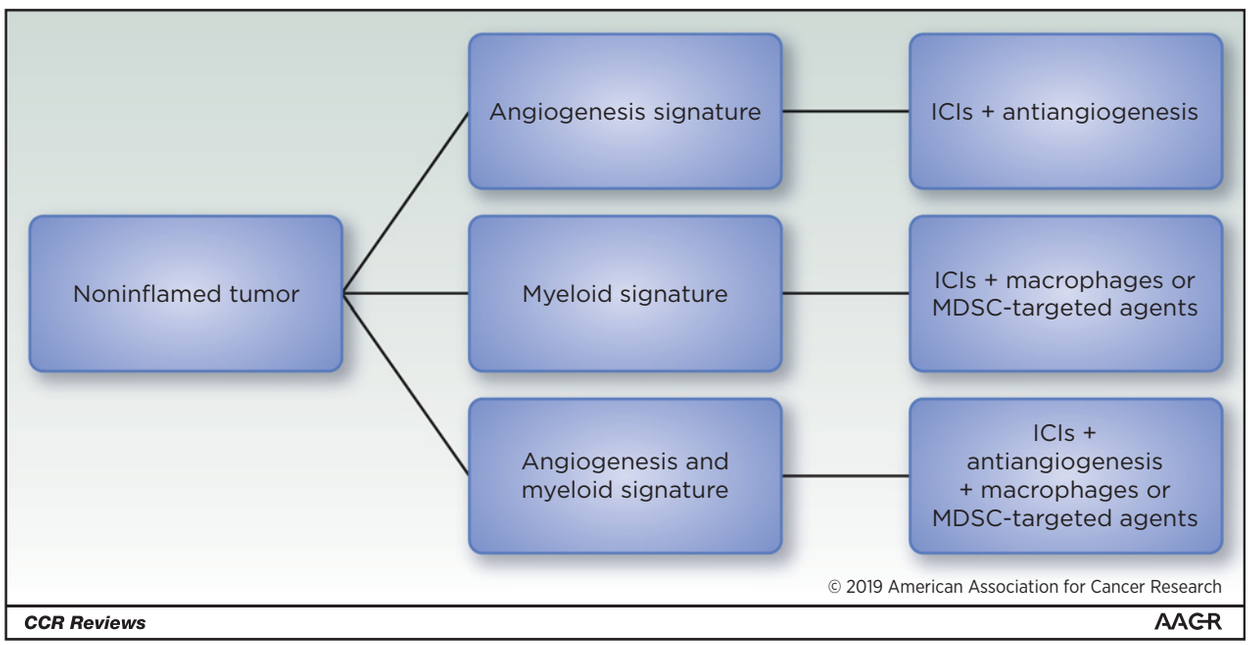

Figure 2.
Suggested biomarker-based trial design. Pretreatment samples are sequenced to identify noninflamed tumors with myeloid and angiogenesis signature expression. Those patients could be then treated with the combination of ICIs and antiangiogenesis if their gene signature is consistent with angiogenesis expression or macrophages/MDSC-targeted agents if they lack the angiogenesis signature, but they express the myeloid signature. If they express both the angiogenesis and myeloid signatures, triplet combination could be used including ICIs, antiangiogenesis, and myeloid-targeted agents.

currently ongoing randomized studies with longer follow up. As described above, angiogenesis has been targeted in combination with ICIs using both antibodies and TKIs; however, the clinical activities of these two approaches have not been compared head to head. Angiogenesis-targeted TKIs block multiple VEGF receptors, while bevacizumab, for example, is directed to a single receptor (VEGFA). Therefore, TKIs may provide a broader biological activity against angiogenesis. This mechanism of action raises the question of resistance to antiangiogenesis, which makes the approach of targeting multiple pathways including VEGFA-D, PDGF, and Tie-Ang-2 an appealing one. Furthermore, combinatorial approaches raise safety concerns and questions regarding the unique mechanism of action of each agent, and the need to identify the optimal dose, sequence, and duration of therapy.

It is important to note that most of the promising data of ICIs/antiangiogenesis combination has been mainly generated in RCC, a tumor with both high angiogenic and immunogenic properties. RCC is highly immunogenic due to relatively high mutational load and predominant tumor-infiltrating immune cells (69). In addition, RCC is a highly vascularized tumor with high expression of VEGF (70), which is associated with tumor progression and poor outcomes (71) and a highly responsive to antiangiogenesis. Accordingly, it is not surprising that RCC is one of the first tumors where the ICIs/antiangiogenesis combination was validated. Indeed, it remains to be determined whether this combination would be proven to be as effective in other tumor types.

Interestingly, the addition of bevacizumab to atezolizumab improved outcomes in tumor subgroups with low effector and high immune suppressive myeloid signature, a subset of tumors that are unlikely to respond to ICIs alone. This observation confirms the role of targeting angiogenesis to overcome primary or secondary resistance to ICIs and pave the way for future combinational studies incorporating tumor biomarkers prospectively. One approach maybe a prospective biomarker-based trial design where pretreatment samples are sequenced to identify noninflamed tumors with myeloid and angiogenesis signature expression. Those patients could be then treated with the combination of ICIs and antiangiogenesis if their gene signature is consistent with angiogenesis expression or macrophages/MDSCs-targeted agents if they lack the angiogenesis signature, but they express the myeloid signature. However, if they express both the angiogenesis and myeloid signatures, triplet combination could be used including ICIs, antiangiogenesis, and myeloid-targeted agents (Fig. 2).

The effect of ICIs/antiangiogenesis could be either synergistic or additive. Given the interplay of angiogenesis and immune suppression as discussed above, it is highly likely that the effectiveness of the combination is due to synergy of the two approaches. On the other hand, it is also possible that the combination targets two different tumor cell populations—one that responds to ICIs and another that respond to antiangiogenesis. The later hypothesis is supported by the Checkmate 214 trial, which demonstrated the effect of nivolumab and ipilimumab in intermediate and poor risk RCC group, while the group with good risk benefited from sunitinib rather than ipilimumab and nivolumab (52).

While many of the clinical study efforts have combined with chemotherapy or attempted to demonstrate a superiority to anti-VEGFA alone, the potential for multiple antiangiogenesis combinations with immune therapy has not been explored to a significant degree to improve mechanistic understandings in patients. The rationale of using chemotherapy as a backbone for those combinations has been speculated as "inducing immunogenic cell death." However, the effect of chemotherapy on the tumor immune microenvironment and angiogenesis is not well studied and may vary from chemotherapy to another. Neoadjuvant design may help answer these questions and provide a rationale to which chemotherapy to be used as backbone in future trials.

With countless ongoing clinical trials using PD-1/PD-L1 or CTLA-4 inhibitors as a backbone in combination with another immunotherapy, chemotherapy, or antiangiogenesis, the only way we could identify a valuable combination to improve efficacy is by deep understanding of how each of these targets change the biology of the tumor microenvironment. Indeed, angiogenesis is a crucial element of this microenvironment that we yet need to explore and understand more.

Disclosure of Potential Conflicts of Interest

O.E. Rahma is a consultant/advisory board member for Merck, Celgene, Alcimed ASA, GFK, Five Prime, Defined Health, PRMA Consulting, Puretech, Leerink, and Genentech. F.S. Hodi reports receiving commercial research grants from Bristol-Myers Squibb and Novartis (to Dana-Farber Cancer Institute), is a consultant/advisory board member for Bristol-Myers Squibb, Merck, Genentech, EMD Serono, Sanofi, Takeda, Surface, Compass Therapeutics, Apricity, Bayer, Pfizer, Pionyr, Verastem, 7 Hills Pharma, and Torque, and has a patent for methods for treating MICA-related disorders (#20100111973) with royalties paid, patents issued for therapeutic peptides (#9402905) and tumor antigens and uses thereof (#7250291), and patents pending for angiopoiten-2 biomarkers predictive of anti-immune checkpoint response (#20170248603), compositions and methods for identification, assessment, prevention, and treatment of melanoma using PD-L1 isoforms (#20160340407), therapeutic peptides (#20160046716, #20140004112, #20170022275, #20170008962), and methods of using pembrolizumab and trebananib. No other potential conflicts of interest were disclosed.

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