

# Angiogenesis and Anti-tumor Immunity in the Tumor Microenvironment: Opportunities for Synergism in Intervention

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**Angiogenesis inhibition and immunomodulation are successful stories of cancer therapy. However, primary and acquired resistances are common. Will combination of the two improve response rate and duration of response through synergy? In addition to blocking neovascular formation, angiogenesis inhibitors (AI) help deliver more effective cytotoxic T lymphocytes to the tumor by improving vascular perfusion. Recent studies also showed that AI not only increased the efficacy of effector immune element but also decreased the number and function of suppressor immune cells such as T-regulatory cells, myeloid-derived suppressor cells or tumor-associated macrophages. In this review, we focus on AI and their effects on antitumor immunity in the tumor microenvironment and their potentials in boosting the efficacy of immunotherapy. In the clinical arena, trials are at the early stage to gauge the feasibility and preliminary signs of synergy.**

Angiogenesis inhibitor | Tumor Microenvironment | Anti-tumor immunity | Immunotherapy | Synergism

## Introduction

Tumor angiogenesis induction refers to a process of new blood vessel formation in malignancy. It is well recognized as one of the ten hallmarks of cancer.(1) Central to this process is activation of the VEGF/VEGFR pathway, which promotes blood vessel growth and survival.(2-4) Blockade of this pathway not only inhibits angiogenesis and destroys tumor vasculature, but also normalizes tumor blood vessels.(4-6) Indeed VEGF blockade is fundamental to most of AI currently in use for cancer treatment including lung, renal, colon, pancreatic neuroendocrine tumors.(7) However, like many other inhibitors used in cancer therapy, efficacy is generally short-lived.

Immune checkpoint mechanisms involving CTLA-4 / B7-1/2 and PD-1/PD-L1 / 2 ligation (8) are used by the cancer cells to achieve immune evasion, another hallmark of cancer.(1) Inhibitors against CTLA-4, PD-1, PD-L1 proved to be effective in a variety of cancer types including melanoma, lung, renal cell, bladder, colon, lymphoma, head and neck etc. However, with single agent treatment, the response is less than 20% with most of them not durable except melanoma. Although the low response rate can at least be partially improved by further immune checkpoint combination use of CTLA-4 and PD-1 inhibitors, the response rate for the combination ranged between 25-60% (9, 10) in lung cancer and melanoma. It is still not clear how long the response will be maintained. However this combination produces an unacceptable level of grade 3 and 4 toxicities in many patients.(9, 10) Other immunosuppressive forces in the tumor microenvironment might explain the low response/escape and short duration of response. With emerging evidences pointing out to a role of immunosuppression by the pro-angiogenic forces, we highlight in this review how AI might contribute to the formation of a microenvironment favoring antitumor immunity and potentially boost immunotherapy.

## Suppressive Immune Cells in the Tumor Microenvironment

Tumor microenvironment (TME) describes a tissue environment in which a tumor exists. Inside the TME, tumor cells interact with the host immune system and stromal surroundings.(11) Tumor growth is also affected by metabolites that influence the pro- or anti-tumor forces surrounding the tumor cells. TME is both impacted by and has impacts on anti-neoplastic therapies including immunotherapy. Here we focus on the immunosuppressive cells that exert crucial influence over antitumor immunity. The cell population mentioned here include T-regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), and the M2 (tumor-tolerant) tumor associated macrophages (TAMs), which act under the direction of the tumor to assist in suppression of antitumor immunity, and allow for propagation of the tumor.(12)

## T Regulatory Cells

Tregs are a population of CD4<sup>+</sup> CD25<sup>+</sup> FoxP3<sup>+</sup> T cells that suppress the proliferation of the activated immune system and maintain immune tolerance to self-antigens. Tregs participate in antitumor immunity, allowing the tumor evasion in epithelial malignancies.(13) Tregs activities are upregulated in the TME, where increased number of Tregs correlated with a poor prognosis in epithelial cancers. Increased numbers of Tregs in the peripheral blood and Treg infiltration in the tumor have also been observed in murine tumor models and in patients with cancer.(14, 15) Tregs depletion dramatically enhances the effect of immunotherapy in murine tumor models but similar approaches have not been effective in patients with cancer.(16) Tregs inhibit the function of tumor specific T cells, particularly in the TME, and their elimination allows antigen-specific T cells to proliferate more robustly. Treg-mediated immune suppression may in part explain the poor clinical response of some cancer patients undergoing immunotherapy. There is a positive correlation in murine models and in patients treated with ipilimumab between the CD8 effector to Tregs cell ratio in the TME and tumor response (17-20). Agents that increase this ratio are associated with improved tumor control.

In three models of lung cancer (a Kras mutation, a carcinogen-driven and a transgenic model), depletion of Tregs with rapamycin, or antibody or genetic ablation, reduced lung tumorigenesis by 90%, 80% and 75% respectively(21). It was observed that Tregs exhibited high suppressive activity and this activity increased with tumor stage of non-small cell lung cancer (NSCLC).(22) In a retrospective analysis of 87 surgically resected NSCLC specimens, it was found that patients with high Tregs counts had significantly worse prognosis in terms of relapse free survival (RFS) and overall survival (OS).(23) Compared with

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healthy individuals, NSCLC patients have increased number of Tregs in their peripheral blood. The increase in Tregs number correlated with stage, with higher levels of Tregs observed in more advanced stages (IV>III>II).(24).

### Myeloid Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells differentiated from common myeloid progenitor cells, which also give rise to granulocytes, dendritic cells and monocytes. Phenotypically, they are CD11b<sup>+</sup> CD14<sup>-</sup> CD15<sup>+</sup> HLA-DRlow/CD33<sup>+</sup> (PMN-MDSC) or CD11b<sup>+</sup> CD14<sup>+</sup> CD15<sup>-</sup> IL4Rα<sup>+</sup> HLA-DRlow CD33<sup>+</sup> (MO-MDSCs) in humans and are of CD11b<sup>+</sup> Ly6C<sup>-</sup> Ly6G<sup>+</sup> (PMN-MDSCs) or CD11b<sup>+</sup> Ly6C<sup>+</sup> Ly6Glow<sup>-</sup> (MO-MDSC) in mouse.(25) Identified in the mid-1980s', they were found to inhibit innate and adaptive immunity, suppress T cell proliferation and generation of cytotoxic T lymphocytes in an antigen independent manner.(26)

Inflammation, whether therapy-induced or tumor-associated, has been known to promote carcinogenesis and tumor growth. Developed under the influence of pro-inflammatory cytokines such as IL-1β, IL-6 and PGE2, MDSCs provide a link between inflammation and carcinogenesis/tumor growth.(27, 28) Tumor and host cells in the TME are able to produce various pro-inflammatory mediators. These mediators activate MDSCs and drive MDSC accumulation and suppressive activity. MDSC can also secrete VEGF and VEGF drives MDSC accumulation forming a positive feedback loop.(29, 30) ROS inside the MDSC drives expression of VEGFR by MDSC. Increased expression of VEGFR further serves as chemo-attractant for MDSCs and ligation of VEGFR receptor also promotes angiogenesis.(31)

MDSCs utilize a variety of mechanisms to suppress T cell activation, induce immune-suppressive cell populations, and regulate inflammation in the TME.(25) Infiltration of MDSCs also promotes tumor cell epithelial to mesenchymal transition (EMT). EMT is an important mechanism that fosters metastatic potential and allows for vascular mimicry. Vascular mimicry refers to the development of VEGF-independent microvascular structures that are not composed of endothelial cells, but rather tumor cells, and interconnect with the tumor vascular circuit to allow an alternative mechanism of oxygen and nutrient delivery.(32, 33)

MDSCs have been isolated from patients with solid tumors. Tumor subtypes ranged from breast cancer,(34, 35) head and neck squamous cell carcinoma,(36) non-small cell lung cancer(37) colon and rectal cancer,(38) renal cell carcinoma,(39) bladder cancer,(40) gastrointestinal cancer,(41) pancreatic adenocarcinoma,(42) esophageal cancer,(43) prostate cancer,(44) to urothelial tract cancer(36) among others. Patients with multiple myeloma and non-Hodgkin's lymphoma also exhibit elevated levels of MDSCs in their blood.(45, 46) Their MDSC levels directly correlated with clinical cancer stage and metastatic burden. Importantly, increased populations of MDSC were predictive of poor initial response to conventional treatments, have demonstrated shortened progression free survival (PFS), and predicted poor outcomes, with a recent meta-analysis showing significant effects on overall survival (OS) in patients with solid tumors.(47-49)

### Tumor Associated Macrophages

Tumor associated macrophages (TAM) refer to macrophages present in the TME. Blood monocytes become macrophages once outside the blood vessel at the site of inflammation or tumor. Despite their normal role of promoting both innate and adaptive immunity and phagocytosis of dead cells and cell debris, they can

be converted by the tumor to a phenotype that promotes tumor growth and metastasis. When this happens, macrophage M1 phenotype is converted to M2. Therefore M1 is the classically activated macrophage and they are pro-inflammatory, anti-tumor and pro-immunity whereas M2 is alternatively activated macrophages that are anti-inflammatory, pro-tumor and immune-suppressive in nature.(50)

Tumor cells entry into the blood stream is facilitated by TAMs in contact with the blood vessels thus TAMs promote tumor metastasis.(51) TAMs and MDSCs have also been shown to be able to directly incorporate into the endothelial wall to allow an alternative mechanism for vessel formation that does not rely on endothelial cells.(33) TAMs promote tumor growth by down-regulating both adaptive and innate immunity through secretion of many immunosuppressive cytokines or metabolites such as TGF-β, IL-10, arginase 1 and nitric oxide(52) and through interaction with MDSCs. The Tie2 expressing TAMs are pro-angiogenic. They promote tumor growth and recovery from cancer treatment with chemo/radiation.(53, 54) The extent of TAM infiltration into the tumor negatively affects the outcome of cancer.(55) TAM dampens the efficacy and response of cancer treatment by promoting angiogenesis and suppressing antitumor immunity.(53) Cancer treatment including chemo/radiation promotes accumulation of bone marrow MDSC. MDSC then differentiate into TAMs in treated tumors.(56-58) Depleting TAM by suppressing conversion of M1 to M2 phenotype or blocking entry of macrophages into the tumor improves cancer treatment response.(59)

### VEGF and Hypoxia in the Tumor Microenvironment

VEGF plays a central role in angiogenesis. In addition, it has complex interactions with the immune environment via hypoxia mediated signaling effects,(60) by direct impairment of antigen presentation mechanisms and dendritic cell maturation, by preventing propagation of the inflammatory signals and VEGFR ligation on MDSCs, Tregs, TAMs, and CTLs, to further support an immunosuppressive tumor microenvironment.(61)

Hypoxia has been shown to promote immune checkpoint molecule programmed-cell-death-ligand-1 (PD-L1) expression to allow further protect the tumor from the immune system.(62) It also causes PI3K/Akt/mTOR activation via HIF-1α mediated intracellular accumulation of adenosine, which ultimately leads to upregulation of immunosuppressive cytokines and immune checkpoint markers, while also stimulating angiogenesis.(63)

### Effects of AI on Antitumor Immunity Immunogenic Modulation

The mechanisms for the immunomodulating effects of anti-angiogenic therapies are emerging, and many studies described beneficial effects of AI and immunotherapy, using either vaccine or checkpoint inhibitor therapy to re-ignite the immune system.(64-66)

Small molecule TKIs have been shown to have immunomodulating effects due to their effects on immunosuppressive MDSCs as well as their anti-angiogenic effect to functionally normalize tumor vasculature, providing an improved environment for an active immune response. (67, 68) The improved T-cell response appears to be a dual response to blockade of VEGF binding to Tregs, directly influencing Tregs apoptosis to reduce their immunosuppressive potential(69) while also releasing suppression of NFκB to restore transcription of pro-inflammatory mediators and chemokines that in turn causes an increase in cytotoxic T lymphocytes (CTL) infiltration and activity.(70) This same effect has been observed with bevacizumab.(71) Interestingly, inhibition of Treg function

rendered previously resistant tumors sensitive to treatment with anti-VEGF antibodies again.(72) Importantly, the decrease in Tregs was shown to have a beneficial effect on overall survival.(73)

Treatment with a VEGFR2 antibody was found to increase the population of M1 (immunoactive) TAMs and tumor infiltrating lymphocytes (TILs), and when it is combined with vaccine therapy, lead to increased CTL killing in both immunogenic and immune exhausted cancer models.(64) Other studies showed that treatment with an anti-Gr1<sup>+</sup> antibody targeting MDSCs in combination with an anti-VEGF antibody showed improved tumor inhibition than either agent alone.(74)

While these AI may cause infiltration of immune cells initially, the CTLs that are activated are counteracted by increased expression of PD-L1 in the TME leading to exhaustion.(75) Dendritic cells (DC) with an enhanced immunologic phenotype were also found to be down-regulated by the tumor immune environment to a tolerant phenotype.(76) Since immune checkpoint inhibitors do well in reversing such tolerant state, combination use of AI with immune checkpoint inhibitors may curb AI resistance. Both sunitinib (77) and sorafenib (78) have been shown to decrease expression of these checkpoint inhibitors in certain tumor populations to allow for an active immune response (see below).

#### **TME conditioning**

The functionally and structurally abnormal tumor vasculature is characterized by leaky pericyte coverage, allowing for increased tumor cell transmigration, increased interstitial fluid pressure, and with tortuous, irregular, and blunted branch points creating a turbulent and heterogeneous flow that allows for maintenance of the hypoxic and immunosuppressive environment and limits delivery of therapeutics.(79)

In addition to the direct effects on the immune system, anti-angiogenic treatment causes changes in this abnormal vessel structure, permeability, and stability. All these can improve the hypoxic conditions of the TME,(80) reverse the impaired immune response and help the delivery of therapeutics to the tumor site,(81) including improvement to utility of adoptive immune transfer and vaccine therapy.(82) One of the many studies investigating this compared both bevacizumab and pazopanib (a VEGFR2 TKI) in colorectal cancer. The interventions yielded decreased interstitial fluid pressure to allow for increased chemotherapy penetration to the tumor.(83)

#### **Effects of individual antiangiogenic agents on antitumor immunity**

##### **Bevacizumab**

Bevacizumab is a humanized monoclonal antibody against VEGF and approved by the FDA for metastatic NSCLC, colon cancer among others. In an orthotopic xenograft model of breast cancer, antibodies that blocked VEGF binding to VEGFR2 were associated with decreased infiltration of TAM and reduced tumor microvessel density.(84) Similarly in a breast cancer model, low dose anti-VEGFR2 antibody normalized tumor vascularity and changed macrophage polarity from the M2 phenotype to M1 and facilitated CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration whereas high doses did not produce this effect.(64) This study suggests that lower dose might be preferred rather than high dose given the fact that high dose did not result in improved overall survival in combination with chemotherapy in breast cancer. Bevacizumab reduced VEGF levels in a malignant pleural effusion. It was also associated with a decrease in Treg that expressed high levels of VEGFR2. Additional confirmatory work demonstrated that VEGFA/ VEGFR blockade with bevacizumab or sunitinib

inhibited tumor induced Treg proliferation in the CT26 mouse model of colorectal cancer.(85) Colon cancer patients treated with bevacizumab and chemotherapy showed lower Treg numbers following treatment than those treated with chemotherapy alone. Bevacizumab was also tested for its effects on Tregs in peripheral blood of patients with metastatic renal cell carcinoma (mRCC) in combination with IL-2 and chemotherapy.(86) Among the 51 patients treated in the phase II part, it was found that Tregs rapidly increased after IL-2 treatment in all patients, and bevacizumab decreased their number but only in patients who had clinical benefit with no disease progression (including stable disease, 37% or partial response, 29.5%). In colon cancer treated with bevacizumab and FOLFIRI, responders had greater decrease in Tregs and decreased Treg frequency was associated with better PFS and OS.(87) In stage IV NSCLC patients, MDSC counts in the peripheral blood were significantly lower in those who had received chemotherapy with bevacizumab compared to those with chemotherapy only. On disease progression, MDSC numbers were again much higher, suggesting that bevacizumab efficacy correlates well with downregulation of MDSC.(88) This appears to be true also in colon cancer treated with bevacizumab with the FOLFOX regimen,(89) where accumulation of MDSC predicted the efficacy of FOLFOX-Bev regimen among the 25 patients treated. The control group had 20 healthy individuals.

##### **Sunitinib**

Sunitinib is a multi-targeted tyrosine kinase inhibitor of VEGFRs and PDGFR. Like axitinib, sunitinib synergized with folate-hapten mediated immunotherapy in 3 murine models of cancer through blocking TAM, MDSC which secrete VEGF and promote neovascularization.(90) Sunitinib treatment also reprogrammed TAM towards classically activated macrophages in a RCC model.(91)

Sunitinib also down-regulates the immunosuppressive effects mediated by the MDSC in man and animal models of cancer. Comparison of the number of peripheral blood MDSC in RCC patients showed that sunitinib treatment reduced MDSC and caused reversal of type 1 T cell response suppression. Type-1 T cell response (IFN- $\gamma$ ) is critical for the generation of effective antitumor immunity along with a reduction in the development of a type 2 cytokine bias.(92) The scenario could be reproduced in vitro by depletion of MDSC. In a RCC model, sunitinib pretreatment improves TIL expansion by reducing intratumoral MDSC (68). The reduction of MDSC in response to sunitinib treatment also correlated with a reduction of Treg cells.(93) Importantly immune checkpoint molecules CTLA-4, PD-1 expression in both CD4 and CD8 T cells and PD-L1 expression on MDSC and DC were also significantly reduced by sunitinib treatment.(77) In the human glioma model, treatment with sunitinib decreased the infiltration of MDSC in the tumor and prolonged the survival of tumor carrying animals.(70, 94) In the melanoma B16F10 and fibrosarcoma T241 models, sunitinib enhanced the antitumor response of CD40 antibody by reducing MDSC and improving endothelial cell and T cell recruitment.(70) Sunitinib also enhanced the effects of therapeutic cancer vaccine and stereotactic radiation therapy through modulation of MDSC.(95) A decrease of intratumoral and peripheral blood Tregs correlated with OS in mRCC treated with sunitinib.(96) VEGFA/VEGFR blockade inhibited tumor induced Treg proliferation in colon cancer.(85) Hence, sunitinib can be a valuable tool for boosting antitumor effects where MDSC/Treg mediated immunosuppression is crucial.

Results of a randomized controlled trial COMPARZ showed that subjects with RCC receiving sunitinib and pazopanib had significant shorter OS (15.1m vs 35.5m for sunitinib and 15.3 m

vs 27.8 m for pazopanib) when increased PD-L1 expression is found in the tumor,(97) suggesting that increased PD-L1 expression might be associated with resistance and PD-1 blockade might improve the outcome of these patients with increased PD-L1 expression.

To test potential synergy between sunitinib and tremelimumab, the two agents were combined in a phase 1 clinical trial. (98) Tremelimumab was given in three cohorts of 6mg/kg, 10 mg/kg, and 15 mg/kg every 12 weeks, and sunitinib was given either continuously at 37.5 mg daily or at 50 mg for four weeks and off for two weeks. Unexpectedly, too much toxicities including one sudden death were observed in all cohort. Rapid onset renal failure happened in 9/21 patients receiving 10 mg/kg of tremelimumab and 37.5 mg daily of sunitinib. Further investigation of this combination in human was deemed too toxic. In a phase 2 trial clinical trial involving metastatic renal cell carcinoma, OS was found to be associated T cell response to IMA901, a ten tumor associated peptide vaccine. Potential synergy between sunitinib and the vaccine was tested in a phase 3 trial named IMPRINT.(99) A total of 339 patients having either locally advanced or metastatic clear cell carcinoma were randomized to receive either sunitinib plus IMA901 (N=204) or sunitinib (N=135) alone. With a median follow up of 33.27 months, no difference was observed between the two groups in terms of mOS, which was the primary end point (HR 1.34).

To study the effects of angiogenesis inhibitor on immunosuppressive TME, researchers studied 33 healthy kidneys, 41 untreated primary RCCs, and 42 bevacizumab-pretreated and 39 sunitinib-pretreated primary RCC(75). In comparison to normal healthy kidney tissue, RCC have significantly more CD3<sup>+</sup>, CD4<sup>+</sup>, CD45RO<sup>+</sup>, CD8<sup>+</sup> lymphocytes and CD68<sup>+</sup> macrophages infiltration. The infiltration of lymphocytes and macrophages was even higher in sunitinib-treated compared to control RCC specimens. Bevacizumab-treated kidneys had increased lymphocytes but not macrophages. Interestingly, CD4<sup>+</sup> / CD8<sup>+</sup> T-lymphocyte infiltration was inversely correlated with OS and PFS in patients treated with AI therapy. In patients who were not treated, no obvious correlation was observed between CD4<sup>+</sup> T-lymphocyte infiltration and OS or PFS. These results collectively indicate that a preexisting immunosuppressive TME inhibited or subverted the antitumor function of T cells. When T lymphocytes infiltration increased, Treg infiltration also increased. Interestingly; Treg can attract CD8<sup>+</sup> T lymphocytes to their vicinity and suppress CD8<sup>+</sup> T lymphocytes function (100). CD8<sup>+</sup> T-lymphocyte infiltration correlated very well with Treg infiltration (R=0.799). Furthermore, PD-L1 expression increased with antiangiogenic therapy. The correlation between CD8<sup>+</sup> T-lymphocyte infiltration and PD-L1 expression in sunitinib-treated samples was also high (R=0.725). This study suggests that antiangiogenic therapy may be insufficient to mount an effector T-cell response capable of overcoming the immunosuppressive TME and combination use of sunitinib with checkpoint inhibitors or drugs that inhibit Tregs may boost the efficacy of antiangiogenic maneuver.

### Sorafenib

Sorafenib is a multi-targeted tyrosine kinase inhibitor of VEGFR/PDGFR. It also target RAF/MEK/ERK pathway. In a murine hepatocellular carcinoma (HCC) model, MDSC and Treg increased with tumor burden. Sorafenib decreased the immunosuppressive MDSC and Tregs.(101) Low dose of sorafenib was sufficient to suppress Treg function and promote the CD4<sup>+</sup> effector cells.(102) In 45 patients treated with sorafenib for 4 weeks, Tregs were decreased in patients taking 400 mg and 800 mg a day while no changes in T effector cells and Th1 cells

were found.(103) In a study that included 19 patients with advanced HCC, researchers found high levels of immunosuppressive cell infiltrate in HCC patients compared with healthy controls. Sorafenib reduce the number of Tregs, MDSC, and PD-1<sup>+</sup> exhausted T cells.(78) Decrease in PD-1 expression on T cells and Treg number were correlated with improved OS in patients following sorafenib treatment. The high pretreatment levels of immunosuppressive cell infiltration significantly correlated with achievement of better OS in patients, suggesting that higher pretreatment numbers of these cells represent predictive immune correlates of responsiveness to sorafenib treatment. The study result suggests that sorafenib may enhance the therapeutic effects of checkpoint blockade. In another study, sorafenib enhanced the antitumor effects of anti-CTLA-4 antibody by inhibiting MDSC in a murine cancer model.(104)

It is worth mentioning here that although sunitinib and sorafenib seemingly target similar receptors, sunitinib is not effective in cancer harboring KRAS or BRAF mutations (105) whereas sorafenib does target BRAF/KRAS suggesting the subtle difference in target selection maybe behind the effects on immunosuppression Tregs and PD-1 expression in clinical trials.

### Axitinib

Axitinib targets VEGFR1, 2, 3. Axitinib treated recurrent GBM is associated with increased Tregs and T cell exhaustion with increased expression of PD-L1 in CD4 and CD8 T cells on progression from treatment, pointing out PD-L1 expression as possible mechanism of escape from angiogenic inhibition.(106) In mouse model of melanoma, axitinib decreased MDSC and increased CD8 T cells without affecting Tregs.(107) Similarly Axitinib together with CTLA-4 antibody improved intratumoral DC function and CD4<sup>+</sup> CD8<sup>+</sup> T Cell function at the same time causing MDSC suppression in a melanoma brain metastasis mouse model.(108) Axitinib and sunitinib were able to synergize with folate-hapten mediated immunotherapy in three syngeneic murine model of renal cell (Renca), NSCLC (M109) and lymphocytic leukemia (L1210A) through blocking of TAM, MDSC releasing/secretory VEGF, which promote neovascularization.(90)

### Cabozantinib

Cabozantinib is a kinase inhibitor targeting VEGFR2/MET and one of the two drugs tested as AI in prostate cancer.(109) Combination of cabozantinib or BEZ235 with PD-1 and CTLA-4 inhibitors had remarkable synergy compared to cabozantinib or PD-1 and CTLA-4 inhibitors alone which had minimal impact on tumor growth in a chimeric mouse model of castration refractory prostate cancer model (mCRPC).(110) MDSC targeting was felt to be the key.

### Conclusion

Immunotherapy with the checkpoint blockade has emerged as an effective way to restore antitumor immunity and terminate escape in the TME in a variety of cancers. However, innate and adaptive resistances are common and postulated to be related to the immune suppressive cells among others. Angiogenesis inhibition, another effective treatment for advanced cancer by targeting VEGF/VEGFR and restore normal vasculature, has the added benefit of immunoediting in the TME through down-regulating the function of suppressive immune cells. It is expected that combined use of these two classes of drugs will delay emergency of resistance, improve response rate and hopefully survival. Clinical trials addressing the tolerability of the combination and the efficacy are under way (refer to table 1 for ongoing clinical

trials combining pembrolizumab with AI registered in clinicaltrials.gov).

**Abbreviations**

AI: angiogenesis inhibitor; Bev: Bevacizumab; mCRPC: metastatic castration refractory prostate cancer; CTLA-4: cytotoxic T lymphocyte associated antigen-4; CD: Cluster of differentiation; DC: dendritic cells; EMT: epithelial to mesenchymal transition; ERK: extracellular regulated kinase; FDA: food and drug administration; FOLFOX: fluorouracil leucovorin oxaliplatin; FOLFIRI: fluorouracil leucovorin irinotecan; FOX-P3: fork head box P3; GBM: Glioblastoma multiforme; HCC: hepatocellular carcinoma; HIF-1 $\alpha$ : hypoxia

inducible factor-1 $\alpha$ ; IL: Interleukin; MDSC: myeloid derived suppressor cell; MEK: mitogen activated extracellular response kinase ; MO: monocytic; MET: mesenchymal epithelial transformation (receptor tyrosine kinase); OS: overall survival; PD-1: programmed death-1; PD-L1 Programmed death ligand-1; PFS: progression free survival; PGE: prostaglandin E; PI3K: Phosphatidylinositol-3-kinase; PMN: polymorphoneutrophil; RAF: Raf gene; mRCC: metastatic renal cell carcinoma; RFS: relapse free survival; ROS: reactive oxygen species; TAM: tumor associated macrophage; TIL: tumor infiltrating lymphocytes; TKI: tyrosine kinase inhibitor; TME: Tumor microenvironment; mTOR: mammalian target of rapamycin; Treg: T regulatory cells; VEGF(R): vascular endothelial growth factor (receptor).

**Table I. Ongoing clinical trials in combination with pembrolizumab**

AI in combo	Patient#	Cancer type	Phase	Primary Objective	NCT#	Status
Bev	53	Melanoma NSCLC	2	BMRR	02681549	Recruiting
Bev/XRT	42	H.G. Glioma	1	MTD	02313272	Recruiting
Bev/CPE	40	Ovarian; Fallopan, Peritoneal	2	PFS, Tolerability	02853318	Recruiting
+/-Bev	82	GBM	2	PFS, MTD	02337491	Not Rec
Bev	61	RCC	1b/2	MTD, Efficacy	02348008	Not Rec
Axitinib	60	RCC	1b	DLT	02133742	Not Rec
Axitinib	840	RCC	3	PFS, OS	02853331	Recruiting
Axitinib	30	Alveolar soft part, STS	2	PFS	02636725	Recruiting
cabozantinib	55	RCC	1/2	ORR	03149822	Not yet

BMRR: Brain metastasis response rate; CPE: Cyclophosphamide; DLT: dose limiting toxicity; H.G. High grade; MTD: maximum tolerated dosage; Not Rec: active, not recruiting; ORR: overall response rate; STS: soft tissue sarcoma; XRT: radiation therapy.

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