

Antiangiogenic therapy and immune checkpoint blockade go hand in hand

Luis Felipe Campesato, Taha Merghoub

Swim Across America and Ludwig Collaborative Laboratory, Immunology Program, Parker Institute for Cancer Immunotherapy, Memorial Sloan Kettering Cancer Center, NY, USA

Correspondence to: Taha Merghoub, PhD. Swim Across America and Ludwig Collaborative Laboratory, Immunology Program, Parker Institute for Cancer Immunotherapy, Memorial Sloan Kettering Cancer Center, NY 10065, USA. Email: merghout@mskcc.org.

Provenance: This is a Guest Editorial commissioned by Section Editor Wan Wang, PhD (Medical Technology School, Xuzhou Medical University, Xuzhou, China).

Comment on: Allen E, Jabouille A, Rivera LB, *et al.* Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. *Sci Transl Med* 2017;9:eaak9679.

Submitted Oct 06, 2017. Accepted for publication Oct 13, 2017.

doi: 10.21037/atm.2017.10.12

View this article at: <http://dx.doi.org/10.21037/atm.2017.10.12>

Immune checkpoint blockade (ICB) therapies, such as the ones targeting cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) or programmed cell death protein 1 (PD-1), have displayed durable clinical responses in various cancers and are now approved by the FDA for a growing list of cancer types (1). Although these new immunotherapies have had a notable effect on cancer treatment, multiple mechanisms of immune resistance have been described. Tumors considered immunologically “cold”, with reduced or even absent infiltration of T-cells, are a major hurdle in the cancer immunotherapy field, and strategies to overcome this resistance mechanism constitute an unmet need. Recent efforts have focused on combining this immunotherapy approach with other therapies to maximize its potential. However, the candidate for these combination therapies are numerous and therefore the need for mechanism based and rationally designed therapies is needed.

In this manuscript by Allen *et al.*, they propose to combine ICB with antiangiogenic therapy. The interest in targeting tumor vasculature has been pursued by multiple groups and the role of blood vessels in tumor progression has been investigated for more than a century (2). The requirement of a blood supply in order to sustain tumor development and its capacity to metastasize is a critical feature of cancer (3). However, tumor vasculature is generally known to be unevenly distributed and chaotic, leading to blood flow through tumors that do not follow a constant, unidirectional path. This creates zones of

ischemia and ultimately necrosis as tumors outgrow their blood supply (4). So far, more than 40 molecules have been identified to play a critical role in angiogenesis (the process of sprouting of new vessels from pre-existing ones) but most studies to date have focused on vascular endothelial growth factor (VEGF) and its receptors (5). Since 2004, ten drugs that target VEGF or its receptors have been approved for the treatment of various malignant diseases (6). Unfortunately, these agents provide modest survival benefits in some tumor types and have had no efficacy in others. The mechanism underlying this limited antiangiogenic therapy responses are the subject of intense research and will hopefully enable more efficient therapeutic interventions. Nevertheless, numerous studies demonstrate that resistance mechanisms can be regulated by immune cells, which provide a critical source of chemokines and cytokines that promote neovascularization, immunosuppression, and other tumor hallmarks (7,8).

A dysfunctional angiogenic vasculature, such as the one found in many solid tumors, prevents the extravasation of tumor-reactive T cells and fosters an immunosuppressive microenvironment that allows tumors to escape from immunosurveillance. Tumor endothelium for instance can express several inhibitory molecules that can limit the antitumor immune response. The inhibitory checkpoints Programmed cell death ligand 1 (PD-L1) and PD-L2 can be expressed by endothelial cells. Indoleamine 2,3-dioxygenase (IDO1) and T cell immunoglobulin domain and mucin

domain protein 3 (TIM3) are expressed by the tumor endothelium (9-11). There is mounting evidence that sustained angiogenesis as well as immunosuppression are interconnected processes which are facilitated by shared regulators in cancer as well as under normal physiological conditions (3). The accumulation of high levels of VEGF in many cancers has been associated with impaired immune cell trafficking and antitumor immunity (3,12). VEGF has been shown to decrease immune cell-endothelial interactions by down-regulating the expression of cell adhesion molecules such as ICAM-1 and VCAM-1 in angiogenic vessels, VEGF also has been shown to directly inhibit dendritic cell (DC) maturation and to activate antigen-specific regulatory T cells (T-reg) (13).

In addition, there is a growing list of haematopoietic cell types that, when appropriately polarized, can promote both immunosuppression and angiogenesis through production of VEGF and other factors such as bFGF, CCL2 and ANGPT2 (3). This list includes tumor-associated macrophages, myeloid-derived suppressor cells, TIE2+ monocytes, immature DCs, and T-reg cells which have been shown to have this dual capacity (3,14-16). A recent study described an involvement of phosphatidylinositol 3-kinase (PI3K) activation in myeloid cells in resistance to antiangiogenic therapy (7) and, more recently, two papers showed the role of a myeloid-specific isoform of PI3K (PI3K gamma) that when inhibited switched the activation state of myeloid cells from an immunosuppressive to a pro-inflammatory phenotype, sensitizing tumors to ICB (17,18).

In this publication by Allen and collaborators (19) they sought to investigate additional immune-related mechanisms of resistance to antiangiogenic therapy and described the effects of combining the inhibition of angiogenesis and ICB. The authors made use of three syngeneic tumor models with different responses to VEGF/VEGFR inhibitors (sorafenib and the anti-VEGFR2 antibody DC101): RIP1-Tag2 pancreatic neuroendocrine tumor model, an MMTV-PyMT mammary carcinoma model and an NFpp10-glioblastoma (GBM) model. Given that Rip-Tag2 mice are refractory to DC101 treatment at about 4 weeks of treatment; it is a good model to evaluate mechanisms associated with therapy resistance. Their findings demonstrate a substantial increase of the immune checkpoint molecule PD-L1 expression, particularly in refractory pancreatic and breast cancer models. The up-regulation of PD-L1 after antiangiogenic treatment, although more pronounced, was not restricted to tumor cells, but also observed in immune cells (CD45+) and endothelial cells (CD31+) in the tumor microenvironment

of pancreatic and breast tumors. GBM tumors, although resistant to antiangiogenic therapy, also presented with a modest increase in PD-L1 expression particularly on immune cells. Altogether, Allen *et al.* findings suggest that PD-L1 upregulation is likely not a mechanism of resistance to antiangiogenic therapy *per se*, but rather a bystander effect resulting from the induction of an inflammatory response. It would be interesting to explore effect of antiangiogenic therapy on PD-L1 expression in a tumor-independent context.

Given that PD-L1 is an important interferon (IFN)-responsive gene that is often highly expressed in inflamed tumors (20), the authors investigated whether IFN γ was differentially expressed on CD4+ and CD8+ T cells after antiangiogenic treatment. The rationale is based on preliminary evidences indicating that targeting the tumor vasculature enhances T cell trafficking into the tumor microenvironment (21). Interestingly, treatment increased the frequency of intratumoral IFN γ -expressing T cells as well as Granzyme B-expressing CD8+ T cells in all tumor models, suggesting that an immunostimulatory function is indeed occurring during VEGF/VEGFR blockade. The study would have benefited from functional assays evaluating if the noted changes of Granzyme B and IFN γ expression reflect better effector function of CD8+ T cells in the context of antiangiogenic therapy. Moreover, the authors showed augmented expression of three other IFN-responsive genes (*CXCL10*, *Mx1*, *Ifit3*) in PD-L1+ cells after therapy to support the claim that PD-L1 up-regulation is IFN-mediated. Notably, the most pronounced effects are observed in pancreatic and breast cancer models, and, despite still being present, are very modest in GBM. Given that the data is based on correlation between VEGF/VEGFR and IFN-mediated PDL1 expression the message could be strengthened with the use of IFN γ , IFN γ R-KO and/or immunodeficient mouse models to better define a causal link between them.

The authors further examined the combination of VEGF/VEGFR inhibitors with anti-PDL1 therapy and showed notable responses in terms of reduced tumor burden and prolonged survival in pancreatic and breast cancer models, but anti-PDL1 therapy was insufficient to sensitize GBM to antiangiogenic therapy. It is important to note that a number of clinical trials are now evaluating similar combinatory approaches of VEGF/VEGFR and PD-1/PD-L1 inhibition for a number of cancer types such as renal cell carcinoma, colorectal, ovarian cancer and recurrent glioblastoma (NCT03024437, NCT02659384,

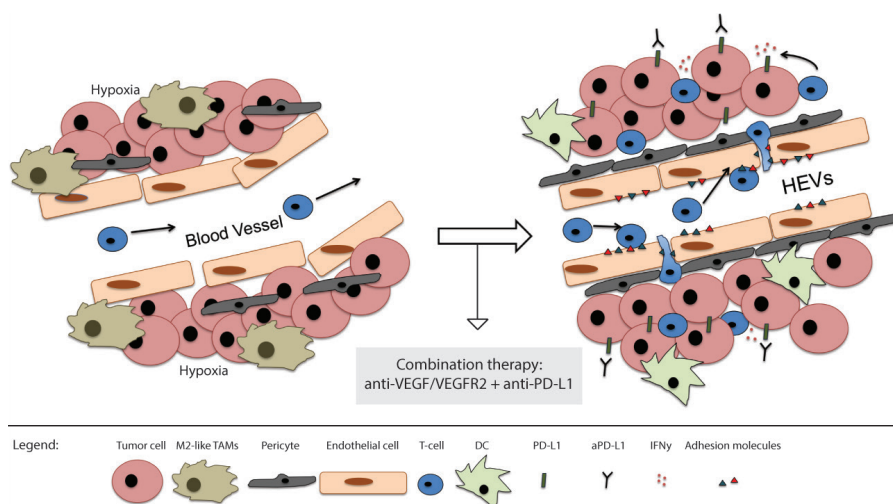


Figure 1 Illustration of proposed mechanism for combination therapy with anti-vascular endothelial growth factor (VEGF)/VEGFR2 and immune checkpoint blockade (ICB). On the left: tumor with abnormal vessels, characterized by a defective structure, poor pericyte coverage, areas of hypoxia and impaired T cell infiltration. On the right: tumor after antiangiogenic therapy with a normalized vasculature; enhanced pericyte coverage, expression of adhesion molecules and increased T cell infiltration. The formation of HEVs leads to more efficient T cell influx into tumors. IFN γ -mediated expression of immune inhibitory checkpoint molecules, such as PD-L1, can be counteracted by the use of ICB promoting more efficient tumor control.

NCT02873962, NCT02017717), with hopefully promising results for cancer patients as well. Interestingly, the combination with anti-PDL1 reversed the accumulation of PI3K activated myeloid cells (previously associated with both antiangiogenic and immunotherapy resistance) found in anti-VEGFR2 refractory pancreatic as well as breast tumors and GBMs. Moreover, the combination facilitated the infiltration of effector CD8⁺ T cells and DCs in pancreatic and breast tumors, but did not in therapy-resistant GBMs. The weak stimulation of both innate and adaptive immune cells and lack of significant T cell infiltration after combination treatment could potentially explain the therapeutic resistance observed in GBMs. The presence of tumor-infiltrating T cells is known as a rate-limiting step for therapeutic success in many tumor types. Therefore, it would be interesting to see how tumor-bearing immunodeficient mouse models respond to this combinatorial approach.

Based on the premise that the synergistic effect of antiangiogenic/anti-PD-L1 therapies is immune-dependent, the authors decided to investigate in more detail if alterations of the tumor vasculature and consequently immune cell trafficking could be responsible for the distinct outcomes of antiangiogenic/immunotherapy in the different tumor models. Interestingly, combination therapy

stimulated blood vessel normalization—characterized by reduced vessel density and permeability as well as increased pericyte coverage, leading to homogeneous blood flow—in all three tumor types, but it was less prominently in GBMs. Most importantly and possibly as their major finding, the authors demonstrate that morphological changes resembling features of high endothelial venules (HEVs) occur in several areas of the tumor vasculature of pancreatic and breast tumors during combination therapy. HEVs are specialized postcapillary vascular sites, rich in adhesion molecules—such as GlyCAM-1 ICAM-1, CD34 as well as in T cell chemokines such as CCL21—that mediate T cell adherence and transendothelial migration into secondary lymphoid organs (22). The authors provide confirmatory data, by immunohistochemical analysis using a HEV-specific MECA79 antibody, that antiangiogenic/anti-PD-L1 therapy induces the formation of HEVs only in pancreatic and breast tumors, but not in the resistant GBMs. Also, the MECA79 + HEV tumor vessels had an increased number of T and B cells, which is a common feature of normal HEVs. All together, their findings suggest that the beneficial effects of the combination therapy are likely dependent on intratumoral HEV formation, which consequently facilitates T-cell trafficking (Figure 1).

In their final results, the authors present some interesting

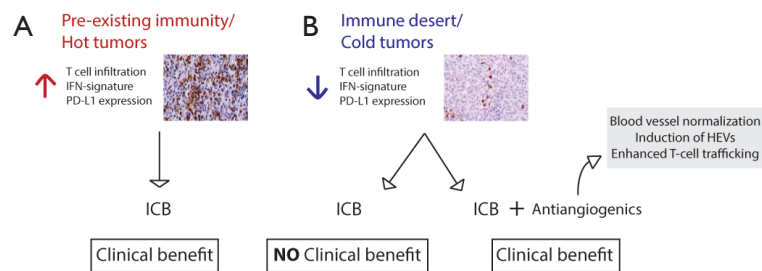


Figure 2 Proposed scheme for rational combinatorial therapy strategies based on characteristic of the tumor microenvironment. (A) Tumor with a pre-existing immunity, which are defined by high T cell infiltration, a gene signature associated with interferon (IFN) signaling and high expression of PD-L1, are exemplified in an immunohistochemistry (IHC) staining of CD8 (brown) in a tumor section. Tumors presenting these features are associated with good response to Immune checkpoint blockade (ICB) interventions alone. (B) Tumors lacking T cell infiltration (as exemplified in the IHC staining of CD8) and other inflammatory features, such as IFN-signature and PD-L1 expression, are generally not good responders to ICB therapy alone. Combinatorial strategies using antiangiogenic drugs, which promote normalization of the blood vessel structure and potentially more efficient immune cell trafficking into the tumor tissue, can work on sensitizing immune “cold” tumors to the use of ICB therapy.

data showing the induction of several lymphotoxin β receptor (LT β R)-regulated genes, including its ligand LIGHT, in HEV+ tumors upon antiangiogenic/anti-PD-L1 therapy. Lymphotoxin and LIGHT, known inducers of LT β R, are expressed by a number of activated immune cells such as DCs, Natural Killer cells, B and T cells (23,24) They proceeded to test whether stimulating LT β R signaling directly with the use of agonists or antagonists could promote HEV formation. Indeed, LT β R-activation alone and in combination with antiangiogenic/immunotherapy implicated in the formation of HEVs, increased lymphocytic infiltration and tumor protection in all tumor models, including GBMs. Further studies aiming to understand the biology behind the formation of intratumoral HEVs as well as the therapeutic potential of LT β inducers, such as LIGHT, are needed and will certainly enable the development of novel targets and treatment options for cancer patients.

In summary, overcoming immunotherapy resistance is a major focus of research in the scientific community and the identification of mechanisms based on means to facilitate blood vessel normalization and enhanced T cell trafficking has important implications on the design of more effective rationally designed combinatorial strategies. Also, understanding what makes certain tumors, such as GBMs in this study, intrinsically more resistant to the formation of HEVs and antiangiogenic/immunotherapy combination is a promising avenue of research. Successful immunotherapy must offer rational combinatorial strategies that take into consideration tumor features in a personalized manner, in order to effectively overcome resistance mechanisms

(Figure 2). Switching “cold” into “hot” tumors, based on tumor T-cell infiltration and inflammatory gene-expression signature, is an attractive modality, given the requirement of T cell infiltration in different therapeutic modalities for efficient eradication of tumor cells. The findings presented by Allen *et al.* and Schmittnaegel *et al.* (19,25), published in the same edition, move forward in that direction and introduce a proof of concept for exploring therapies targeting the tumor vasculature to enhance T cell trafficking and to sensitize tumors which are resistant to ICB.

Acknowledgements

We would like to thank Swim Across America, Ludwig Institute for Cancer Research, Parker Institute for Cancer Immunotherapy and the MSKCC Core Grant (P30 CA008748) for their support. We would also like to thank Sadna Budhu and Aditi Gupta for their critical review of this manuscript.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Khalil DN, Smith EL, Brentjens RJ, et al. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat Rev Clin Oncol*

- 2016;13:394.
2. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249-57.
 3. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat Rev Immunol* 2011;11:702-11.
 4. Nagy JA, Chang S-H, Dvorak AM, et al. Why are tumour blood vessels abnormal and why is it important to know? *Br J Cancer* 2009;100:865-9.
 5. Jayson GC, Kerbel R, Ellis LM, et al. Antiangiogenic therapy in oncology: current status and future directions. *Lancet* 2016;388:518-29.
 6. Jain RK. Antiangiogenesis Strategies Revisited: From Starving Tumors to Alleviating Hypoxia. *Cancer Cell* 2014;26:605-22.
 7. Rivera LB, Meyronet D, Hervieu V, et al. Intratumoral Myeloid Cells Regulate Responsiveness and Resistance to Antiangiogenic Therapy. *Cell Rep* 2015;11:577-91.
 8. Shojaei F, Wu X, Malik AK, et al. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol* 2007;25:911-20.
 9. Huang X, Bai X, Cao Y, et al. Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. *J Exp Med* 2010;207:505-20.
 10. Riesenber R, Weiler C, Spring O, et al. Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. *Clin Cancer Res* 2007;13:6993-7002.
 11. Rodig N, Ryan T, Allen JA, et al. Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytotoxicity. *Eur J Immunol* 2003;33:3117-26.
 12. Dirx AE, Oude Egbrink MG, Kuijpers MJ, et al. Tumor angiogenesis modulates leukocyte-vessel wall interactions in Vivo by reducing endothelial adhesion molecule expression. *Cancer Res* 2003;63:2322-9.
 13. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 1998;92:4150-66.
 14. Yang L, DeBusk LM, Fukuda K, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 2004;6:409-21.
 15. Riboldi E, Musso T, Moroni E, et al. Cutting edge: proangiogenic properties of alternatively activated dendritic cells. *J Immunol* 2005;175:2788-92.
 16. Facciabene A, Peng X, Hagemann IS, et al. Tumor hypoxia promotes tolerance and angiogenesis via CCL28 and Treg cells. *Nature* 2011;475:226-30.
 17. De Henau O, Rausch M, Winkler D, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3K γ in myeloid cells. *Nature* 2016;539:443-7.
 18. Kaneda MM, Messer KS, Ralainirina N, et al. PI3K γ is a molecular switch that controls immune suppression. *Nature* 2016;539:437-42.
 19. Allen E, Jabouille A, Rivera LB, et al. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. *Sci Transl Med* 2017;9:eaak9679.
 20. Gajewski TF, Schreiber H, Fu Y-X. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 2013;14:1014-22.
 21. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol* 2015;33:55-63.
 22. Girard JP, Springer TA. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunol Today* 1995;16:449-57.
 23. Wang Y, Zhu M, Miller M, et al. Immunoregulation by tumor necrosis factor superfamily member LIGHT. *Immunol Rev* 2009;229:232-43.
 24. Girard JP, Moussion C, Förster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat Rev Immunol* 2012;12:762-73.
 25. Schmittnaegel M, Rigamonti N, Kadioglu E, et al. Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. *Sci Transl Med* 2017;9:eaak9670.

Cite this article as: Campesato LF, Merghoub T. Antiangiogenic therapy and immune checkpoint blockade go hand in hand. *Ann Transl Med* 2017;5(24):497. doi: 10.21037/atm.2017.10.12