Neoangiogenesis in Melanoma: An Issue in Biology and Systemic Treatment

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Neoangiogenesis is a recognized hallmark of cancer, granting tumor cells to dispose of metabolic substrates through a newly created vascular supply. Neoangiogenesis was also confirmed in melanoma, where vascular proliferation is associated with increased aggressiveness and poorer prognosis. Furthermore, melanoma cells show the so-called vascular mimicry, consisting in the assumption of endothelial-like features inducing the expression of pro-angiogenic receptors and ligands, which take part in the interplay with extracellular matrix (ECM) components and are potentiated by the ECM remodeling and the barrier molecule junction alterations that characterize the metastatic phase. Although neoangiogenesis was biologically proven and clinically associated with worse outcomes in melanoma patients, in the past anti-angiogenic therapies were employed with poor improvement of the already unsatisfactory results associated with chemotherapeutic agents. Among the novel therapies of melanoma, immunotherapy has led to previously unexpected outcomes of treatment, yet there is a still strong need for potentiating the results, possibly by new regimens of combination therapies. Molecular models in many cancer types showed mutual influences between immune responses and vascular normalization. Recently, clinical trials are investigating the efficacy of the association between anti-angiogenetic agents and immune-checkpoint inhibitors to treat advanced stage melanoma. This paper reviews the biological bases of angiogenesis in melanoma and summarizes the currently available clinical data on the use of anti-angiogenetic compounds in melanoma.

Keywords: neoangiogenesis, melanoma, combination strategy, antiangiogenics, immunotherapy

INTRODUCTION

Melanoma is an aggressive cancer arising from melanocytic precursors, with high potential for locoregional and metastatic spread. As a common hallmark of cancers, when the dimensions of the primary tumoral mass of melanoma reach the threshold for nutrient diffusion, a cancer-specific net of blood vessels is fundamental to provide substrates for cancer cell survival and growth (1). The newborn neoplastic vasculature is aberrant and incomplete with distorted, dilated, and leaky vessels, insufficient pericyte coverage, abnormal endothelial cell proliferation, an uneven distribution within cancer tissues,
and wide fenestrations that ultimately contribute to tumor cell sprouting through the vascular flow (2–5). The process of activation of quiescent vasculature by cancer cells is called “angiogenic switch” and is sustained by a disequilibrium between molecular activators and inhibitors of angiogenesis, in favor of angiogenesis (6). Angiogenesis is essential in growth and progression of cancers, including melanoma (7). Warren first described angiogenesis in a melanoma graft animal model, observing remarkable angiogenic capacity of melanoma tissue (8). Since then, angiogenesis in melanoma has been further investigated to identify a possibly more complex and heterogeneous behavior in the handling of blood vessels by this neoplasm (9). First, melanoma cells induce the spread of new blood vessels by pre-existing ones; secondly, melanoma cells recruit bone marrow progenitors that reach hypoxic areas within the tumor microenvironment, where they can induce vascular formation; finally, melanoma cells themselves can acquire an endothelial-like phenotype in the so-called vascular mimicry phenomenon, directly taking part into the structure of blood vessels (9). So far, inhibitors of angiogenesis have been used in oncology in other cancers including renal, colorectal, and ovarian neoplasms, but data from clinical melanoma research are lacking. The current therapies for advanced stage disease in melanoma are based on the inhibition of either the aberrantly activated BRAF/MEK pathway, or the immune checkpoints PD1 and CTLA4. Despite obtaining previously unexpected outcomes in patients with advanced disease, leading to improved survival rates, these therapies still have potential for further improvements. This paper summarizes the biological bases of angiogenesis in melanoma matching to the most recent development of treatments, and reviews the currently available preclinical and clinical data on the use of anti-angiogenic compounds in patients with melanoma.

INSIGHTS CONCERNING THE MOLECULAR MECHANISMS OF ANGIOGENESIS

The recent evolution of melanoma treatment has led to a primary role of targeted and immune therapy both in the adjuvant and the metastatic setting. Interestingly, the molecular architecture of melanoma microenvironment is far more complex than previously thought, and a strong cross-talk has been clearly demonstrated between the angiogenic and the immune components of cancer stroma, with mutual influences in the molecular compartments that are involved in response to systemic treatments. Angiogenic factors are produced by tumor cells, stromal cells including cancer-associated fibroblasts (CAFs), and inflammatory cells like lymphocytes and macrophages. The hyperproduction of pro-angiogenic factors is induced by several mechanisms, primarily the activation of the hypoxia-induced HIF1α pathway, the oncogene-induced transcription of Vascular Endothelial Growth Factor (VEGF), and the loss of oncosuppressor genes including p53, which both stimulate the production of antiangiogenic factors like thrombospondin-1 and inhibits the expression of proangiogenic factors (10, 11).

Vascular Endothelial Growth Factor (VEGF)

VEGF-A is the archetype and most biologically relevant among proangiogenic factors, with a strong effect on endothelial survival and migration (12), and on vasculogenic mimicking properties in melanoma cells (13). VEGF-A is upregulated by HIF-1α and oncogene signaling pathways (14–16).Remarkably, VEGF-A has also shown immunosuppressive capacity. In particular, the increased production of VEGF-A in cancer leads to inhibition of T cells in several ways: 1) reducing the activity of functional T cells both directly (17) and indirectly through the endothelial PGE2-mediated suppressive action on T cells (18); 2) decreasing neoantigen presentation to lymphocytes by inhibiting the maturation of dendritic cells (DCs) (19), mainly interfering with NF-kB activation (20); 3) recruiting immunosuppressive T regulatory cells (Tregs) into the tumor microenvironment (21, 22); 4) limiting endothelial cytokine response and adhesion molecule expression, hence affecting vascular functional permeability to leukocytes and their peripheral recruitment to cancer microenvironment (23, 24). Relevantly for immunotherapy, VEGF pathway activation also enhances T cell exhaustion mediated by immune checkpoints like PDL1, CTLA4, TIM3, and LAG3 (25). While lowering the immunogenic compartment of immunity, VEGF potentiates the counteracting immunosuppressive microenvironment (26) both by recruiting immune-suppressive Tregs (27) and myeloid derived stromal cells (MDSCs) (28), and activating tumor-associated macrophages (TAMs) at the tumor site (29).

To confirm this role of VEGF, the anti-VEGF antibody Bevacizumab induces DC maturation and a reduction in Tregs and MDSCs recruitment to cancer sites (22, 30, 31). VEGF can be produced by cancer cells and immune cells from tumor microenvironment, mostly from Tregs and in smaller proportions from TAMs, MDSCs and DCs (22), creating cellular communications that either directly or indirectly convey on the inhibition of cytotoxic T lymphocytes (33).

Angiopoietin-2 (ANG-2)

ANG-2 is an antagonist cytokine of the Angiopoietin-1/Tie2 pathway that acts as a facilitator of VEGF-dependent angiogenesis (34). ANG-2 has long been considered an exclusive product of endothelium, but more recently Pari and colleagues demonstrated that it can also be produced by melanoma (35). ANG-2 levels in sera are increased in stage III and IV melanoma patients, but not in stages I and II (36). Consistently with this evidence, ANG-2 is produced by melanoma cells themselves, especially by metastatic sites (35). Differently from the stromal-derived ANG-2, melanoma-derived ANG-2 was not shown to increase the microvessel density of melanoma microenvironment but rather showed a protection of tumor cells from oxidative stress and a role in reactive oxygen species associated metastatization to the lungs in a mouse model (35). High serum ANG-2 levels were correlated with worse overall response rate to immunotherapy in melanoma (37). ANG-2 contributes to immune microenvironment composition, by acting on the Tie-2 expressing subpopulation of circulating monocytes, that are recruited by ANG-2 and converted to...
M2-like macrophages (38) and secrete IL10, which is a known promoter of Treg expansion and inhibitor of effector T cell activity (39).

**Toll-Like Receptors (TLRs)**

TLRs are a family of pattern recognition receptors involved in antimicrobial immunity, apoptotic cell clearance, and cancer. Among all family members, TLR-4 is expressed in 90% of primary and 93% of metastatic melanomas, where it plays a role in the aggressive behavior of cancer cells (40). TLR-4 signaling involves the activation of signal transducer and activator of transcription 3 (STAT3), which on turn promotes melanoma growth and aggressiveness associated features including angiogenesis and epithelial to mesenchymal transition (41). During melanomagenesis, ultraviolet radiation recruits and activates neutrophils in a TLR4-mediated mechanism, inducing an inflammation that facilitates angiogenesis and favors melanoma angiotropism (40). Moreover, STAT3 has also been associated to immunosuppression in melanoma (42).

**IMMUNE CELLS IN MICROENVIRONMENT AND ANGIOGENESIS**

In cancer microenvironment, there is a constant dynamic cross-talk between all resident cells which is far beyond the mere activity of cancer cells alone: the dynamic interaction between all cell components is responsible for the biological behavior of cancer. Accordingly, angiogenesis in cancer is not only induced by cancer cells themselves: the immune cells in tumor microenvironment can sustain angiogenesis in cancer (43). Globally, tumor cells can influence immune infiltrates towards an immune permissive phenotype. VEGF, for example, is produced by TAMs, tumor-associated neutrophils, regulatory DCs, myeloid derived suppressor cells, NK cells, and γδT17 cells (43). VEGF-R1 and -2 are expressed on DCs, which can promote angiogenesis (44). Neutrophils and TAMs secrete proangiogenic factors including VEGF, TNFα, IL8, and chemokines (45), together with matrix metalloproteases, which are essential to remodel the extracellular matrix during angiogenesis and metastatization (46). During melanomagenesis, an angiogenic stimuli such as TNFα and IL8 can serve as recruitment signals for myeloid cells to the tumor microenvironment (43, 44). Moreover, neutrophils can release proangiogenic factors such as VEGF (45). A reciprocal cellular interaction consisting in the so-called angiocrine signaling that is essential in normal organ development (50) and can be exacerbated in cancer. Tumor cells can then induce endothelial activation mediated by CKs including Angiopoietin-2, which are responsible for the autocrine induction of STAT3 signaling in the endothelium, followed by the expression of chemokines (CCL2) and adhesion molecules (ICAM1) that recruit CCR2+ macrophages to the cancer site (37). Endothelial cells take part in granulocyte differentiations in physiology and pathology, given the common developmental origin between endothelial cells and hematopoietic cells (37): the endothelium secretes CKs (SCF, CXCL12) that contribute to the quiescence of hematopoietic cells in the bone marrow, but can also promote granulopoiesis in case of inflammation (51). The hypoxia-regulated Endothelin B receptor on tumor endothelium acts as an obstacle to T cell adhesion and has been identified in some cases of resistance to immune therapy (52).

**MANIPULATION OF ANGIOGENESIS BY THERAPEUTIC AGENTS IN MELANOMA**

Antiangiogenics are a class of kinase inhibitors that bind either angiogenic factors or their receptors. The first antiangiogenic agent to be developed was Bevacizumab, an anti-VEGF monoclonal antibody, which is still indicated in the treatment of cancers including colorectal, ovarian, or uterine carcinomas. Many other agents were synthesized furtherly, presenting a wider spectrum of pharmacodynamic targeting, including Sunitinib, Pazopanib, Ramucirumab, Regorafenib, Sorafenib, Aflibercept, being so far approved in daily practice either alone or in combination. Antiangiogenics agents model the irregular and leaky vessels of cancer to create an almost normalized intratumoral vascular network, at least transiently. Such improved vascular efficiency of cancer microcirculation is thus the main responsible for a more efficient transport of chemotherapeutic agents to cancer cells, and also the molecular background to the association of antiangiogenic therapies with traditional chemotherapies (53). Part of the effects of antiangiogenics in cancer may also be attributed to the modulation of immune cell composition in tumor microenvironment, triggered by the reduction in the tissue hypoxia that is associated with the immature cancer vasculature. The response to hypoxia favors the polarization of the tumor microenvironment towards an immune-suppressive phenotype in terms of increase in Tregs and M2-TAMs, reduction of DC activity, and increase in PD-L1 expression on endothelial cells, TAMs, DCs, and cytotoxic lymphocytes (53). Antiangiogenics can then interfere with both CD8+ T cells trafficking and TAMs repolarization, inducing an immunostimulatory milieu (37). Anti-VEGF-A agents also improve immune responses (54). In a preclinical model of antiangiogenic-driven vascular normalization in melanoma and other primary cancers, Schmittnaegel et al. demonstrated that the administration of a bispecific anti-VEGF-ANG2 antibody was associated with increased recruitment and
receptors can prolong the maintenance of sensitivity to transformed into Vemurafenib-resistant, and silencing of such melanoma cell lines express higher VEGF-receptors when Vemurafenib (67). As evidenced by Aztori and colleagues, proliferation and macrophage-mediated angiogenesis (62). Furthermore, the pathway of VEGFR-1 has recently been identified as an escape mechanism to the BRAF-inhibitor Vemurafenib (67). As evidenced by Aztori and colleagues, melanoma cell lines express higher VEGF-receptors when transformed into Vemurafenib-resistant, and silencing of such receptors can prolong the maintenance of sensitivity to Vemurafenib (67). Together with targeted therapy for BRAF mutated patients, the backbone of treatment in advanced melanoma is represented by the immune therapy with either anti-CTLA4 or anti-PD1 agents, that remove immune checkpoint inhibition to potentiate the immune response to melanoma. Despite an overall improvement in the outcomes of treatment, still immune therapy is often associated to secondary resistance and progression and, yet more rarely, to early resistance. Among the possible mechanisms underpinning these resistances, recent evidence also identified some vascular-related mechanisms. In particular, high serum ANG-2 levels correlated with worse overall response rate to ICI therapy in melanoma, possibly because ANG-2 can recruit monocytes and induce PDL1 expression in M2-macrophages (37). Wu and colleagues identified a subset of melanoma patients characterized by a significant tumor infiltration of CD68+ macrophages that particularly responded to treatment with Ipilimumab and Bevacizumab with a neat decrease in ANG-2 expression (68). Allen and colleagues demonstrated that the combination therapy of antiangiogenics and anti-PD1 agents induced an increase in intratumoral high endothelial venules responsible for selective leukocyte infiltration and for the switch of microenvironment towards immunosensitive features (69). A bispecific anti-VEGFA and anti-ANG2 was also shown to potentiate the efficacy of an anti-PD1 treatment (55).

**DISCUSSION: EVIDENCE FROM CLINICAL TREATMENT OUTCOMES AND POSSIBLE PERSPECTIVES**

The first trials involving antiangiogenetic drugs in melanoma date back to early experiences two decades ago, when chemotherapy was the only available treatment for advanced stage disease, with palliative intent and dramatically poor outcomes. Most of these studies are phase 1 or 2 trials for stage III unresectable or stage IV melanoma, either in single or double arm of treatment, with small cohorts of recruited patients, usually 20–30 (Table 1). Despite preclinical data suggesting the advantage of a more regular vascular network in the distribution of chemotherapics to cancer cells, these trials did not provide satisfying results from the association of antiangiogenics with common chemotherapics, showing no statistically significant improvement in the outcomes of traditional chemotherapy schedules, therefore they have never been investigated in wider phase 3 clinical trials and have never been adopted in everyday practice. More recently, immune therapy became the new gold standard for systemic therapy, together with anti-BRAF and anti-MEK targeted therapy for BRAF mutated patients, and was tested in the association with antiangiogenetic agents, given the evidence of efficacy from the combination of antiangiogenics with ICIs not only in preclinical, but also in the clinical settings for other cancers including renal clear cell or non-small cell lung cancer. Hodi and colleagues performed an investigational phase I trial in 46 patients with advanced melanoma without brain metastases receiving a first (37%) or second (63%) line treatment with Ipilimumab and Bevacizumab: the best overall response rate (ORR) was 19.6% with a disease control rate of 67.4% and a median time to progression of 9 months (78). The immunohistochemical analysis of serial biopsies of target lesions revealed changes in melanoma-associated endothelium with increased expression of E-selectin (78). In a more recent phase IB/II trial, Taylor and colleagues treated patients with advanced solid tumors including melanoma with the association of Pembrolizumab and Lenvatinib, an inhibitor of multiple kinases including VEGFR. Among the 21 patients of the melanoma subcohort, the ORR was 33% and also...
These encouraging data are now furtherly being investigated in a randomized phase III trial specifically dedicated to advanced melanoma. Moreover, other studies are currently ongoing, mostly still in the recruitment phase, for treatment associations of antiangiogenics with anti-PD1 (Nivolumab or Pembrolizumab) or anti-PDL1 (Avelumab) inhibitors (Table 2). As previously described, angiogenesis plays a major role in the natural history of melanoma, from its intrinsic aggressiveness to some forms of resistance to systemic therapy. Despite widely intertwined

| Reference | Phase | Clinical setting | Line of treatment in metastatic setting | Arm 1 | Arm 2 | Primary endpoints | Secondary endpoints | Further analyses |
|-----------|-------|------------------|----------------------------------------|-------|-------|-------------------|---------------------|------------------|
| Del Vecchio et al. (70) | II | Stage IV cutaneous melanoma. | 1st line | Bevacizumab + Fotemustine (20 pts) | None | CR 1/20 | TTP 8 m | OS 20 m | Reduction of VEGF levels post-therapy |
| Tarhini et al. (71) | II | Stage III unresectable or stage IV cutaneous melanoma. No active brain metastases. | 1st line or further | Alllhercept (40 pts) | None | ORR 7.5% | PFS 4 m | OS 16 m | Hypertension correlated with OS |
| Von Moos et al. (72) | II | Stage IV cutaneous melanoma. No brain metastases. | 1st line | Bevacizumab + Temozolomide (62 pts) | None | SD 52% | ORR 16% | PFS 4 m | OS 10 m |
| Kim et al. (73) | II | Stage IV melanoma. No brain metastases. | 1st line | Carboplatin + Paclitaxel + Placebo (71 pts) | Carboplatin + Paclitaxel + Bevacizumab (143 pts) | PFS 4 vs 5 m | OS 9 vs 12 m | OR 11/67 vs 36/141 |
| Schuster et al. (74) | II | Stage IV melanoma. No brain metastases. | 2nd line | Bevacizumab (35 pts) | None | DCR 31% | OS 9 m | OS 16 m | Toxicity 7/11 pts who had disease control developed hypertension |
| Minor et al. (75) | II | Stage IV melanoma. No active brain metastases. cKit mutated. | 2nd line or further. No prior immunotherapy. | Sunitinib (10 pts) | None | ORR 3/4 in mutated cKIT pts; 1/6 in amplified or overexpressed cKIT pts. | PFS 8 m | G3-G4 toxicity 43% | Low VEGF values correlated with longer PFS |
| Mahalingam et al. (76) | II | Stage III unresectable or stage IV cutaneous melanoma. No active brain metastases. | 2nd or 3rd line | Bevacizumab + Sorafenib (14 pts) | None | ORR 0% SD 21% | PFS 8 m | G3-G4 toxicity 43% | |
| Ferrucci et al. (77) | II | Stage IV cutaneous melanoma. No brain metastases. | 1st line | Bevacizumab + Dacarbazine (40 pts) | None | ORR 19% | TTP 5 m Discontinuation 92% G3-G4 toxicity 22% | Recruitment completed. (24 enrolled pts vs 176 initially designed) |
| NCT02158520 | II | Stage IV melanoma. No brain metastases. | 1st line or further | Nab-Paclitaxel + Bevacizumab (12 pts) | Ipilimumab (12 pts) | PFS 129 vs 94 days | OS 18 vs 27 m ORR (2 vs 0 CR; 1 vs 1 PR) G3-G4 Toxicity (9 vs 7) | |

TABLE 1 | Clinical trials of the association of antiangiogenic treatments with old drugs in advanced melanoma.

Included 1 complete response, while the disease control rate was 81% (79). Recently, Arance and colleagues presented the preliminary results of the phase 2 LEAP004 trial investigating the association of Lenvatinib and Pembrolizumab in 103 patients with advanced melanoma progressing on immunotherapy in second or further line of treatment. The median progression-free survival was 4.2 months with a median overall survival of 13.9 months and a 21.4% response rate, supporting a possible role in overcoming resistance to immunotherapy by Lenvatinib (80).
mechanisms between angiogenesis and immunity, the efficacy of antiangiogenic therapies is currently insufficient. Hence, much interest is addressed to the ongoing clinical trials of combined antiangiogenic and immune therapies, to pursue better outcomes in the therapy of advanced melanoma.

**REFERENCES**

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013

2. Cooke VG, Le Bleu VS, Keskin D, Khan Z, O’Connell JT, Teng Y, et al. Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. Cancer Cell (2012) 21(1):66–81. doi: 10.1016/j.ccell.2011.11.024

3. Nagy JA, Chang SH, Shih SC, Dvorak AM, Dvorak HF. Heterogeneity of the tumor vasculature. Semin Thromb Hemost (2010) 36(3):321–31. doi: 10.1055/s-0030-1253454

4. Baluk P, Hashizume H, McDonald DM. Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev (2005) 15(1):102–11. doi: 10.1016/j.gde.2004.12.005

5. Jain RK. Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. Cancer Cell (2014) 26(5):605–22. doi: 10.1016/j.ccell.2014.10.006

6. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell (1996) 86(3):353–64. doi: 10.1016/s0092-8674(00)08010-7

7. Cho WC, Jour G, Aung PP. Role of angiogenesis in melanoma progression: update on key angiogenic mechanisms and other associated components. Semin Cancer Biol (2019) 59:175–86. doi: 10.1016/j.semcancer.2019.06.015

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.
21. Li B, Lalani AS, Harding TC, Luan B, Koprivnikar K, Huan Tu G, et al. Distinct...  
28. Huang Y, Chen X, Dikov MM, Novitskiy SV, Mosse CA, Yang L, et al. Effect of...  
17. Ziogas AC, Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Terpos E, et al. VEGF directly suppresses activation of T cells from ovarian cancer patients.

16. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer.

15. Mac Gabhann F, Popel AS. Systems biology of vascular endothelial growth factor.
51. Boettcher S, Gerosa RC, Radpour R, Bauer J, Ampenberger F, Heikenwalder M, et al. Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. *Blood* (2014) 124(9):1393–403. doi: 10.1182/blood-2014-04-70762

52. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, Balint K, et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat Med* (2008) 14(1):28–36. doi: 10.1038/nm1699

53. Dewhirst MW, Second WM. Transport of drugs from blood vessels to tumour tissue. *Nat Rev Tumour* (2017) 17(12):738–50. doi: 10.1038/nrct.2017.93

54. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. *Front Immunol* (2018) 9:978. doi: 10.3389/fimmu.2018.00978

55. Schmittmagen M, Rigamonti N, Kadioglu E, Cassara A, Wyser Rmll C, Kialainen A, et al. Dual angiopeptin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. *Sci Transl Med* (2017) 9(385):eaak9670. doi: 10.1126/scitranslmed.aak9670

56. De Palma M, Buiato D, Petrucco TV. Microenvironmental regulation of tumor angiogenesis. *Nat Rev Cancer* (2017) 17(8):457–74. doi: 10.1038/nrct.2017.51

57. De Almeida PE, Mak J, Hernandez G, Jesudason R, Herault A, Javinal V, et al. Anti-VEGF Treatment Enhances CD8+ T-cell Antitumor Activity by Amplifying Hypoxia. *Cancer Immunol Res* (2020) 8(6):806–18. doi: 10.1158/2326-6066.CIR-19-0360

58. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, et al. Kinetics of vascular normalization by VEGF/VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopeptin-1, and matrix metalloproteinases. *Cancer Cell* (2004) 6(6):553–63. doi: 10.1016/j.ccr.2004.10.011

59. Huang Y, Yuan J, Righi E, Kamoun WS, Anczkiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci U S A* (2012) 109(43):17561–6. doi: 10.1073/pnas.1215397109

60. Šenbabaoglu Y, Gejman RS, Winer AG, Liu M, Van Allen EM, de Velasco G, and the copyright owner(s) are credited and that the original publication in this journal is reproduced is permitted which does not comply with these terms.

61. Donnel T, Reynolds AR, Kuczynski EA, Gatter K, Vermeulen PB, Kerbel RS, et al. FOXP3+ T regulatory lymphocytes in primary melanoma are associated with previously untreated advanced melanoma. *J Clin Oncol* (2012) 30(34):34–41. doi: 10.1200/JCO.2012.43.6270

62. Schuster C, Eikesdal HP, Puntervoll H, Geisler J, Geisler S, Heinrich D, et al. Phase II study evaluating the efficacy, safety, and pharmacodynamic correlative study of dual antiangiogenic inhibition using bevacizumab in combination with sorafenib in patients with advanced malignant melanoma. *Cancer Chemother Pharmacol* (2014) 74(1):77–84. doi: 10.1007/s00280-014-2479-8

63. Ferrucci PF, Minchella I, Mosconi M, Gandini S, Verrecchia F, Coccoricchio E, et al. Dacarbazine in combination with bevacizumab for the treatment of unresectable/metastatic melanoma: a phase II study. *Melanoma Res* (2015) 25(3):259–65. doi: 10.1097/CMR.0000000000000146

64. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res* (2014) 2(7):632–42. doi: 10.1158/2326-6066.CIR-14-0053

65. Taylor MH, Lee CH, Makker V, Rasco D, Dutcus CE, Wu J, et al. Phase I/II Trial of Lenvatinib Plus Pembrolizumab in Patients With Advanced Renal Cell Carcinoma, Endometrial Cancer, and Other Selected Advanced Solid Tumors. *J Clin Oncol* (2020) 38(11):1154–63. doi: 10.1200/JCO.19.01598

66. Arance Fernandez AM, O’Day SJ, de La Cruz Merino L, Petrella T, Jamar R, Ny L, et al. Lenvatinib (len) plus pembrolizumab (pembro) for advanced melanoma (MEL) that progressed on PD1 or PDL1 inhibitor: initial results of an ongoing randomized phase II study in patients with previously untreated melanoma. *Clin Cancer Res* (2020) 26(1):34–40. doi: 10.1158/1078-0432.CCR-19-1057