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## Normalizing Tumoral Vessels to Treat Cancer: An Out-of-the-Box Strategy Involving TIE2 Pathway

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### Angiogenesis and Cancer

The idea of targeting vessels for cancer therapy, termed antiangiogenesis, was coined by Folkman in his pioneer report in 1971 (1, 2). Folkman's seminal contribution to cancer biology was the early description of what we now understand as the tumor microenvironment (2). In this manuscript, he postulated that tumor growth depends on vessel recruitment and that the growth of vascular and tumor cells are interdependent. Per instance, he stated that the maintenance of the mitotic index of the two cell populations depends on each other, and that the secretion of diffusible factors from tumor cells influences the formation of tumor capillaries (1). Furthermore, he described that the blockade of pro-angiogenic signals resulted in the regression of the new blood vessels. In addition, he hypothesized that central tumor necrosis is the consequence of poor perfusion due to increased internal pressure and decreased of the blood flow at the tumor site (1, 2). This hypothesis was tested by Rakesh Jain who demonstrated that Folkman's assumption was correct, and further suggested that increased interstitial fluid pressure might impede the delivery of large anticancer agents to tumors (2, 3).

### Antiangiogenic Therapy

The conceptual basis of antiangiogenic therapy was based on eradicating tumors by destroying their vascular structures and, subsequently, depriving cancer cells of nutrients and oxygen. Although the description of a blood vessel growth stimulator factor was dated in 1939 (4), the discovery of vascular endothelial growth factor, VEGF, was what constituted

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the first tangible milestone toward an antiangiogenic therapy (5, 6). Years of exciting research in this area culminated with the development of bevacizumab, a recombinant humanized VEGFA-specific monoclonal antibody. Bevacizumab was subsequently approved by the FDA in 2004 as the first-line treatment for metastatic colorectal cancer (7), and later approved in 2009 for a second line treatment for glioblastoma (8). These advancements inspired the development of many other VEGFA signaling pathway inhibitors such as sunitinib, sorafenib, aflibercept, and ramucirumab (4). Despite the initial excitement and some modest benefits observed in clinical trials, the administration of antiangiogenic therapy as single agents did not yield long-term survival benefits. For instance, treatment with bevacizumab did not improve survival in two major studies of patients with glioblastoma (8). For that reason, ongoing studies are in place to further understand the molecular mechanisms of resistance of solid tumors to anti-VEGF therapy.

## Normalization of Tumoral Vessels

While angiogenesis is a hallmark of tumors, tumor vascular structures are architecturally and functionally abnormal. These vessels are tortuous with increased permeability that results in higher interstitial fluid pressure, further challenging the arrival of oxygen and nutrients. Despite this fact, it is worthy to note that antiangiogenic therapy administered in combination with chemotherapy in patients with metastatic colon cancer produced an unprecedented increase in survival (7). Multiple mechanisms might be behind this effect, such as the combinatorial therapy being able to target multiple compartments within the tumor including endothelial and cancer cells (9). However, Jain's group has extensively worked to test an alternative, and perhaps a more relevant, hypothesis. According to this group of investigators, by applying antiangiogenic agents to the tumor, the vasculature will be normalized to allow proficient delivery of oxygen and drugs to the cancer cells (10, 11). In agreement with this model, VEGFR2 inhibition transiently normalized glioma vasculature resulting in a reduced intracranial edema and improved response to radiotherapy of mice bearing gliomas (12, 13). Further supporting Jain's hypothesis, results from phase II trials showed an association between parameters related to vascular normalization, such as increased perfusion and oxygenation, with survival. Thus, treatment with cediranib induced transient vessel normalization in patients with recurrent gliomas, measured as decreased microvessel diameter and permeability, effectively reducing edema (11, 14, 15). The vessel normalization effect was not restricted to brain tumors and was also observed in other kind of cancers. For instance, tumors from patients with HER2-negative breast cancer treated with bevacizumab showed signs of vascular normalization, such as increased pericyte-covered microvascular density and decreased interstitial fluid pressure (16).

## TIE2 Pathway as an Alternative Target Approach to Normalize Tumoral Structures

The time window of opportunity to utilize (i.e. administer additional therapies) the vessel normalization phase after anti-VEGF/VEGFR2 is too narrow and therefore other strategies are being explored. These approaches are mainly focused on targeting the TIE2/Angiopoietin pathway. TIE2 is a tyrosine kinase receptor initially described in the

membrane of endothelial cells and hematopoietic precursor (17), that has later been found to be expressed in other non-endothelial cells, such as cancer cells, pericyte precursors, and specific monocytic populations (18-20). TIE2 signaling is regulated upon binding to its ligands, angiopoietins, such as Angiopoietin1 (ANG1) and 2 (ANG2) which play a dynamic role in vessel formation, maintenance, and permeability. Thus, ANG1 stimulation of TIE2 tightens endothelial junctions resulting in blood vessel stabilization. ANG2 is often upregulated in gliomas, and plays a role in vessel destabilization in low VEGF levels conditions, or a pro-angiogenic role in high VEGF levels conditions (20). Interestingly, ANG2 has been reported to be overexpressed in tumors after anti-angiogenic therapies (14, 21), and it is known to act as a chemoattractant of TIE2-expressing monocytes (21). Overrepresentation of this specific monocytic population might be related to the tumor escape to antiangiogenic therapies (18). In addition, ANG1/ANG2 ratio correlated positively with vascular normalization (14).

Several reports have previously tested the possibility of normalizing tumor vasculature to treat cancer by targeting both the VEGF and the TIE2/ANG pathway. In this regard, the dual inhibition of VEGF by cediranib and ANG2 by a neutralizing antibody, MEDI3617, proved to prolong the survival of mice bearing malignant gliomas (22). Recently, Park and colleagues (23) explored the effect of an antibody, ABTAA, which exerts a dual action on TIE2 pathway (ANG2-binding and TIE2 activating antibody) on three murine models of cancer: orthotopic implantation of gliomas, Lewis lung carcinoma, and spontaneous mammary cancer. The authors reported that TIE2 activation induced vascular normalization in the tumors, which resulted in enhanced blood perfusion. Of interest, chemotherapeutic drug delivery was increased and resulted in reduced tumor growth and metastasis. While an ANG2 blocking antibody (ABA) has some effect on these models, it was more moderate than the effect achieved using the (TIE2-ANG2) dual targeting. Thus, administration of ABTAA or ABA (10mg/kg) to glioma bearing mice resulted to a 39% or 17% reduction of tumor volume, respectively. Park and colleagues suggest that the superiority of ABTAA versus ABA is due to the inability of ANG2 blocking agents to decrease tumor hypoxia, which significantly contributes to the increase in the levels of angiogenic factors and the invasive pattern of tumors observed after antiangiogenic therapies (21, 24). Interestingly, when ABTAA was combined with temozolamide, the standard chemotherapy for glioma treatment, the tumor volume was inhibited 75%, suggesting that ABTAA enhanced drug delivery. In lung and breast cancer models, the effect of the new agent was also abscopal, decreasing metastasis formation (23). Another interesting aspect of this work lies in the changes observed on the immune cell infiltration. Park and colleagues also described in their manuscript that the treatment with ABTAA promotes the tumor infiltration and polarization of M1-like tumor associated monocytes due to the reduction of ANG2 and lactate, suggesting the production of a favorable anti-tumor microenvironment (23). This is of significance because escape and resistance to antiangiogenic therapy has been linked by several studies to the infiltration of tumor-associated macrophages (18, 25).

Interestingly, these authors previously reported the use of ABTAA in treating sepsis (26), a disease, like cancer, associated to inflammation, vascular leakage, and increased ANG2 levels (27, 28). Their data, using a novel model of murine severe sepsis (high-grade cecal ligation and puncture, CLP), showed that treatment of these animals with 10mg/kg of

ABTAA at 6 and 18 hours after CLP reduced cytokine storms and vascular leakage, and strengthened the endothelial glycocalyx. The antibody was also tested in other models of sepsis, such as intraperitoneal *S. aureus* inoculation or LPS injection. The use of ABTAA reduced significantly organ damage and sepsis mortality in the tested models (26). In summary, targeting the TIE2/ANG axis results in vascular normalization and reduces inflammation in sepsis.

We have recently reported the nuclear translocation of TIE2 upon ANG1 binding. Nuclear TIE2 seems to act as a resistance factor to genotoxic stress by modifying the epigenetic pattern of tumor cells (29). In this regard, nuclear localization of TIE2 is related to enhanced DNA repair and radioresistance (29). Further investigation is needed to understand if nuclear TIE2 is a key player also in the resistance to DNA-damaging chemotherapeutic agents, and if this function will be jeopardized by the administration of ABTAA or similar TIE2-targeting agents.

## Conclusions

For several decades, researchers have searched for drugs that will decrease the blood flow of tumors with the goal of depriving tumoral cells from oxygen and nutrients. The development of new biological agents that might normalize tumoral vasculature to improve the delivery of chemotherapeutic drugs into the core of tumors is a more recent strategy that needs to be further tested in the clinical settings. The possibility of reducing metastasis while ‘helping’ the tumor to obtain oxygen and nutrients seems counterintuitive but merits further investigation. Maybe, as Mina Bissell’s theory of “dynamic reciprocity” states, in a healthy micro-environment tumor cells with abnormal genomes should be capable of becoming normal (30), and thus, reverting ‘tumoral’ vessels to ‘normal’ vessels to target the core of the tumor should be right way of proceeding. To achieve these objectives efficient manipulation of the TIE2/ANG pathway seems to be critical.

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