Hypertension

REVIEW

Bevacizumab-Induced Hypertension in Glioblastoma Patients and Its Potential as a Modulator of Treatment Response

Kaitlin G. Scheer, Lisa M. Ebert, Michael S. Samuel, Claudine S. Bonder, Guillermo A. Gomez

ABSTRACT: Glioblastoma invasion is the primary mechanism responsible for its dismal prognosis and is the direct result of interactions between glioblastoma cells and the tumor vasculature. The dysregulated microvasculature in glioblastoma tumors and vessels co-opted from surrounding brain tissue support rapid tumor growth and are utilized as pathways for invasive cancer cells. Attempts to target the glioblastoma vasculature with antiangiogenic agents (eg, bevacizumab) have nonetheless shown limited and inconsistent efficacy, and the underlying causes of such heterogeneous responses remain unknown. Several studies have identified that patients with glioblastoma who develop hypertension following treatment with bevacizumab show significant improvement in overall survival compared with normotensive nonresponders. Here we review these findings and discuss the potential of hypertension as a biomarker for glioblastoma treatment response in individual patients and the role of hypertension as a modulator of interactions between tumor cells and cells in the perivascular niche. We suggest that a better understanding of the actions of bevacizumab and hypertension at the cellular level will contribute to developing more effective personalized therapies that address glioblastoma tumor cell invasion.

Key Words: bevacizumab ■ biomarkers ■ brain ■ glioblastoma ■ hypertension ■ invasion

Glioblastoma is the most common and aggressive form of brain cancer, with <5% of patients achieving 5-year survival.[1,2] Despite concerted research efforts over the past 3 decades, overall survival (OS) for glioblastoma has increased by only 3 months, extending median survival from 12 to 15 months following introduction of the Stupp protocol in 2005.[3,4] This protocol remains the standard of care for patients with glioblastoma and combines maximal surgical resection of the primary tumor and postoperative radiotherapy with concurrent Temozolomide administration.[4] Unfortunately, patients treated with the Stupp protocol invariably experience therapy resistant tumor recurrence.[5]

Interactions between cancer cells and the microenvironment, particularly those cells comprising the vessels within the tumor, contribute to therapy resistance.[6] Glioblastoma vascularization is a prognostic marker predictive of patient survival, with increased tumor microvessel density correlating with poorer OS.[7–9] Angiogenesis in glioblastoma is predominantly attributed to the upregulation of VEGFA (vascular endothelial growth factor A), which stimulates endothelial cell (EC) survival, proliferation, and migration as well as EC progenitor differentiation.[10–12] Of note, the existing brain vasculature is insufficient to sustain rapidly growing glioblastoma, which inevitably results in the generation of hypoxic gradients within the tumour.[13] Hypoxia-induced VEGFA expression subsequently stimulates the formation of new blood vessels[13,14] (Figure 1A), which are tortuous, leaky, and poorly structured in comparison to vessels in the healthy brain.[15] Thus, although glioblastoma tumors are highly

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Nonstandard Abbreviations and Acronyms

EC	endothelial cell
eNOS	endothelial NO synthase
NO	nitric oxide
NOX1	NADPH oxidase 1
OS	overall survival
ROS	reactive oxygen species
VEGFA	vascular endothelial growth factor A

vascularized, the poorly formed neovessels that originate within the tumors do not alleviate hypoxia. As a result, cancer cells in hypoxic regions of the tumor often switch from a proliferative to a more migratory and invasive mesenchymal phenotype.[16,17] These cells then not only escape and migrate away from hypoxic regions by invading the surrounding healthy tissue but also undergo transcriptional changes that additionally increase their resistance to therapy.[18]

Blockade of specific vascular development pathways with bevacizumab, a VEGFA inhibitor, was anticipated to be an effective treatment for glioblastoma.[19] However, while bevacizumab showed promise in early clinical trials, it has not consistently yielded an OS benefit for patients.[20] Indeed, treatment with bevacizumab is associated with increased tumor invasion.[21–23] Intriguingly, a subset (≈40%) of patients who develop hypertension following bevacizumab treatment exhibit significant survival benefits compared with normotensive patients.[24–27]

Despite the limited success and its association with an invasive phenotype, bevacizumab is being broadly adopted to treat recurrent glioblastoma. This reliance on bevacizumab emphasizes the importance of unravelling the mechanisms contributing to disparate patient responses to this agent. In particular, understanding the role of tumor-vessel interactions and the development of hypertension is likely to be critical for identifying glioblastoma patients who benefit the most from receiving bevacizumab.

HYPERTENSION AS A BIOMARKER OF RESPONSE TO BEVACIZUMAB TREATMENT IN GLIOBLASTOMA

Antiangiogenic agents were tested with the goals of normalizing aberrant vasculature (and thus reducing edema), disrupting the glioblastoma perivascular niche, and improving access of other chemotherapeutics to the tumor.[28] A large majority of antiangiogenic compounds tested for efficacy against glioblastoma target VEGFA signaling. This is through inhibition of VEGFA ligand, its principal receptor VEGFR2/KDR, or downstream signaling molecules.[20] For a detailed review of antiangiogenic drugs trialed for glioblastoma, see Anthony et al.[20] The most extensively studied drug, bevacizumab, is a recombinant humanized monoclonal antibody that binds VEGFA, preventing receptor interaction and blocking VEGFA-mediated proangiogenic signaling.[29] In 2009, the United States Food and Drug Administration approved bevacizumab treatment for recurrent glioblastoma based on the promising results in 2 phase II clinical trials.[30] Elsewhere, applications for bevacizumab treatment of glioblastoma met with resistance due to insufficient evidence of efficacy.[31,32] Approval was eventually granted in some countries[33,34] and it is used off-label in others,[35] despite limited evidence of consistent or substantial efficacy.[32,34] The improvement in progression-free survival commonly reported over the preceding 10 years of clinical trials is now referred to as a pseudoresponse.[36] This is because bevacizumab stabilizes the vasculature by inhibiting VEGFA-induced vessel permeabilization,[37] and thereby restores blood-brain barrier integrity, leading to skewed magnetic resonance imaging readouts due to marked decreases of hyperperfusion, edema, and contrast enhancement.[36]

The most reported adverse event following administration of bevacizumab is hypertension (grade 2–3), which affects ≈40% of treated patients[24–27,38,39] (Figure 1B). Interestingly, with the exception of 1 small prospective study,[38] patients who developed hypertension following bevacizumab treatment demonstrated prolonged progression-free survival and OS, with statistically significant increases in OS ranging from 5 to 9 months compared with normotensive patients.[24–27] Furthermore, bevacizumab-induced hypertension was shown by multivariate analysis to independently predict improved OS.[24–27,39] It is, therefore, possible that the inconsistencies of reported bevacizumab efficacy across different clinical trials may be a result of the large proportion of nonresponders statistically masking the benefit experienced by the smaller cohort of responders. Given these findings, it is now urgent to identify which patients will derive the most benefit from bevacizumab.

BEVACIZUMAB AND HYPERTENSION CAUSE MICROVASCULAR DEFECTS

It is thought that the vascular changes induced by bevacizumab confer the survival benefit in responsive patients, with these changes causing hypertension in many patients.[40] As bevacizumab causes a reduction in both vascular density (rarefaction) and production of the vasodilator nitric oxide (NO) production,[41] these are suspected to be causal factors in hypertension pathogenesis following treatment.[42,43] Microvascular rarefaction is also common early in the development of hypertension[44]; however, it is unclear to what extent this contributes to the onset of increased blood pressure. Similarly, EC dysfunction and vascular constriction due to reduced availability of NO, an essential vasodilator, can be both cause

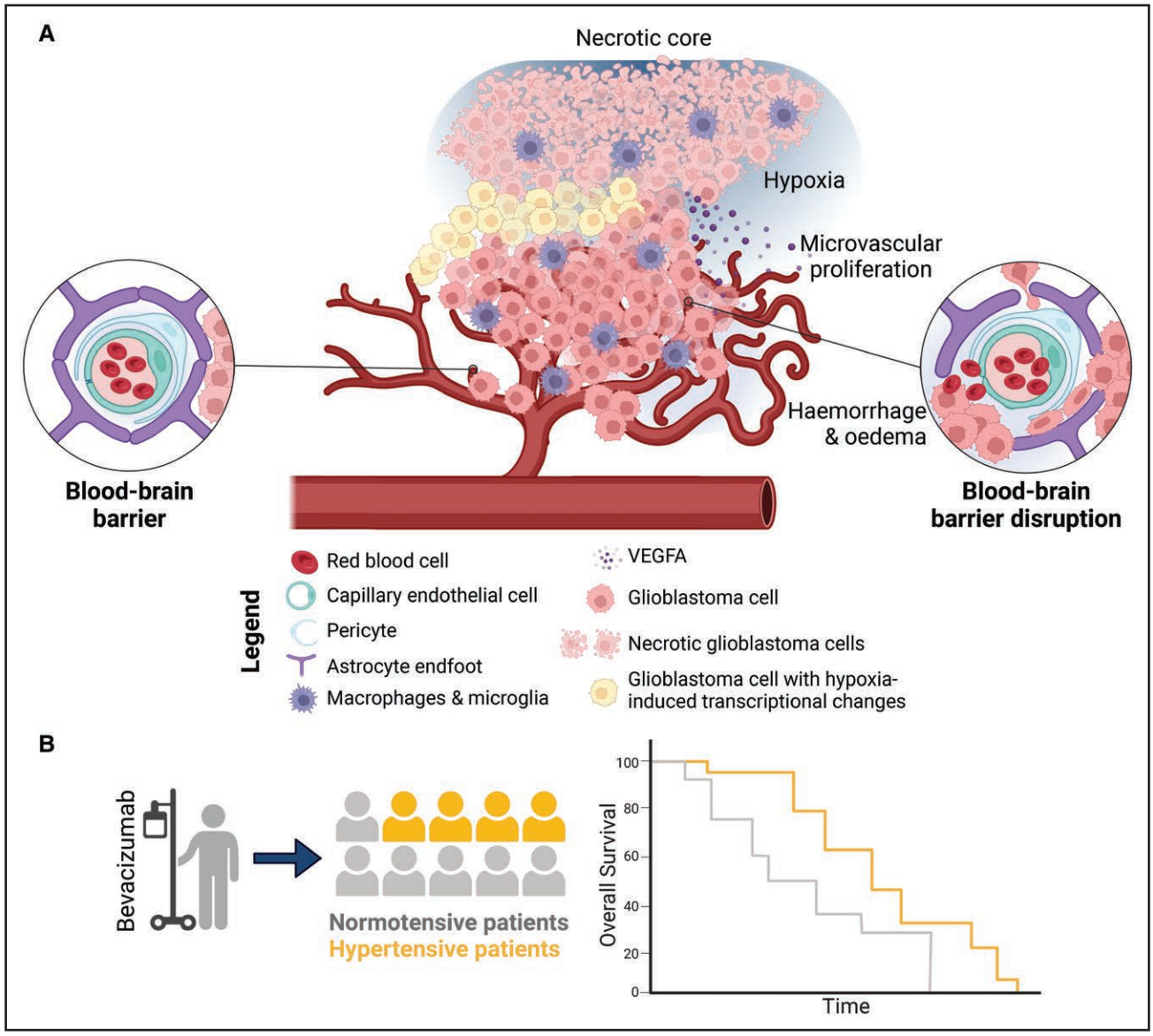

Figure 1. Glioblastoma microvasculature and the correlation between the development of hypertension and survival for glioblastoma patients treated with bevacizumab.

A, Glioblastomas commonly feature regions of hypoxia and necrosis. Tumor cells in the hypoxic areas express VEGFA (vascular endothelial growth factor A), inducing the formation of numerous new poorly formed and leaky blood vessels. The blood-brain barrier is often disrupted in the central tumor, causing edema and hemorrhage, while on the tumor periphery, this barrier variably remains intact. **B**, Roughly 4 of every 10 patients treated with bevacizumab will develop hypertension following treatment. Patients who develop hypertension have improved overall survival. This figure was created using BioRender.com.

and effect of hypertension.[45–47] Under normal conditions, VEGFA stimulates activation and upregulation of eNOS (endothelial NO synthase), subsequently inducing or increasing NO release[42,48] (Figure 2). Conversely, upregulation of angiotensin-II in hypertension causes increased production of reactive oxygen species (ROS) such as superoxide via NOX1 (NADPH oxidase 1), which then combines with NO to form peroxynitrite ($ONOO^-$).[45,47,49] Besides this reducing available NO, peroxynitrite destabilizes eNOS, inducing further superoxide production rather than NO in a feed-forward loop, which drives a reduction in the bioavailability of vasodilators alongside an increase in constricting factors, a state known as endothelial dysfunction.[45,47,49] Therefore, while bevacizumab reduces NO release through VEGFA inhibition, hypertension likely perturbs several alternate NO regulating pathways alongside increased ROS production,[45] thus likely increasing EC dysfunction and impairing the tumor vasculature.

It is, therefore, possible that hypertension contributes further to microvascular rarefaction independently of bevacizumab. Furthermore, development of hypertension may indicate sensitivity to endothelial dysfunction, whereas in other patients inhibition of VEGFA is insufficient to cause dysfunction-induced hypertension.

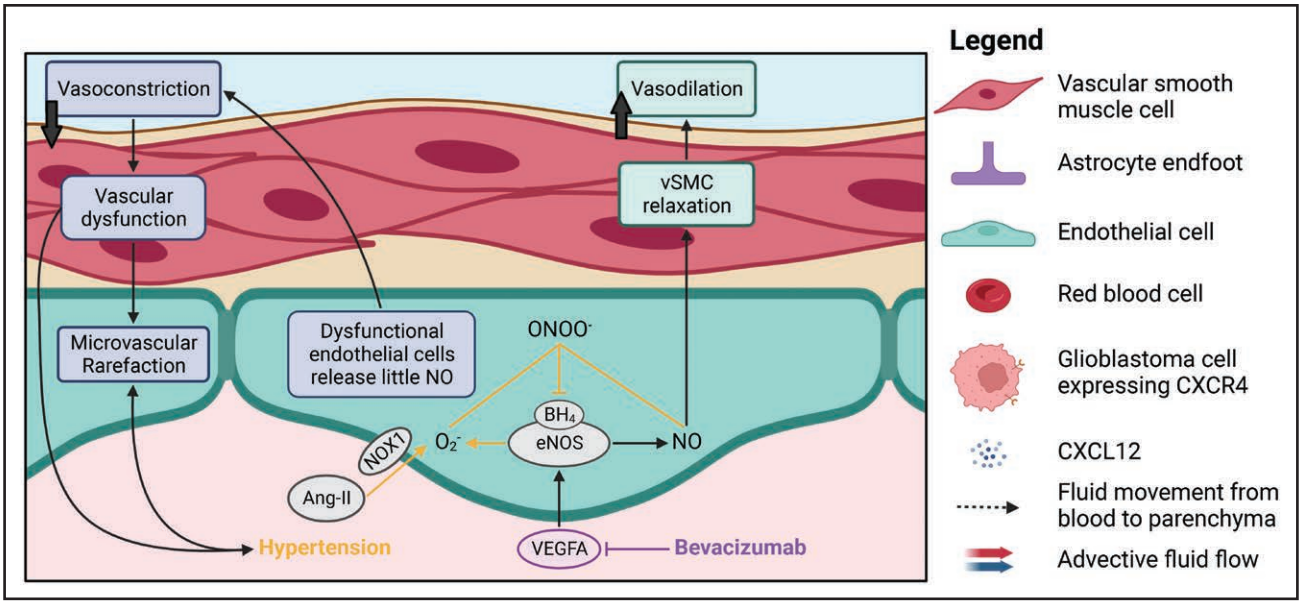

Figure 2. Modulators of vascular tone and endothelial dysfunction in the perivascular niche.
Vascular tone is maintained by a careful balance of vasodilators and vasoconstrictors. VEGFA (vascular endothelial growth factor A) induces upregulation and activation of eNOS (endothelial nitric oxide synthase), which generates the key vasodilator NO. Inhibition of VEGFA signaling by bevacizumab reduces NO production. Under conditions of hypertension, angiotensin-II (Ang-II) signaling induces production of superoxide by NOX1 (NADPH oxidase 1), which combines with available NO to form peroxynitrite. This reactive oxygen species (ROS) then destabilizes eNOS by reducing the cofactor tetrahydrobiopterin (BH_4), causing eNOS to switch to production of superoxide, driving endothelial dysfunction through the loss of NO. This figure was created using BioRender.com.

Indeed, certain eNOS polymorphisms have been linked with significantly elongated progression-free survival for patients with metastatic colorectal cancer treated with bevacizumab, with a trend between polymorphisms associated with improved outcome and patients who experienced higher grades of hypertension.[50] However, little is known about whether hypertension itself could influence glioblastoma progression. Two studies showed no difference in survival between normotensive and hypertensive patients,[51,52] which contrasts with a recent unbiased mortality study that showed a decreased risk of any cancer death in hypertensive participants.[53] Letourneur and colleagues[54] investigated the effects of chronic hypertension on glioma growth in a spontaneously hypertensive rat model. Intriguingly, tumors progressed slower and were significantly smaller at the experiment end point in spontaneously hypertensive rat compared with control Wistar-Kyoto rats.[54] This was similar to the reduction in tumor size observed in rodents treated with antiangiogenic agents.[55,56] The authors postulated that slower glioma growth in spontaneously hypertensive rat may be due to the known effects of hypertension on the vascular system, such as inhibition of angiogenesis.[54,57] Microfluidic in vitro models of angiogenesis have demonstrated that neovessel growth is stimulated by increased interstitial flow on the basal side of EC monolayers.[58–60] In contrast, flow in the direction of sprouting (apical/luminal side) was conversely shown to inhibit angiogenesis and even cause vessel regression.[59] More recently, interstitial flow was confirmed to induce angiogenesis by mechanotransduction independent of VEGFA, although physiologically normal network formation required a balance between the 2.[58] Taken together, these results suggest that increased hydrostatic pressure against the lumen of blood vessels can modulate angiogenesis independently of VEGFA blockade by bevacizumab.

INTRATUMOR HETEROGENEITY DRIVEN BY CHANGES IN TUMOR CELL TRANSCRIPTIONAL STATES IS RELIANT ON A DELICATE BALANCE OF NO AND ROS

While control of rampant blood vessel growth reduces the availability of nutrients to the rapidly growing tumor, interactions between tumor and vascular cells within perivascular niches will remain, and these are of critical importance in other aspects of glioblastoma biology. Glioblastoma cells exhibiting stem-like properties have commonly been shown to reside in close contact with brain vasculature within a perivascular niche.[20,61,62] Much like neural stem cell niches,[63] these are spatially distinct microcompartments, where heterotypic interactions of stem-like glioblastoma cells with the surrounding microenvironment and nontumor cells can induce stem cell maintenance or differentiation and clonal selection.[18,64] ECs provide cues to maintain stemness and self-renewal of tumor cells in the perivascular space, in addition to facilitating protection from radiation.[62,65–68] Indeed, a high degree of cancer cell plasticity has been reported

to be a feature of glioblastoma, with cancer cells shifting between different transcriptional states depending on the local tumor microenvironment.[18,64] Reference to glioblastoma cells in this review should thus be considered to encompass various subsets of tumor cell including those typically referred to as glioblastoma stem cells.

The vascular niches occupied by glioblastoma cells are suggested to be predominantly arteriolar,[69] in a relatively hypoxic microenvironment with low levels of ROS, conditions known to maintain cancer cell stemness and self-renewal.[70] These conditions have been likened to those in adult stem cell niches[69,71] and a delicate balance of hypoxia/ROS is indeed critical for stem cell maintenance. Quiescent or self-renewing stem cells display low levels of ROS, intermediate levels of ROS induce stem cell differentiation, and high ROS levels cause senescence and cell death.[72] In the case of glioblastoma cancer cells, induction of intracellular ROS reduces tumorigenicity, decreases capacity for self-renewal, and induces differentiation.[73] NO is also an important regulator shown to variably maintain cancer cell stemness, enhance invasiveness, or induce apoptosis under different conditions.[66,74,75] In patient biopsies, eNOS is upregulated in ECs adjacent to perivascular glioblastoma cells expressing nestin, a stem cell marker.[66] PDGF-induced gliomas in mice with reduced NO due to eNOS knockdown had delayed tumor growth and improved survival.[66] Since this early study, a large volume of research has consolidated NO synthases in glioblastoma as potential therapeutic targets.[75] As previously mentioned, hypertension causes downregulation of NO with concomitant increases in ROS production, eNOS destabilization, and EC dysfunction (Figure 3).[45–47,49] Whether hypertension may disrupt the perivascular glioblastoma cell niche by perturbing the balance of ROS and NO remains to be elucidated.

CONCLUSIONS

The prevailing wisdom is that hypertension may be a useful marker of patient responsiveness to bevacizumab to determine whether therapy is worth continuing. Here, we have reviewed the literature on the relationship between glioblastoma cancer-related outcomes and the development of hypertension after VEGFA inhibition using bevacizumab. So far, multiple reports suggest a positive correlation between the development of hypertension and a favorable tumor response to bevacizumab treatment. However, the causality of this relationship needs to be confirmed as, to date, no study has demonstrated whether effects mediated by hypertension increase the efficacy of these drugs or ameliorate adverse effects. This is an important distinction, because if hypertension improves treatment outcome, this may alter how hypertension is managed in patients treated with bevacizumab, particularly given the evidence for increased microvascular density and heightened risk of

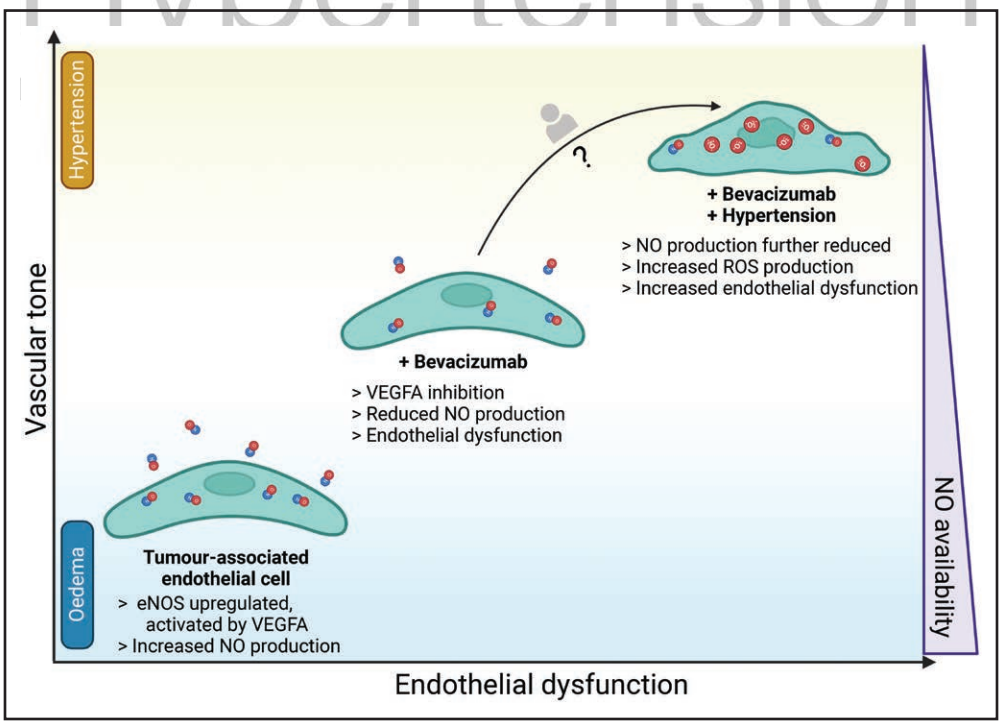

Figure 3. VEGFA (vascular endothelial growth factor A) inhibition by bevacizumab normalizes vascular tone by reducing endothelial nitric oxide synthase (eNOS) production of NO.
Certain patients experience such a change in peripheral resistance due to capillary rarefaction and increase in vascular tone that they develop hypertension following treatment. These patients may experience further endothelial dysfunction due to the compounding effects of hypertension on NO availability. This figure was created using BioRender.com.

(any) cancer-related death following effective control of hypertension with antihypertensives.[76,77] Furthermore, discovery of how hypertension affects survival may identify patients who will most benefit from bevacizumab treatment, or pathways that could be targeted in combination with bevacizumab.

No significant investigative effort has yet been made into the role of hypertension in glioblastoma, and extrapolation from currently available data is difficult due to continuing controversy in the literature. However, evidence gleaned from studies of hypertension demonstrate its potential impact on glioblastoma invasion—as discussed in the Supplemental Text[21–23,55,56,78–106]—and stem cell maintenance, which could enhance patient response to bevacizumab. In this regard, the recent development of spatial omics approaches (spatial transcriptomics[107] and proteomics)[108] applied to patient tumor tissue (resected or postmortem) could help to elucidate the relationships between the onset of hypertension and response, including the potential influence of the immune system in hypertension development. Furthermore, it may be possible to identify new biomarkers that can in the future be used for the stratification of patients likely to respond to bevacizumab treatment, at the time of diagnosis.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Supplemental text
Figure S1
References 78–106

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