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Is the blood–brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data

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Abstract

The blood–brain barrier (BBB) excludes the vast majority of cancer therapeutics from normal brain. However, the importance of the BBB in limiting drug delivery and efficacy is controversial in high-grade brain tumors, such as glioblastoma (GBM). The accumulation of normally brain impenetrant radiographic contrast material in essentially all GBM has popularized a belief that the BBB is uniformly disrupted in all GBM patients so that consideration of drug distribution across the BBB is not relevant in designing therapies for GBM. However, contrary to this view, overwhelming clinical evidence demonstrates that there is also a clinically significant tumor burden with an intact BBB in all GBM, and there is little doubt that drugs with poor BBB permeability do not provide therapeutically effective drug exposures to this fraction of tumor cells. This review provides an overview of the clinical literature to support a central hypothesis: that all GBM patients have tumor regions with an intact BBB, and cure for GBM will only be possible if these regions of tumor are adequately treated.

Key words

blood brain barrier | drug therapy | glioblastoma | magnetic resonance imaging

The prognosis for newly diagnosed glioblastoma (GBM) remains dire despite years of intensive basic, translational, and clinical research. Over the past 30 years, there have been only 4 FDA approvals for systemically administered therapies for GBM: lomustine, carmustine, temozolomide, and bevacizumab. The first 3 drugs are simple alkylating agents approved over a decade ago with partial brain penetration, while bevacizumab is a monoclonal antibody that binds vascular endothelial growth factor (VEGF) and prevents VEGF receptor activation on capillary endothelial cells. Bevacizumab is effective at controlling edema in some GBM patients, but clinical trials have not demonstrated a convincing impact on patient survival.[1–3] In contrast, in the last decade there has been tremendous progress in developing highly effective, targeted therapies for most other non-CNS solid malignancies. Specifically, 28 molecularly targeted agents have gained FDA marketing approval since 2006 for breast ($n = 8$), lung ($n = 13$), and melanoma ($n = 7$) (https://www.cancer.gov/about-cancer/treatment/drugs/cancer-type). While there are many potential factors that contribute to the striking lack of progress in developing effective therapies for GBM, we propose that limited and heterogeneous drug delivery across the blood–brain barrier (BBB) is a major cause of treatment failure for otherwise promising novel therapies in GBM.

The BBB provides both physical and biochemical barriers to drug delivery into normal brain (Fig. 1).[4,5] Continuous tight and adherens junctions between brain capillary

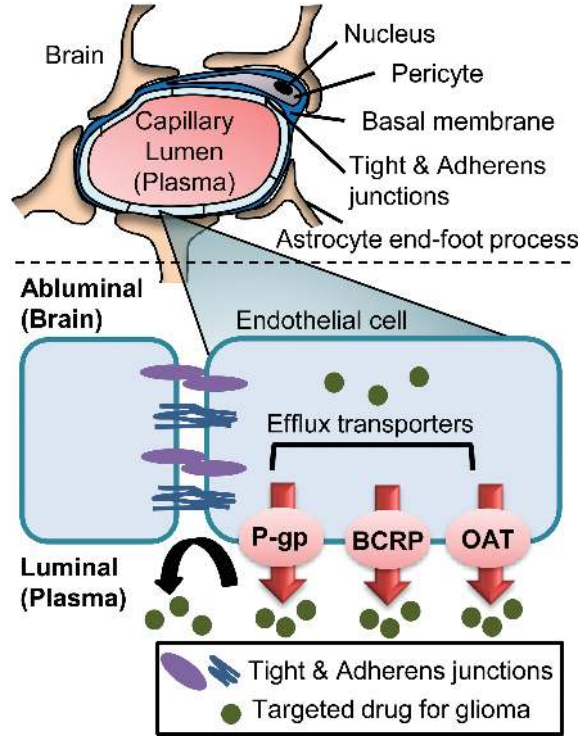

Fig. 1 Illustration of key components of the BBB that provide physical (tight and adherens junctions) and biochemical (transporter-mediated efflux) barriers to brain penetration of antiglioma agents.

endothelial cells prevent paracellular diffusion, and as a result, molecules in the bloodstream can enter the brain only by transiting across endothelial cell luminal and abluminal plasma membranes.[6,7] This physical barrier markedly limits brain distribution of many oncologic drugs, including monoclonal antibodies, antibody-drug conjugates, and hydrophilic molecules that do not readily cross lipid bilayers. For lipophilic molecules that readily diffuse across plasma membranes, various transmembrane efflux transporters in endothelial cells function as a biochemical barrier by actively transporting drugs into the capillary lumen. P-glycoprotein, breast cancer resistance protein, and organic anion transporters are especially important efflux pumps within the BBB that limit accumulation of small-molecule targeted therapies.[7,8] Between the biochemical and physical barriers presented by the normal BBB, many anticancer agents have significantly impaired distribution into normal brain parenchyma (Table 1). Thus, the BBB is unequivocally important for exclusion of the vast majority of approved and experimental oncologic drugs from normal brain.

A variety of pathologic conditions, including brain tumors, can disrupt the integrity of the BBB. This BBB dysfunction is most commonly detected on conventional contrast-enhanced MRI following intravenous administration of gadolinium-based contrast agents. In regions of physically disrupted BBB, the hydrophilic contrast molecules diffuse out of the vessel lumen and accumulate within the extravascular extracellular space, manifesting as contrast-enhancing hyperintense regions on T1-weighted (T1W) sequences in nearly all GBM.[9] These contrast-enhancing regions are associated with dense tumor and are the typical target for surgical resection. However, beyond the contrast-enhancing region, essentially all GBM have a region of non-enhancing edema that is evident on imaging as increased signal intensity on T2-weighted (T2W) or T2W fluid attenuation inversion recovery (FLAIR) imaging. This imaging feature reflects a combination of cellular infiltration and vasogenic edema.[10–13] The presence of vasogenic edema reflects a more subtle dysregulation of the BBB that allows abnormal accumulation of fluid within the brain parenchyma but is insufficient to allow accumulation of contrast. As discussed in detail below, there is unequivocal evidence that all GBM have tumor cells infiltrating this edema volume and that these cells have a profound influence on the ultimate efficacy of therapy.

Numerous studies support the importance of maximal surgical resection to prolong survival of patients with GBM.[13–16] Indeed, some have proposed resecting a margin of surrounding "normal" brain from non-eloquent regions, if clinically feasible, to minimize the residual disease burden.[13,17] Nevertheless, GBM is ultimately not a surgically curable disease. Even with complete resection of all radiographic abnormality (both T1W contrast-enhancing and T2W FLAIR volumes), recurrence is inevitable. This sobering reality has been appreciated since the early twentieth century, when pioneering work by Scherer demonstrated infiltration of glioma cells into otherwise normal brain,[18,19] and Dandy and colleagues demonstrated that even removal of the entire ipsilateral hemisphere (hemispherectomy) was followed by recurrence in the contralateral hemisphere.[20,21] In conjunction with this natural history, several image-guided surgical sampling studies have demonstrated significant tumor cell infiltrates present in 80%–100% of biopsies obtained in regions of T2W/T2W FLAIR abnormality (reviewed by Matsuo et al[22]). Sampling one or more centimeters beyond the T2W/T2W FLAIR abnormality also demonstrates a smaller fraction of biopsies containing tumor cells.[11,12] Collectively, these surgical data provide indisputable evidence that a clinically meaningful tumor burden exists beyond tumor volume defined radiographically by contrast enhancement and support the concept of GBM as a whole-brain disease.[23] To achieve significant improvement in progression-free survival, and especially to achieve a cure, at least some component of a multifactorial approach to therapy must address the non-contrast-enhancing tumor burden infiltrating the brain.

While conventional T1W contrast-enhanced MRI provides gross qualitative assessment of BBB disruption, this technique fails to resolve the degree to which the BBB is disrupted, which can vary from patient to patient and within different regions of the same tumor. Instead, advanced techniques such as dynamic contrast enhanced (DCE)-MRI can quantitatively measure the transport constant of contrast molecules across different contrast-enhancing regions using pharmacokinetic modeling and dynamic imaging acquisition to estimate vascular permeability.[9,24] Numerous studies have shown extensive intratumoral heterogeneity of DCE parameters within

Table 1 Heterogeneous brain distribution of antiglioma agents in clinical and preclinical studies*

Drug	Tumor Tissue-to-Plasma Ratio[a]			Efflux Transporter Substrate Status (e.g., P-gp, Bcrp)	References
	Contrast Enhancing	Non-Enhancing	Normal ("distant") Brain		
Temozolomide	—	0.20	(0.41)	Yes	65–67
Methotrexate	0.30	0.063	(0.11)	Yes	68,69
Carboplatin	0.054 to 0.49	—	0.17 (0.031)	—	49,70,71
Cilengitide	3.39	—	—	—	72
Erlotinib	0.35 (0.51)	(0.10)	(0.02 to 0.09)	Yes	73,74
Imatinib	1.35	—	(0.16)	Yes	75,76
Gefitinib	26.4	—	(0.10)	Yes	77,78
Estramustine	15.8 (4.6)	—	(3.5)	—	79,80
Idarubicin	15.6	3.75	—	Yes	81,82
Ranimustine (MCNU)	2.54	—	0.16	—	70
Tauromustine (TCNU)	0.51	0.56	—	—	83
Liposomal daunorubicin	2.16 to 7.11	2.02 to 7.88	1.1 to 4.55	—	84,85
Mitoxantrone	34	—	(0.25)	Yes	86,87
Paclitaxel	7.35	—	(0.5)	Yes	88–90
Etoposide	0.19 to 0.36	0.13	—	Yes	91–94
Teniposide	0.19 to 2.39	0.029 to 0.19	—	—	91,95,96
Temsirolimus	1.43	—	—	Yes	97,98

*The review article by Pitz et al[64] previously reported the tissue-to-plasma ratios observed in human brain tumors (high-grade glioma). This table provides additional data, including the preclinical brain penetration data (in parentheses) and efflux transporter substrate status.
Abbreviations: P-gp, P-glycoprotein; Bcrp, breast cancer resistance protein. Blank areas (—), not reported in the literature.
[a]Determined from the area under the concentration-time curve ratio and/or a single-time concentration ratio. Data provided are clinical or preclinical.

T1W contrast-enhancing tumor regions reflecting varying degrees of BBB disruption and vascular permeability.[25–27] For tumor regions that are devoid of contrast, other advanced and emerging MRI techniques are needed to characterize tumor extent using tumoral imaging phenotypes that are independent of BBB integrity,[28] such as tumor cell density on diffusion-weighted imaging (DWI),[29] white matter infiltration on diffusion tensor imaging (DTI),[30,31] or diffusion kurtosis imaging (DKI),[32] metabolic profiling on MR spectroscopy (MRS),[33,34] and microvessel volume on dynamic susceptibility-weighted contrast-enhanced (DSC) MRI.[35,36] Highlighting the issue of non-contrast-enhancing tumor volumes, an analysis of 21 previously untreated GBM patients by DWI demonstrated a significant volume of hypercellularity extending beyond regions of T1W contrast enhancement (mean volume 7.3 cc, minimum volume 0.2 cc, maximum volume 59.8 cc) in all patients.[37] Similar radiotherapy planning studies using MRS demonstrate significant extension of metabolically detectable tumor beyond T1W contrast-enhancing regions.[38–40] Finally, consistent with the Response Assessment in Neuro-Oncology guidelines, isolated progression of the T2 signal on serial head MRI is a strong predictor of subsequent radiographic progression within the contrast-enhancing volume.[41,42] Collectively, these observations reinforce the fact that contrast enhancement is not an accurate delimiter of gross tumor burden in GBM.

Complementary PET techniques have been used to delineate the tumor extent in GBMs. Several large neutral amino acids (^{11}C-methionine; ^{18}F-fluoro-ethyl tyrosine [FET]; 3,4-dihydroxy-6-^{18}F-fluoro-L-phenylalanine [FDOPA]) are actively transported into the brain across the BBB and preferentially accumulate in brain tumor tissue.[43,44] Using PET imaging of these tracers, several studies demonstrate that a significant fraction of the PET-defined tumor volume (59%–71%) extends beyond the contrast-enhancing lesion in the majority (68%–100%) of GBM patients (Fig. 2).[22,45,46] Collectively, these MR and PET imaging studies demonstrate that a majority of GBMs have gross tumor burden with an intact BBB that extends beyond the contrast-enhancing tumor volume. Combined with the surgical experience, these data support our central contention that *all GBM have a clinically significant tumor burden "protected" by an intact BBB*.

In conjunction with evidence that all GBM have regions of microscopic and gross tumor burden with an intact BBB, there are significant clinical data demonstrating the negative impact of inadequate drug distribution on control of microscopic tumor burden in the context of brain metastases. Small-cell lung cancer is highly sensitive to doublet

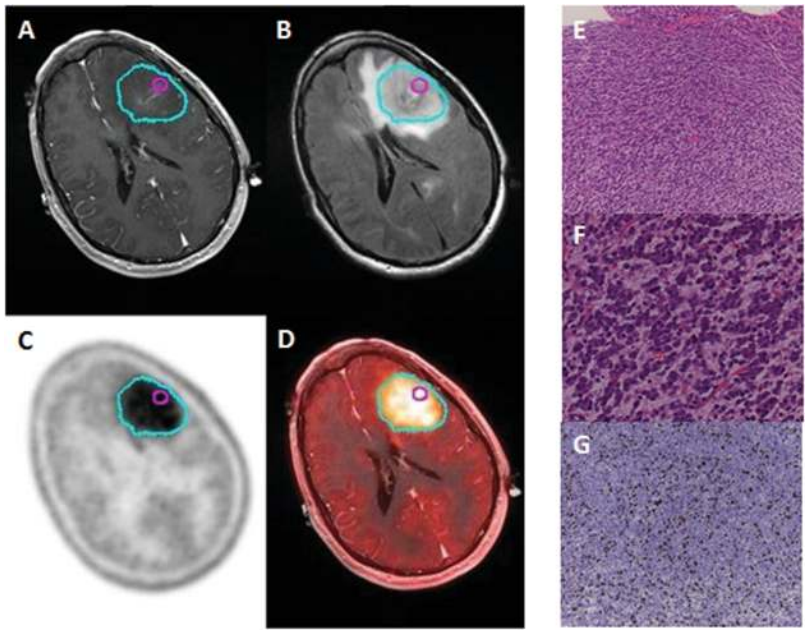

Fig. 2 Illustrative case for a patient with significant tumor burden beyond contrast-enhancing regions. Sequential imaging with (A) T1W + contrast, (B) T2W FLAIR, (C) FDOPA PET (cyan contour), and (D) PET/CT fusion demonstrate significant regions of an FDOPA-positive GBM without contrast enhancement on MRI. Location of a stereotactic biopsy is marked with a magenta contour. Samples were processed for photomicroscopy of hematoxylin and eosin at (E) 100x magnification and (F) 400x magnification showing hypercellularity. (G) Ki-67 staining of the same sample, imaged at 100x magnification shows a high proliferative index (>20%).

chemotherapy with cisplatin and etoposide, and in limited stage disease treated with cisplatin/etoposide and localized chest irradiation, approximately 25% of patients can be cured.[47] However, small-cell lung cancer has an exceptionally high propensity to metastasize to the brain,[48] and because neither cisplatin nor etoposide has significant distribution into normal brain,[49–51] the ultimate brain failure rate is as high as 80%. As a result, several large randomized clinical trials have demonstrated that prophylactic cranial irradiation in patients with no evidence of disease in the chest or brain at completion of chemo/radiotherapy reduces the risk of failure in the brain by over 50%.[52,53] Similarly, cranial or craniospinal irradiation has been used in pediatric patients with high-risk acute lymphoblastic leukemia to prevent central nervous system tumor recurrences before modern intrathecal chemotherapy regimens were developed.[54–56] In the context of precision medicine strategies, similar patterns of brain-only failure have been observed in patients with human epidermal growth factor receptor 2–amplified breast cancer and anaplastic lymphoma kinase–translocation lung cancers treated with highly effective but brain impenetrant targeted therapies.[57–59] These clinical experiences, with both cytotoxic and targeted therapies, demonstrate that poor brain distribution can result in an inadequate treatment of subclinical deposits of tumor cells in the brain. While there are no direct clinical data demonstrating the impact of poor drug delivery on patterns of failure in GBM, extrapolation of the clinical data in brain metastases suggests that poor drug delivery into regions of GBM with an intact BBB will limit efficacy of therapy in these regions. Stated differently, a central tenet of oncology is that a cure is possible only if an effective therapy is delivered with adequate exposure to the entire population of targeted cells, and failure to adequately deliver therapies into regions of GBM that have an intact BBB will preclude a chance for cure.

In this context, is there any role for testing drugs with poor penetration across an intact BBB in patients with GBM? Following standard of care surgery, radiation, and temozolomide therapy, the predominant failure pattern for GBM patients is within the high-dose radiation volume that is centered on the region of contrast enhancement.[60–62] Anecdotal reports of drugs with very limited brain distribution (vemurafenib, ABT-414) demonstrate that at least a subset of GBM patients may benefit from such therapies (Gan et al, ASCO meeting 2015).[63] While we would predict that these agents would be ineffective in lesions with extensive tumor infiltration beyond the contrast-enhancing region, there are a subset of "nodular" GBM with a dominant contrast-enhancing lesion and limited surrounding edema volume (Fig. 3).[11,13] If therapeutic levels of a poorly brain penetrant but otherwise effective drug are effectively delivered into tumor regions with a disrupted BBB, we might speculate that those patients with a nodular imaging phenotype, with the dominant tumor burden contained within the contrast-enhancing region, may derive greater tumor control benefits than those patients who have a greater tumor burden outside of the enhancing region. However, these strategies ultimately

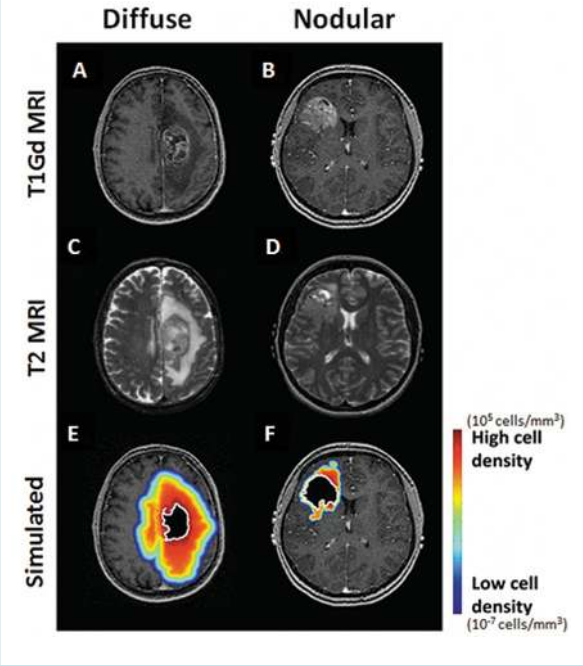

Fig. 3 Patient-specific simulations of tumor cell distribution and density for both a diffuse and a nodular newly diagnosed GBM. T1W + gadolinium (Gd) contrast and T2W MRIs for a diffuse (A, C) or nodular (B, D) tumor. A simulation estimating glioma cell extent is overlaid on the T1Gd MRI with red and blue indicating high and low (but nonzero) glioma cell density, respectively (E, F).

will fail without an effective approach to address the non-contrast-enhancing portion of the tumor.

Accepting the importance of drug distribution across an intact BBB into brain is a critical first step in developing effective therapies for GBM and must be a key consideration in any clinical trial design for newly diagnosed or recurrent GBM. Acknowledging interspecies differences in the biochemical functions within the BBB, pharmacokinetic and pharmacodynamic analyses of drug delivery into normal rodent brain and corresponding relevant orthotopic tumor models may provide initial data regarding potential limitations of drug delivery encountered in patients. Within phase I tolerability studies, embedding phase 0 clinical designs to assess drug distribution and pharmacodynamic effects using either image-guided surgical sampling or functional imaging assessments can specifically address drug penetration and efficacy in tumor regions with an intact BBB (T2W FLAIR) versus disrupted BBB (T1W + contrast). Decisions to move forward with clinical efficacy testing in phase II/III trials then can be made based on a combined understanding of mechanism of action, drug potency, and intra- and intertumoral heterogeneity of drug distribution. Especially for drugs with relatively poor brain distribution, consideration of intra- and inter-patient heterogeneity in BBB disruption, as approximated by contrast enhancement, and the fractional tumor burden within and beyond regions of contrast enhancement, will be instrumental in failure analysis of negative clinical trials and for identifying regimens that are effective in subsets of patients. Moreover, the clinical realities of the contribution of the BBB to treatment failure in GBM argue for renewed efforts to develop BBB-penetrating agents, optimize BBB-disruption technologies, and refine implantable drug delivery technologies that bypass the BBB and deliver therapeutic concentrations throughout an infiltrating tumor volume.

Key Conclusions

- GBM is highly infiltrative and is a whole-brain disease.
- All GBM have clinically significant regions of tumor with an intact BBB.
- Failure to deliver an effective therapy to all regions of GBM will result in treatment failure.

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