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Targeted delivery of antibody-based therapeutic and imaging agents to CNS tumors: Crossing the blood-brain-barrier divide

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Abstract

Introduction—Brain tumors are inherently difficult to treat in large part due to the cellular blood-brain barriers (BBB) that limit the delivery of therapeutics to the tumor tissue from the systemic circulation. Virtually no large-molecules, including antibody-based proteins, can penetrate the BBB. With antibodies fast becoming attractive ligands for highly specific molecular targeting to tumor antigens, a variety of methods are being investigated to enhance the access of these agents to intracranial tumors for imaging or therapeutic applications.

Areas covered—This review describes the characteristics of the BBB and the vasculature in brain tumors, described as the blood-brain tumor barrier (BBTB). Antibodies targeted to molecular markers of CNS tumors will be highlighted, and current strategies for enhancing the delivery of antibodies across these cellular barriers into the brain parenchyma to the tumor will be discussed. Non-invasive imaging approaches to assess BBB/BBTB permeability and/or antibody targeting will be presented as a means of guiding the optimal delivery of targeted agents to brain tumors.

Expert Opinion—Pre-clinical and clinical studies highlight the potential of several approaches in increasing brain tumor delivery across the blood-brain barrier divide. However, each carries its own risks and challenges. There is tremendous potential in using neuroimaging strategies to assist in understanding and defining the challenges to translating and optimizing molecularly-targeted antibody delivery to CNS tumors to improve clinical outcomes.

Keywords

Immunotargeting; CNS; brain cancer; blood-brain barrier; blood-brain tumor barrier; imaging

1. Overview

Despite the tremendous advances in understanding the molecular and cellular basis of cancer biology, as well as the development of targeted therapies to treat malignancies, there remain critical challenges in treating primary and metastatic disease found within the central nervous system (CNS). An estimated 25,000 new cases of primary malignant brain cancer, and 200,000 secondary metastatic brain tumors are expected to be diagnosed in the USA in 2013 [1,2]. Gliomas broadly refer to all tumors arising from the glial cells of the brain (e.g., astrocytes, oligodendricytes, ependymal cells), and represent 80% of all primary malignant brain tumors [1,2]. In children, brain tumors are the second-leading cancer-related cause of death [3]. In adults, glioblastoma multiforme (GBM), representing ~50% of all gliomas, has a dismal prognosis and is responsible for a large percentage of deaths related to CNS malignancies [4]. Despite aggressive treatment strategies including surgical resection to remove bulk disease, and recent advances in standard-of-care treatment of radiation therapy combined with temozolomide (TMZ) chemotherapy, the overall median survival of GBM patients remains only 14.6 months from diagnosis [5]. Thus, reality demands that we achieve a better understanding of CNS tumors and how best to treat them.

Many investigational agents have demonstrated pre-clinical success, yet none have to-date provided substantial clinical benefit. This could be due to genetic heterogeneity of GBM, complexity of cancer signaling pathways and their redundancy, and inadequate drug delivery to the CNS tumor. The latter factor is accentuated by the fact that primary and metastatic brain cancers reside within the CNS sanctuary formed by the blood-brain barrier (BBB), a cellular barrier found across species that protects the brain from exposure to toxins, both endogenous and exogenous. The BBB serves as a major impediment to effective delivery of many therapies from the bloodstream into the brain parenchyma [6]. Some chemotherapies, including TMZ, can effectively cross the intact BBB, but most drugs cannot pass through the BBB to access the CNS tumor. The conventional wisdom that has evolved in the last decade is that targeting agents to specific vascular determinants in the BBB may boost delivery to the CNS. In recent years, monoclonal antibody (mAb)-based therapy has achieved remarkable success in treating both hematologic and non-CNS solid tumors due to their inherent targeting specificity [7]. Engineered antibodies (Ab), either alone or in combination with other therapeutic agents, represent more than 30% of biopharmaceuticals and biologics currently in clinical trials [8]. Advances in mAb-directed therapy against systemic and extraneural cancer have resulted in ever-increasing number of clinical trials attempting to translate these findings to CNS disease. Understanding and advancing targeted drug delivery approaches to these inaccessible and invasive tumor cells may substantially improve the therapeutic outcomes of patients with CNS tumors.

Here we will review the physiology of the BBB and the pathophysiology of the blood-tumor barrier, and highlight the molecular markers that have reported potential as therapeutic targets against CNS tumors. Examples and strategies for delivery of molecular-targeted agents, namely antibodies as diagnostics and therapeutics, across the BBB will be presented. Finally, noninvasive imaging methods will be presented as a means of guiding the optimal delivery of targeted agents to improve our knowledge and management of brain tumors.

2. The blood-brain barriers for antibody delivery to CNS tumors

2.1 The blood-brain barrier (BBB)

The brain is a highly vascularized organ with an extensive capillary network. Brain capillary endothelial cells (BCECs), pericytes, astrocytes and neurons, are intimately involved in creating the BBB, to control permeability and other functions of the brain microvasculature (Figure 1). BCEC form a tight continuous monolayer lining the microvasculature and serve as the primary interface between blood and the brain parenchyma. The endothelial basal lamina does not generally constitute a diffusion barrier to molecules [9]. Pericytes, which stabilize and monitor the BCECs, send out cellular projections, which penetrate the basal lamina and cover approximately 20-30% of the microvascular circumference [10]. Astrocytes are glial cells that line more that 99% of the abluminal side of the BBB endothelium by astrocytic end-feet through the basal lamina [11]. Neurons contribute to the BBB by innervating the capillary [12]. BCEC is arguably the main diffusion restrictive component of the barrier, protecting the brain from toxic and damaging substances, while selectively permitting delivery of various nutrients, and efflux or removal of toxins and metabolites from the brain.

The selective permeability properties of the BBB can be attributed to specific characteristics of the BCEC. Tight junctions between these cells form a continuous monolayer to block pericellular transport to the brain [13-15], whereas transcellular passage is also generally ineffective due to lack of caveoli and fenestrations [16-20]. Lastly, the permeability of the BBB is partially under the control of astrocyte end-foot processes via release of chemical factors and signals that modulate the permeability of the brain endothelium [21]. The selective transport across an intact BBB is through transcellular mechanisms such as concentration-driven facilitated and passive diffusion, and active transport, which requires ATP-energy to move molecules across a gradient. However, the BBB hinders drug penetration into the CNS: virtually no large-molecule drugs and fewer than 2% of small-molecule drugs cross the intact BBB [22].

There are additional types of barriers in the brain in addition to the BBB: the blood–cerebrospinal fluid (BCSF) barrier formed by the epithelial cells of the choroid plexus, and the meningeal or arachnoid barrier in the subdural space surrounding the brain (Figure 1) [16]. Although outside the scope of this review, there are surgical-based techniques for drug delivery to CNS tumors that include the use of: 1) Intracerebro-ventricular therapeutic infusion into the choroid plexus, 2) intrathecal administration into the CSF under the arachnoid membrane of the brain or spinal cord, 3) Convection-enhanced delivery directly into tumor, and 4) polymer-drug implant systems (e.g., wafer loaded with bischloroethylnitrosourea (BCNU)) which directly release therapeutics into the CNS tumor. There are several limitations with these delivery approaches; most significantly, the diffusion of drug in the brain parenchyma is very low, thus placement of catheters or implants must be precisely mapped for optimal efficacy, or the tumor must be close to the BCSF or meningeal barriers.

2.2 The blood-brain tumor barrier (BBTB)

The brain tumor endothelium is, distinct from normal brain tissue due to abnormalities in the tumor microenvironment and is thus often described as a “blood-brain tumor barrier” (BBTB) rather than a BBB [23-25]. The tumor vessels in the BBTB of highly angiogenic brain tumors, such as GBM, can have different morphologies ranging from greatly enlarged, thin-walled, and tortuous vessels, to vascular glomeruli that are tangles of microvessels lined with hyperplastic endothelial cells, smooth muscle cells and pericytes. The later are characterized by sluggish and aberrant blood flow, and susceptibility to occlusion which results in diminished perfusion to the tumor tissue. Endothelial cells in BBTB are often hypermeable due to disrupted, leaky tight junctions [26], increased fenestrations and vesicular transcellular transport [27], and an abnormal over expression profile of membrane transporters, such as the drug efflux pumps P-glycoprotein (P-gP), and multi-drug resistance-associated proteins MRP1, MRP3, and the potassium channel K_{Ca} and K_{ATP} [28]. These changes within the BBTB can enhance passage of low molecular weight compounds, including standard chemotherapeutics, compared with the BBB.

However, these pores can be narrow enough to prevent the effective passage of materials larger than 12 nm (including certain proteins and nanomaterials) [23,29,30]. In addition, drug delivery is impeded by increased interstitial fluid pressure (IFP) in brain tumors, as seen in both patients [31,32] and animal tumor models [33]. Although not fully understood, viable tumor tissue with increased permeability and high resistance to fluid flow in the interstitial space, produces a pressure gradient that moves drugs along with interstitial fluid into necrotic tumor tissue [33], the tumor periphery, or into the surrounding normal brain tissue [31,33-36].

Clearly, the complex and marked heterogeneity of the CNS tumor microenvironment, and the tumor interaction with the BBB/BBTB can have significant implications for achieving sufficient drug delivery to the tumor (particularly the viable component of the tumor) to effect therapeutic response. The BBTB is generally considered compromised in the situation of large brain lesions (> 2 cm), and invasive and malignant gliomas, favoring drug delivery to the tumor tissue. However, there may well be sites in these and smaller tumors, where the functional integrity of the BBB is intact, especially around the tumor edge or around micrometastases, effectively limiting adequate drug delivery to these tumor cells. A recent animal study of experimental brain metastases from human breast cancer cells found that although most metastases showed some degree of BBTB permeability, the concentration of paclitaxel or doxorubicin accumulated within these brain metastases was <15% of that achieved in extracranial metastases [37]. Consequently, the BBTB impedes the accumulation of therapeutic concentrations of both of these agents thereby limiting their effectiveness for treating intracranial tumors. These remnant tumor cells will continue to grow, develop neovascular structures, and continue to progress to a clinically significant size that would require another surgical/therapeutic intervention.

Thus, to address the challenges of targeted drug delivery to CNS tumors, efforts must be focused on drug-delivery technologies that are capable of penetrating both the intact BBB and the pathologically altered BBB and BBTB to maximize clinical outcomes for patients.

3. Molecular targets for antibody-based therapeutics and diagnostics in CNS tumors

Advances in the understanding of GBM biology and pathogenesis have greatly facilitated the development of antibodies targeting antigens typical of the tumor tissues but absent in normal brain. Since the development of the first FDA-approved therapeutic immunoglobulin (IgG) mAb, muromonab-CD3, this area of research has expanded significantly. In addition to humanizing murine IgG antibodies to make them immune-tolerable, and increasing the production efficiency of therapeutic antibodies, recombinant antibody-based systems have been devised, including antibody fragments (Fab), single-chain variable region fragments (scFv), and bispecific antibodies (Figure 2). With advances in molecular genetics and chemical engineering, it is now possible to design antibody-based reagents that are smaller in size, having multivalent modular domain and conjugated with different cargoes and carriers, for various applications. These agents may be exploited clinically because of their endogenous cytotoxic effects or by rendering them cytotoxic by conjugation to cytotoxic drugs, toxins, or radioisotopes. In a similar vein, antibodies can be functionalized with radioisotopes for non-invasive sensitive imaging using positron emission tomography (PET) or single photon emission computed tomography (SPECT), for monitoring drug delivery and tumor status based on the local level of the biomarker.

To date, several clinical trials have evaluated the therapeutic effect of monoclonal antibodies in GBM patients (Table 1). Angiogenesis is a major hallmark of cancer, and is characterized by the formation of new blood vessels required for growth and metastasis of tumors. Brain tumors in particular are highly angiogenic. Therefore, molecularly-targeted interventions designed to normalize tumor endothelium and prevent angiogenesis in systemic cancer may also be effective at controlling brain tumors [38,39]. Vascular endothelial growth factor (VEGF) is a soluble protein (~40 kDa) that serves as the key signaling mediator of angiogenesis and vascular permeability. Bevacizumab, a recombinant human neutralizing VEGF IgG antibody, is the first FDA-approved antiangiogenic agent in systemic cancer treatment. The success of bevacizumab prompted its translation to the treatment of malignant gliomas, Studies released in the past five years reveal favorable radiographic responses and potential survival benefit observed with bevacizumab [39], and resulted in accelerated FDA-approved of bevacizumab in 2009 to treat recurrent GBM. Despite its large molecular size, bevacizumab is effective because it does not rely on disruption of the blood-brain barrier, but rather can work to neutralize VEGF within the lumen of the capillary endothelium. As highlighted in Table 1, anti-VEGF mAb therapy is being extended to newly diagnosed glioblastoma, anaplastic astrocytomas, CNS lymphoma, and secondary cerebral metastases.

The identification of non-responders to bencizumab from the onset of therapy suggests that tumors may be driven by angiogenic factors other than VEGF. Another widely explored angiogenic targets in GBM patients is the cell-surface tyrosine kinase receptor epidermal growth factor receptor (EGFR). Overexpression of EGFR is found in approximately 50–60% of GBM tumor cells. EGFRvIII, a mutant EGFR with deletion of exon 2-7, rendering the receptor constitutively active, is the most common EGFR mutant in GBM and is present

in 24–67% of GBM [40,41]. EGFR is generally considered a favorable candidate for antibody targeting due to high accessibility. Cetuximab is a chimeric IgG1 mAb that can block EGFR activation by binding to the ligand-binding domain, which induces internalization of EGFR thereby preventing downstream signaling [42]. PET imaging of ^{89}Zr-labeled cetuximab has been investigated in several pre-clinical studies, and has potential as a scouting procedure before radioimmunotherapy with ^{90}Y-cetuximab or ^{177}Lu-cetuximab to confirm tumor targeting and allow estimation of radiation dose delivery to tumors and normal tissues [43,44]. Similarly, imaging of EGFR mutant expression might be more useful in selecting the optimal patient population for personalized treatment as well as predicting therapeutic response. This image-based scouting approach for treating brain tumors in patients is currently under-explored. Other candidates under clinical investigation for antibody-based therapy include the growth factor receptor tenascin [45-47], and MRP3 [48]. The initial response of some patients to anti-VEGF therapy soon follows to disease progression with a nonangiogenic and invasive phenotype, suggesting it is necessary to combine anti-angiogenic approaches with anti-invasive therapy.

Currently, 165 anti-tumor mAbs are in malignant glioma clinical studies, including 89 in Phase I; 64 (39%) and 12 (7%) have advanced to Phase II and Phase III, respectively. While nearly 50% of the mAbs used in these studies are intact IgG antibodies, the remainder are non-canonical antibodies that have the capacity of further modification with specific therapeutic or imaging modularity [49]. Antibodies can bind to two adjacent epitopes, which increases the affinity, and induce natural host-mediated cytotoxic effects via the Fc-fragment. Non-canonical antibodies include bispecific antibodies, antibody–cytokine fusion proteins, and immunotoxins.

Bispecific antibodies can bind to two different antigens and can therefore be more specific in therapeutic targeting. Their molecular framework can vary in terms of the type of IgG used and antigen-binding fragment combinations (Figure 2). Although most bispecific antibodies are currently in early phase pre-clinical development, ten different bispecific mAbs are being evaluated in Phase I and Phase II trials. Catumaxomab, a bispecific IgG targeting CD3 and epithelial cell adhesion molecule (EpCAM), has been approved for clinical use in patients with malignant ascites in Europe [50].

Cytokines are natural immunomodulatory agents that induce pro-inflammatory and potentially cytotoxic signaling cascades. Immunotherapy with cytokines, such as interferin (IFN)-α, interleukin (IL)-7, IL-10, IL-12, IL-15, and IL-21, is being used in clinic or is currently in clinical trials for cancer treatment [51,52]. However, systemic cytokine treatment often results in low concentrations of therapeutic cytokines at the target site with severe off-target toxicities. To improve the therapeutic window of cytokines, antibody-cytokine fusion proteins have been developed [53]. The antibody module of an antibody-cytokine fusion protein is to specifically localize the cytokines to the target site, i.e. a tumor. The fusion protein design often involves fusing a therapeutic cytokine with whole IgG, or an antibody fragment such as Fab, scFv, scFv-Fc, or divalent derivatives. Several antibody-cytokine fusion proteins are under clinical evaluation, and some demonstrate encouraging results in terms of clinical benefit and limited off-target toxicity. Challenges in using these

agents in clinical settings include the need to prolong cytokine stability, maintain therapeutic efficacy, reduce immunogenicity, and increase tissue penetration.

An immunotoxin is a conjugate comprised of a targeting moiety (either a natural ligand or an antibody) and a toxin that can kill the target cells once internalized. Several immunotoxins have been evaluated in pre-clinical and clinical trials. For example, Pseudomonas exotoxin mutant (PE) conjugated to the protein cytokine interleukin-4 (IL-4-PE) for targeting the IL-4 receptor on T-cells, is in clinical trials for GBM. The immunotoxin conjugates of anti-TfR (transferrin receptor) mAb with the saporin toxin (anti-TfR-SAP), PE conjugated with mAb DTAT13 directed to urokinase plasminogen activator receptor (uPAR-PE), and antibodies to the receptor-type protein tyrosine phosphatase modified with PE (RPTPβ-PE), are under pre-clinical evaluation [54].

The majority of studies involving these targeted agents involve peripheral and hematologic malignancies. In order to effectively use them in patients with initial or recurrent CNS tumors, the challenging problem of limited CNS delivery needs to be resolved.

4. Approaches to targeted delivery to the CNS

One of the challenges of brain tumor chemotherapy is that, due to specific local mechanisms limiting extravasation described above, carriers improving the pharmacokinetics and local delivery of anti-tumor agents (e.g., antibodies, liposomes, nanocarriers) have no or limited access to the target [55]. Below, we briefly outline current strategies for resolving this problem.

4.1 Antibody delivery across intact BBB

4.1.1 Adsorptive-mediated transcytosis of cationized antibodies—Adsorptive-mediated transcytosis is a non-specific vesicular transport mechanism that can enhance the ability of cationic macromolecules, such as peptides, proteins, and antibodies, to enter the CNS (Figure 1). Covalent modification of the molecule with cationic polyamines, such as hexamethylenediamine or tetramethylenediamine [56,57], raises the isoelectric point (pI) of proteins from pI 4 to greater than 10. Like most cell membranes, the luminal plasma membrane of BCECs is slightly anionic; hence cationic carriers bind to and have a chance to undergo transcytosis across the BBB [58,59]. This mechanism is not mediated by specific recognition of target determinants. Cationic carriers bind to blood elements and endothelium in systemic vasculature- first of all, in the lung after intravenous (IV) injection [60]. Local administration via the cerebral arteries helps to enrich binding in the downstream CNS microvasculature [60].

The enhanced CNS delivery of cationized IgG by absorptive-mediated transcytosis across the BBB was first explored in the late 1980s [58]. Recent studies showed that as compared to native protein, a polyamine-modified $F(ab')_2$ fragment targeted to Aβ plaques has increased uptake in the mouse Alzheimer's brain relative to wild type mouse brain [61]. However, clinically meaningful therapeutic benefits from using cationized antibodies remain to be validated in animal models of CNS cancers. Chemical modification may aggregate carrier proteins and yield heterogeneous molecular species. Attention should be paid to

modification of critical amino acids required for antibody-antigen binding which can result in diminished immunoreactivity and consequent therapeutic efficacy. Functionalization of site-specific tags introduced chemically or using recombinant methods to introduce modifiable amino acids in the carrier protein helps to solve some of these technical issues [62].

4.1.2 Receptor-mediated transcytosis of antibody constructs—Endogenous macromolecules necessary for normal brain function are delivered to the brain by specific receptors expressed on the luminal side of the endothelial cells forming the BBB (receptor-mediated transport, RMT) (Figure 1). Ligand binding and clustering of receptor molecules induces endothelial endocytosis of the complex, which, in some cases traffics via series of intracellular vesicles across the cell. Dissociation of the ligand and receptor presumably occurs during cellular transit or during the exocytotic event. The receptors most often studied for RMT at the BBB are TfR, and insulin receptor (IR) [63]. BCEC also express other receptors capable of RMT: insulin-like growth factor (IGF) receptor [64]; leptin receptor (LEPR) [65]; and scavenger receptors (scavenger receptor, class B, member 1 [SR-B1]), also commonly referred to as the acetylated low-density lipoprotein (LDL) receptor [66].

RMT can occur by different entry points on the plasma membrane depending on the engagement of receptors with specific cytoplasmic domains (e.g., clathrin versus caveolae-coated vesicles) [67]. Each route offers the interesting possibility that the point of entry determines the subsequent fate of a particular internalized cargo molecule. Interestingly, BCEC have the lowest frequency of caveolae, and while there is general agreement that RMT with TfR is clathrin-mediated, the exact mechanism for RMT of other receptors, including IR and LDL remain unresolved [67].

Conjugating therapeutics and drug cargos to ligands (antibodies or peptides) targeted to BBB targets for RMT have long been proposed. However, considerations must be made to how the entities need to be linked to each other. Certain cargos (antibodies or drugs) may not be pharmacologically active following attachment to a BBB-directed ligand for RMT either due to inactivation due to chemical modification or steric hindrance. Thus, it may be necessary to attach the cargoes to the BBB transport ligand either through a cleavable linker (e.g., disulfide bond or acid-sensitive linker) or through a long spacer arm (e.g., polyethylene glycol (PEG) linker), respectively. The choice of a cleavable linker is highly dependent on which chemistry ensure an active cargo molecule following release from the transport vector, and in what intracellular compartment the cargo needs to be released to ensure access to the brain parenchyma on the abluminal side. Acid-sensitive linkers could be cleaved at the lower pH present in late endosomes or lysosomes. Thioredoxin proteins within the compartments endocytic pathway can also create a reducing environment to facilitate cleavage of disulfide bonds. Notably, premature cleavage of the antibody construct from the RMT-directing vector within these endocytic compartments could lead to trafficking of the therapeutic cargoes to lysosomes for degradation rather than transport across the endothelial cell to the target brain cell. Alternatively, a site-directed PEGylation approach could serve to minimize inactivation due to chemical modification or to limit any steric hindrance imparted by the close proximity of the cargo with the BBB-transport vector.

These factors illustrate the various approaches for conjugating CNS tumor targeting cargoes to RMT vectors depending on the specific functional needs of the cargo under consideration.

Proof-of-concept studies of RMT-mediated CNS delivery have been conducted in animal models [22,63,68]. For example, conjugation of the murine TfR-specific mAb OX26 to ^{111}In-labeled EFG-peptide separated by a PEG linker permitted successful ex vivo imaging of a conjugate targeting to intracranial GBM tumors expressing EGFR in rats, whereas EGF peptide alone did not bind to the tumor due to its inability to cross the BBB (Figure 3) [69]. However, widespread expression of these receptors on peripheral organs can limit the capability of RMT for specific brain delivery and, especially for anti-IR antibodies, may present additional toxicity.

More recent work highlights the role of optimal, not necessarily the highest, affinity of targeting antibody [70]. High affinity TfR antibodies do not dissociate from the receptor and thus are retained in brain capillary endothelial cells, whereas a low-affinity counterpart offers more effective delivery into the brain parenchyma [70]. The therapeutic potential of this approach was demonstrated in vivo by targeting BACE 1 (β-amyloid precursor protein cleaving enzyme 1), the key contributor to the production of amyloid-β (Aβ) peptide and eventual Aβ plaque deposition, the latter being a hallmark of Alzheimer's disease (AD). Wild-type mice injected with a bispecific antibody (anti-TfR/BACE1) that binds both TfR and BACE1 with >25-fold lower TfR affinity than the parent TfR mAb, resulted in ~50% reduction of endogenous Aβ peptide brain levels due to more effective inhibition of BACE1 enzyme activity compared to anti-BACE1 mAb alone [70]. These results are suggestive of the potential of RMT in enhancing therapeutic antibody delivery to the brain to reducing Aβ plaque burden in AD.

The potential for antibody efflux in the brain to blood direction must also be taken into consideration when developing antibody-based diagnostic and therapeutic strategies for CNS cancers. Endothelial cells at the BBB express the neonatal Fc receptor (FcRn), which recognizes the constant region (Fc) of IgG antibodies, and mediates its efflux from the abluminal side to the luminal side of the BBB by RMT (Figure 1C) [71]. This efflux mechanism can be avoided when using antibody fragments devoid of Fc regions, including $F(ab')_2$, and scFv though the absence of Fc will also preclude induction of cytotoxicity.

4.2 Enhancing antibody delivery through blood-brain barrier disruption (BBBD)

The transient disruption of the physical barrier presented by BBB and/or BBTB is another approach to improving CNS delivery of agents that would typically be impermeable. The subsequent section reviews current BBBD approaches that may help increase extravasation of macromolecular drugs into the brain parenchyma and facilitate access to CNS tumors. The advantage of this strategy is that it may be applied to diverse drugs and carriers, whereas the safety and practical utility represent its common challenge.

4.2.1. Osmotic BBBD—Transient disruption of the BBB is achievable via intra-arterial (IA) infusion of concentrated hyperosmotic solutions [72]. Early work demonstrated that osmotic disruption of BBB in patients with CNS melanoma metastasis can increase transient brain uptake of ^{131}I-labeled Fab targeted to melanoma antigens, as confirmed by SPECT

imaging [73]. To date, a number of substances have been studied as osmotic BBBD agents, with IA infusion of hypertonic mannitol solution via the internal-carotid artery being the most commonly reported method in pre-clinical and clinical studies [74-76]. These treatments result in osmotic shrinkage of the brain capillary endothelial cells, followed by disruption of tight junctions, and increased permeability for several hours through intercellular pores [77]. Animal studies suggest that this method is able to increase concentrations of various small molecule chemotherapeutic agents in the brain 4 to 90-fold [78]. In the last decade there have been an increasing number of clinical trials evaluating the efficacy of osmotic BBBD and improved drug delivery in primary CNS lymphoma, malignant gliomas and brain metastases [79-81]. Included in this group of trials are those evaluating concomitant delivery of targeted agents, such as bevacizumab [81-83].

The clinical impact, safety and tolerability of this approach needs to be evaluated in larger Phase II and III trials that are currently ongoing at multiple centers. Overall the concerns are regarding the safety and efficacy of clinical osmotic BBBD [75,84]. First, the technique is invasive and its clinical use requires considerable expertise [84]. Second, endothelial tight junction disruption is non-selective, and these transient changes in microvasculature permeability can enhance entry of potentially toxic blood-borne substances throughout the CNS [85]. Finally, increased blood-brain barrier permeability may be limited spatially, thus complicating drug delivery to the whole organ or specific loci [86]. The effectiveness of the procedure can be influenced by different factors, including hemodynamic variables, type of anesthesia and rate of hyperosmolar solution infusion [87,88]. It is thus paramount to monitor the degree of the barrier permeabilization obtained after a procedure, as it can be highly variable from patient to patient, and even with repeated procedures in the same subject. However, applications of IA delivery of chemotherapeutics via the internal carotid approach was introduced into clinical practice decades ago to treat GBM [89]. And, as recently reported, in experienced hands, these procedures can be regarded as clinically safe and effective [81,90].

4.2.2. Biochemical BBBD—There are a variety of biochemical agents that increase the permeability of the brain capillary network by pharmacologic effects on BCECs. This class of vascular disrupting agents requires IA infusion, and can thus present similar challenges as indicated with osmotic BBBD strategies. Bradykinin, a peripheral vasodilator peptide, and the synthetic bradykinin analog RMP-7, can increase tight junction permeability by activating B2 receptors on BCECs through a calcium-mediated mechanism [91,92]. In pre-clinical tumor models, RMP-7 has been found to increase the brain concentration of systemically administered (IA or IV) carboplatin chemotherapy [93]. However, improved efficacy of carboplatin following RMP-7 mediated BBBD could not be demonstrated in Phase II and III clinical trials in pediatric as well as recurrent adult primary CNS malignancies [94,95].

Nitric oxide (NO) donors, leukotrienes, soluble guanylate cyclase activators, and calcium-dependent potassium channels (K_{Ca}) agonists have all been reported to increase BBB permeability to enhance drug delivery to brain tumors in pre-clinical models [28,96-99]. However, current efforts are largely focused towards disrupting the BBB in a more tumor-specific manner to limit neurotoxicity by allowing the BBB to exert its neural protective

effects, and to achieve more selective delivery to the tumor by controlling the duration of BBBD. Dysregulation of K_{Ca} channels has been implicated in a wide spectrum of diseases, including epilepsy [100] and cancer [101]. In pre-clinical models, brain tumors and brain tumor capillaries express significantly higher K_{Ca} levels than normal brain tissue or capillaries [96,102], with expression of K_{Ca} correlated with malignancy and aggressive growth phenotypes [102-104]. The K_{Ca} receptor agonist NS-1619 causes depolarization selectively in murine brain tumor vasculature to enhance BBTB permeability. In a murine brain tumor model, co-administration of NS-1619 with the HER2-targeted mAb trastuzumab significantly improved distribution of the fluorescently-tagged antibody, and mice that received this combination also showed improved median survival [102]. Thus, these selective reagents may represent a safer biochemical mechanism for BBB disruption. However, the effects of potassium channel agonists on BBTB permeability vary between syngeneic and allogeneic animal models due to different expression levels of K_{Ca}. This mirrors observations that the expression of potassium channels in human brain tumors is variable, which may be associated with different tumor permeability to therapeutic agents among patients [105]. Further, while well-established tumors show such neovascularization, invasive cells and nascent tumors, which are the primary cause of CNS tumor recurrence, have not yet formed a tumor vasculature with differential K_{Ca} receptor expression. This could very well limit access of therapeutics to these sites by BBTB-specific disrupting strategies.

4.2.3. Focused ultrasound (FUS) for BBBD—Focused ultrasound (FUS) has been used in pre-clinical investigations as a method for increasing drug delivery to the brain by inducing temporary BBB disruption [106,107]. Development of FUS technology has enabled ultrasound to function not only as a diagnostic tool but also as a therapeutic modality when using gas-filled microbubbles as ultrasound contrast agents. Acoustic waves can be focused deeply into soft tissue without the need for surgical intervention. The mechanical interaction between the ultrasonic wave, the microbubbles, and the vasculature causes a transient disassembly of BBB tight junction proteins [108,109], and can stimulate active transport [110] to create a transient window for drug delivery. This technique has several advantages over other approaches in that it is noninvasive, readily repeatable, and targeted only to desired regions in the brain to where FUS is applied. Animal studies have shown that tight junction disruption increases the permeability of the BBTB [111,112], with the barrier restored within a few hours [106,108,113]. FUS as a BBBD is reportedly is not associated with significant tissue damage [114-116]. Recent work demonstrates that FUS following microbubble administration is capable of enhancing brain tumor delivery of BCNU chemotherapy [117] or trastuzumab [118], improving survival in a rat glioma model [117] and HER2-positive breast cancer brain metastases model, respectively [119].

4.2.4. Radiation-induced BBBD—Radiation therapy (RT), the use of ionizing radiation to damage DNA and instruct cell death, has become a mainstay of treatment for brain tumors. The safety record of RT now spans many decades. RT has become increasingly precise in recent years with innovations such as tumor definition with imaging, and conforming radiation delivery to the tumor by beam shaping, permits focused targeting of RT to tumors while minimizing radiation dose to normal tissue. In addition to having a

direct anti-tumor effect, RT may also enhance drug delivery to brain tumors. Pre-clinical and clinical studies support the premise that RT results in focal disruption of the BBTB while minimizing disruption of the adjacent BBB [120-127]. Early studies with irradiated rat brain found diffuse changes in rat brain when using more therapeutically relevant dosages of 20 to 25 Gy (a dose employed in clinical stereotactic hypofractionated RT), including dilation of blood vessel lumen, thickening of the blood vessel wall, enlargement of endothelial cell nuclei, and hypertrophy of adjacent astrocytes [128]. Others report reduced expression of drug efflux pump P-gP on BCECs by almost 60% [129].

New technologies such as the Small Animal Radiation Research Platform (SARRP) micro-irradiator, enables image-guided focal RT of murine brain tumors for translational investigations of RT-induced BBBD [130,131]. Large molecular weight fluorescent dye (60 kDa) that is impermeable to an intact BBB, accumulates significantly in a mouse brain tumor model following focused RT with SARRP [130]. SARRP irradiation focused on either control mouse brain or a brain tumor, as shown in Figure 4, also results in extravasation of the typically BBB impermeable Evans Blue dye, and systemic IgG [131]. IgG extravasation is considerable higher in irradiated brain tumor relative to irradiated normal brain, suggesting that the BBTB is more sensitive to BBBD relative to an intact BBB. These results together indicate that targeted RT can potentially serve to modulate BBTB permeability to enhance therapeutic drug delivery to CNS tumors.

In summary, improving brain delivery via BBBD is a double-edged sword that requires precise control of extent, location and duration of BBBD avoiding potentially dangerous side effects including the enhanced passage of unwanted substances through the protective barrier.

5. Noninvasive imaging approaches to guide success of molecular CNS tumor therapies

Despite the many candidate molecular markers of CNS cancers, it is increasingly evident that the delivery of immunotargeted therapeutics to these markers in brain tumors is limited in large part due to the cellular barriers of the BBB and BBTB. Noninvasive neuroimaging technologies used clinically, such as MRI, CT, PET, and SPECT, have greatly improved the accuracy of brain cancer diagnosis, are indispensible in pre-surgical planning, and also serve as a means for tumor evaluation during or after therapy. These same imaging techniques, as well as the optical imaging methods more often used in pre-clinical research, can also be used to explore important questions for any targeted drug delivery strategy to the brain. These questions include whether the molecular target is expressed and accessible, how much drug is delivered and to which area of the tumor. Undoubtedly, the development of imaging surrogates (functional and molecular) will allow appropriate patient and treatment selection, define optimum biological dose, predict treatment response, and identify drug resistance. Once validated, these surrogate imaging markers would be of great benefit in facilitating translational research.

5.1 Imaging BBTB permeability

Functional imaging of BBTB permeability in brain tumor patients using high-resolution, noninvasive and quantitative in vivo methods is gaining renewed interest for several reasons. First, BBTB permeability may serve as a surrogate marker for brain tumor angiogenesis, and would be useful for studying the effectiveness of anti-angiogenic drugs on decreasing vessel permeability. Second, understanding the mechanisms of BBTB permeability can identify which therapeutic agents can enter the brain parenchyma and target to tumor tissue. Third, as described in Section 4.2, understanding how increasing permeability results from BBBD methods can help in the optimizing selective permeability of the BBTB to enhance drug delivery. Additionally, the detection of functional changes in BBB/BBTB would serve to limit brain injury, and could permit a more objective assessment of therapeutic benefit on a shorter time scale than is possible with anatomical techniques. Magnetic resonance imaging (MRI) [122], computed tomography (CT) [132], SPECT [133], and PET [134] are translational imaging modalities available to quantify BBB/BBTB permeability noninvasively. However, the measure of BBB permeability in fact depends on the size, charge, and composition of whatever contrast agent is being used to measure BBB function, as defined by status of BCEC tight junctions, transporters, and channels.

In CT and MRI applications, BBB vessel permeability is assessed using intravenous contrast materials, such as iodine-based or gadolinium (Gd)-based agents, respectively [135]. MRI is a practical modality for assessing BBB permeability since it is already widely used clinically to assess vascular changes in tumor growth. MRI images are derived from the detected changes in relaxation properties (T 1 *1* and T_2) of excited hydrogen (H) nuclei in water and lipids of soft tissue generated from an electro-magnetic field. MRI contrast agents containing paramagnetic nuclei, such as Gd, change the chemical bonding of water to increase the rates of ^{1}H relaxivity, thereby altering the signal strength. Most studies for assessing leakiness of the blood–brain barrier in human brain tumors have used T_1-weighted dynamic contrast-enhanced MRI (DCE-MRI) using Gd chelated to diethylene triamine pentaacetic acid (DTPA). It should be noted that changes in MRI signal strength are not linear over the range of concentrations of contrast found in tissue thus measurements are typically made relative to a reference region. The disadvantage with MRI, however, is that MRI protocols and instrument performance have substantial effects on signal strength, making it difficult to compare data obtained with different instruments. With Gd-DTPA having low toxicity and MRI involving non-ionizing radiation, repeat imaging is possible and is limited mainly by instrument availability and expense. Perfusion CT images of BBB permeability are obtained from the x-ray attenuation of tissues containing an iodinated contrast agent, most often iohexol (MW 821 kDa). Data from CT imaging may be more quantitative than that obtained by MRI because the intensity of signal attenuation is directly proportional to concentration of contrast in a given region of interest. In addition, CT has the highest spatial resolution of all imaging modalities. However, the sensitivity of CT is limited since the high concentrations of CT contrast agent required for optimal signal attenuation can be toxic. Together with the relatively high radiation dose imparted to patients, the use of repeated CT scanning is limited.

Many studies have investigated the correlation between the presence of enhancement and the glioma grade [136,137]. These studies have shown that enhancement alone does not clearly distinguish varying grades of tumor malignancy. For example, approximately 30% of grade III anaplastic astrocytomas (AA) have functional BBB/BBTB as the tumors do not display contrast enhancement [138], whereas some higher grade IV GBM do not show enhancement at all. Currently, CT or MR imaging contrast agents used in the clinic are of low molecular weight (~0.5 to 0.9 kDa) and have an average hydrodynamic diameter of 1 nm. Since permeability measurements depend on the leakage of materials from the blood to the interstitial space, it is conceivable that low-molecular-weight contrast agents can leak relatively easily from the blood into the brain parenchyma, which might over-estimate vascular permeability. In a recent pre-clinical MRI study, it was noted that when comparing low molecular weight (0.5 kDa) and macromolecular (3.5 kDa) gadolinium contrast agents, there was differential enhancement in intracranial tumors after RT and anti-angiogenic therapies [121]. This was presumably because of different sizes pores created within the BBTB following RT. Generally, CT and MR imaging contrast agents do not differ much in their molecular weight. However, the disparity in permeability measures between these two modalities could be due to the difference in surface charge of nonionic CT contrast agents as compared with ionic MRI contrast agents.

Imaging of BBB status in patients can also be performed with SPECT, using ^{99m}Tc-DTPA and ^{99m}Tc-glucoheptonate (^{99m}Tc-GH) radiopharmaceuticals. In patients with malignant brain tumors, ^{99m}Tc-GH (physical half-life ($t_{1/2}$) of 6 h) imaging showed that the permeability of the BBB in and around the tumor increases from approximately 20% to 75% following 30 Gy irradiation [120]. However, obtaining quantitative data from SPECT remains challenging. In principle, PET is uniquely capable of quantifying total tracer uptake and determining the kinetics of tracer transport across the BBB with dynamic studies. Both ^{68}Ga-ethylenediamino tetraacetic acid (^{68}Ga-EDTA, $t_{1/2}$ 68 min) and ^{82}RbCl ($t_{1/2}$ 75 sec) have been successfully used to image permeability in a variety of malignancies in the human brain [134]. The permeability values measured with these two tracers are not the same, because of their large differences in chemical properties and molecular size. ^{68}Ga-EDTA measures the passive physical permeability of the BBB as seen in dynamic contrast-enhanced MRI. In patients with primary CNS tumors, ^{68}Ga-EDTA-PET can detect significant changes in tumor permeability; following chemotherapy and reported normalization of tumor vasculature, tumor permeability measurements by PET are comparable to that of the normal brain [139]. $^{82}Rb^{+}$ is a potassium analogue that also crosses the intact BBB, albeit slowly due to its positive charge and significant hydration. Despite its very short half-life, ^{82}Rb-PET can be used to rapidly quantify changes in BBB permeability, for example in patients with intracranial tumors [140], and after mannitol-induced BBBD [141].

Imaging BBB/BBTB permeability with currently available contrast agents is very helpful in identifying areas of leaky vasculature, and determining how and on what time scale BBBD strategies can further increase permeability. Using these techniques, some authors have reported an association between the degree of the BBBD as a surrogate of drug delivery and survival in patients bearing primary central nervous system lymphomas treated with a BBBD procedure [142]. However, these contrast agents with sizes much smaller than Abs,

would only report on the delivery potential of therapeutics of comparable size to enter the brain tumor, and could be a false predictor of mAb delivery and improved clinical benefit. Thus, the arsenal of imaging probes for brain permeability must include CNS-deliverable reporters that should inform drug accessibility and targeting of larger immunotargeted agents across the BBB/BBTB.

5.2 Imaging immunotargeting

Molecular imaging is used to detect characteristic biomolecules, whose concentration is often changed by disease. Detection sensitivity of probes for CT imaging systems is in mmol/L whereas for MRI it is ~10 μmol/L. PET and SPECT are the most sensitive clinical imaging techniques where pmol/L sensitivity is achievable (note that PET is two to three order of magnitude more sensitive than SPECT) [143]. Many drugs and therapeutics can be labeled and thus detected directly by imaging techniques. To date, the FDA has approved four antibodies for the detection of cancer (anti-TAG-72 [tumor-associated glycoprotein 72] mAb ^{111}In-satumomab pendetide, anti-EGP40 [epithelial glycoprotein 40] Fab ^{99m}Tc-nofetumomab merpentan, anti-CEA [carcinoembryonic antigen] Fab' ^{99m}Tc-arcitumomab, anti-PSMA [prostate specific membrane antigen] mAb ^{111}In-capromab pendetide), all of which are radioactively tagged for SPECT imaging [144]. Immuno-PET, which combines positron-emitting isotopes with mAbs, is an attractive approach to detecting mAb targeting due the very high sensitivity and spatial resolution of PET, as compared to SPECT. Hybrid scanners such as PET/CT and PET/MRI add the additional benefit of allowing co-registered anatomical and functional imaging. Further, PET imaging allows repeated measurements over time to obtain pharmacokinetic data on uptake, distribution, and clearance, and is equally applicable in animals and humans. For example, in vivo data can show whether the candidate immunotherapeutic reaches the brain tumor in appreciable concentrations or whether it accumulates excessively in another organ, alluding to drug efficacy and/or toxicity. The potential to quantify molecular interactions makes immuno-PET especially attractive as a scouting procedure prior to therapy.

Imaging of antibodies and their derivatives is a key application in their clinical translation seeing as size is a determinant for biodistribution and clearance kinetics. Typical examples of biological $t_{1/2}$ decreasing with decreasing antibody size include IgG (150 kDa, $t_{1/2}$ ~21 d), minibodies (80 kDa, $t_{1/2}$ 6–11 h), diabodies (55 kDa, $t_{1/2}$ 3–7 h), and, scFv (25 kDa, $t_{1/2}$ 0.5–2.0 h). Antigen affinity, epitope accessibility, and the fate of the probe upon cell binding are highly dependent on antibody design, and imaging can thus inform re-engineering of construct design to optimize antibody-tumor targeting and off-target profiles.

Currently, the information obtained in most neuro-oncological imaging studies in brain tumor patients is limited to anatomic features in addition to a small subset of functional biological processes, such as enhancement, glucose metabolism, and amino acid metabolism (Figure 5). Remarkably, very few studies have evaluated drug concentrations in brain tissue following administration. PET has been used in early clinical drug development trials, as was done in a Phase II trial for ^{11}C-TMZ to assess tracer pharmacokinetics in gliomas and normal brains [145]. However, nuclear imaging of therapeutic mAb delivery to brain tumors following systemic administration is limited to SPECT examples (Figure 5B). The

information obtained from immune-PET imaging studies can be very helpful in guiding the translation of investigational antibody agents from pre-clinical studies to early-phase clinical trials through to late-phase studies.

6. Conclusions

Ultimately, the adequate delivery of therapeutics to brain tumors is critical to kill all residual cancer cells for optimal patient outcomes. As described in this review, the BBB and BBTB presents a significant physical barrier that muse be overcome to achieving adequate therapeutic concentrations in the brain from the systemic circulation. Various approaches have been designed to improve brain delivery of immunotargeted agents. And, while each have the potential to play a significant role in the treatment of CNS disease, *in vivo* pre-clinical and clinical studies systematically quantifying accessibility and delivery of immunotargeted agents to the brain, and measuring therapeutic response and safety in CNS tumors is significantly lacking. Evaluating the efficacy of new treatment paradigms is extremely time-consuming and expensive owing to the standard clinical end points of radiographic response and survival outcomes. Noninvasive imaging with MRI, CT, PET and SPECT can serve to streamline the process by measuring surrogates of treatment response, including biomarkers that are themselves the target of therapy, that are more responsive to phenotypic changes in the tumor status. Patients, providers and payers alike would eagerly await the outcomes of such clinical trials to test the safety and efficacy of these exciting new approaches as definitive brain tumor therapies.

7. Expert Opinion

CNS malignancy remains a challenge in management, particularly in GBM where no currently available treatment is curative and prognosis is poor. Parallel to the search for a definitive treatment for CNS tumors, there is a need to improve the delivery of currently available drugs and those in development to adequately evaluate their therapeutic potential. As reviewed here, antibody-based devices, which are inherently targeted, still suffer from poor delivery across the blood-brain barrier into CNS tumors. Many approaches are being explored to enhance the delivery of these agents across the intact BBB by receptor-mediated transcytosis, or following osmotic or radiation-induced BBBD, for example. While some pre-clinical studies highlight the significant potential of these approaches in achieving improved brain tumor delivery of immunotargeted agents, the ultimate goal is the definitive assessment if the therapeutic value of such strategies and the subsequent translation of these approaches to clinical trials. Thus, current efforts should also be focused on evaluating the efficacy of these approaches to develop newer generations of potent and selective agents for optimal in vivo benefit. It should also be kept in mind that those strategies that increase normal brain exposure to currently approved drugs as compared to tumor exposure may alter the therapeutic index and safety profile of many drugs.

Imaging procedures have made important contributions to drug discovery and drug targeting, particularly in determining drug pharmacokinetics of directly labeled therapeutics, including those that are immunotargeted. Also valuable in the setting of CNS tumor imaging is the assessment of BBB/BBTB permeability, especially in the context of evaluating BBB

disrupting strategies that are being explored clinically. While helpful in delineating the general status of the cellular barriers that can act to restrict access of contrast agents to the brain, one important caveat is that these measures are typically obtained from contrast agents that are less that 3.5 kDa in size. Thus, permeability measures can only be extrapolated for drug delivery of therapeutics of that size, and may not serve as a surrogate measure for delivery potential of antibodies and larger immunotargeted carriers (>25 kDa). Nonetheless, the various imaging techniques are complementary, and multimodal neuroimaging strategies will help to accelerate drug development by assisting in understanding and defining the challenges to translating molecularly targeted agents to the brain and tumors contained within this organ.

Undoubtedly, bevacizumab has had remarkable clinical benefit in brain tumor shrinkage and an increase in 6-month progression free survival. However, antiVEGF-therapy is not curative and can transform tumor growth pattern toward a more invasive phenotype, suggesting that targeting angiogenesis alone is not sufficient for effective tumor control. Given the relative success of bevacizumab, along with the low rate of anti-cancer mAbs that have advanced into Phase III trials, the questions remains as to why numerous other immunotargeted agents developed in the past decade were effective in preclinical laboratory models, but have been “lost in translation”. It could be that current heterotropic and orthotopic xenograft models of CNS cancer fail to recapitulate the complex tumor microenvironment found in human disease. More recent alternative models include genetically engineered mouse models (GEMMs) [146], and cancer stem cell models [147]. Spontaneous canine GBM, which is highly invasive and exhibits the classical patterns of human GBM invasion, also represents a very valuable preclinical model to test not only the efficacy of novel therapies, but also their toxicity to the normal brain.[148]

While newer immuotargeted agents are designed to be more specific and potent with ideal pharmacokinetics to target disease, the first trials were in unselected patient populations and favorable responses to certain therapeutics were diluted since not all patients were positive for the specific molecular abnormality that was targeted. Using noninvasive imaging technologies like PET and SPECT is an ideal approach to identifying the molecular phenotype of patient’s tumors in real time for subsequent treatment stratification with an individualized therapeutic regimen based on their molecular signature. Perhaps under these conditions it will be easier to identify improved overall survival outcomes.

A final barrier to consider for the clinical translation of these new discoveries could potentially be linked to the high cost associated with developing biologics relative to small molecule inhibitors. Alternatives to these non-trivial issues would include the development of smaller antibody fragments and peptides that have comparable targeting specificity and potency but are easier and cheaper to produce as compared to whole IgG antibody-based agents. Ideally, the reduction in development costs would decrease the barrier to patient access to these innovative treatments without incurring costs that can be in excess of $100,000 per year for many biologics.

While the current landscape for developing effective immunotargeted agents with optimal delivery to CNS tumors may seem daunting given the challenges presented, there is

tremendous opportunity for the drug delivery field to make important contributions in identifying definitive brain tumor therapies and make them more accessible to patients to change the course of their disease and improve their overall survival.

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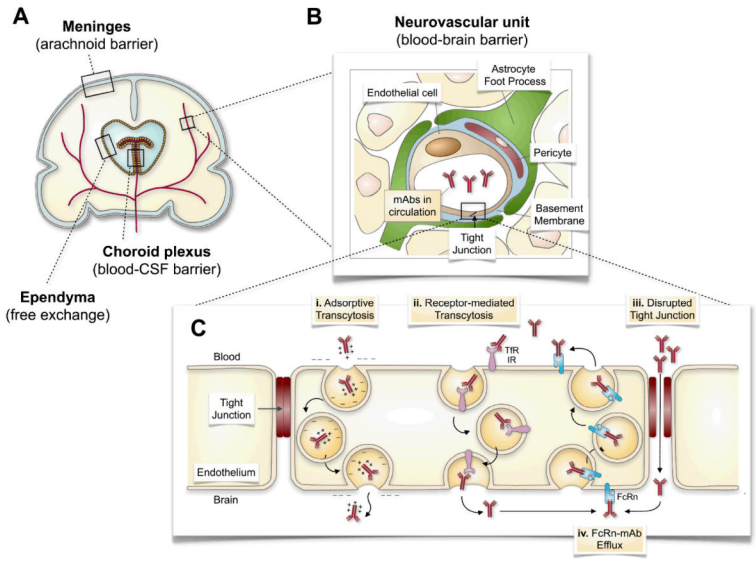

Figure 1.

Routes for antibody delivery across the blood-brain barrier (BBB). (A) The brain is comprised of multiple barriers that control access of administered agents into the brain parenchyma, including the BBB. (B) The BBB is comprised of a neurovascular unit that consists of pericytes, astrocytes, and neurons surrounding brain capillary endothelial cells (and basement membrane). (C) Expansion of the endothelial lining of the BBB shows that intact tight junctions in the normal brain restrict the intercellular diffusion of mAb across the BBB into the brain. (i) Adsorptive transcytosis of cationized mAb can increase transcellular transport across the BBB by their interaction with naturally negatively charged plasma membrane. (ii) MAb engagement of the transferrin receptor (TfR) or the insulin receptor (IR), for example, can also increase mAb transport across the BBB by receptor-mediated transcytosis. (iii) The disruption of tight junctions by pharmacological (osmotic, biochemical) and mechanical (ultrasound, irradiation) can enhance paracellular extravasation of mAbs into the brain parenchyma. (iv) The neonatal Fc receptor (FcRn) serves as an efflux mechanism to remove Fc-containing mAb from the brain and return them to the systemic circulation. Figure 1A and 1B adapted with permission from Elsevier Ltd. (Copyright © 2008): Saunders, N.R. *et al.* Barriers in the brain: a renaissance? Trends in Neurosciences. 31(6): 279-286. Abbreviations: CSF, cerebrospinal fluid; mAb, monoclonal antibody; TfR, transferrin receptor; IR, insulin receptor; FcRn, neonatal Fc receptor.

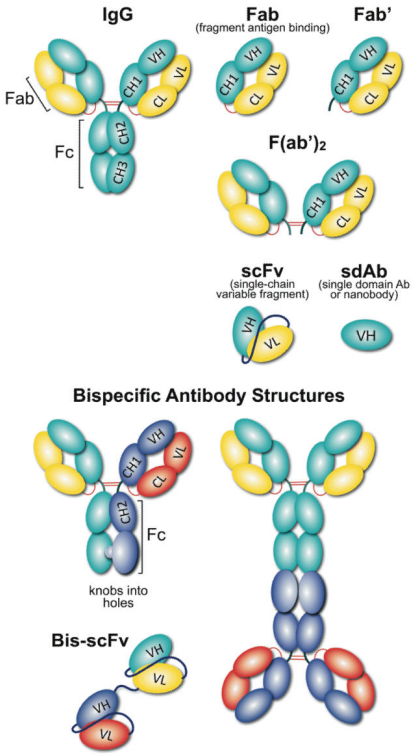

Figure 2.

Schematic representation of different antibody formats. An array of recombinant antibody formats were developed using combinations of antibody domains, joined either with linkers and/or disulfide bonds. Using molecular biology tools, antibody valency and specificity can be tailored into monovalent (sdAb (single domain Ab); Fab and Fab' (fragment antigen binding), divalent ($F(ab')_2$) and even multivalent formats. Formats with antibody constant domains are highly stable, however, single chain antibody formats are popular due to their small size, expression and tissue penetration capabilities. Linker length can also influence the antibody behavior in terms of stability, flexibility and aggregation. A bispecific antibody is a recombinant antibody format that recognize two distinct antigens, using two IgG or scFv, linked together either chemically by covalent conjugation; or genetically with peptide linkers; or disulfide bonds.

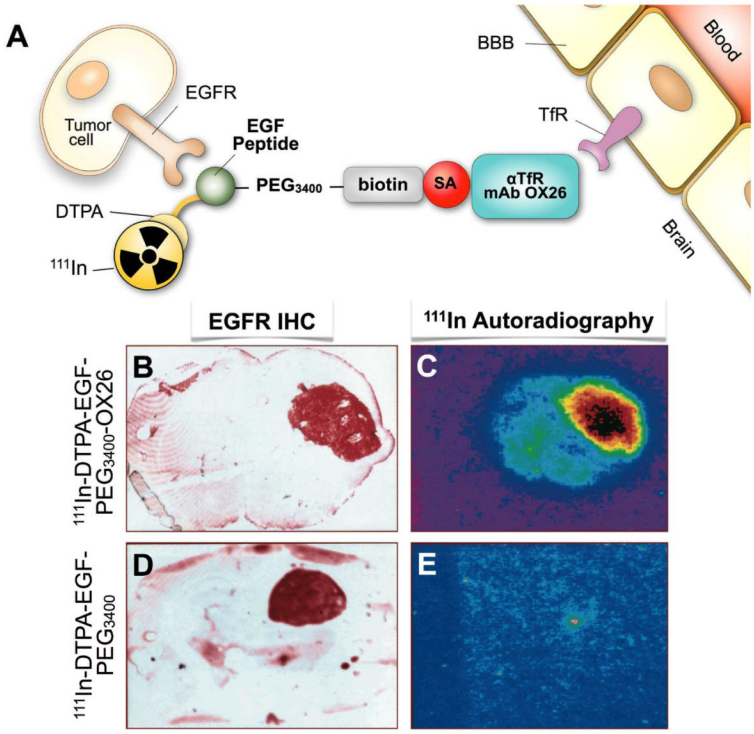

Figure 3.

A, Structure of dual targeted epidermal growth factor (EGF) chimeric peptide conjugated to rat transferrin receptor (TfR) targeted mAb OX26. The EGF is modified with diethylenetriamine pentaacetic acid (DTPA) to chelate the radioisotope ^{111}In. The EGF is attached to a 3400-Da polyethylene glycol linker (PEG_{3400}) terminated with a biotin moiety for conjugation to streptavidin (SA)-OX26 MAb. B, D. Brain sections of U87 human glioma tumor-bearing rats were stained immunocytochemically using an MAb to the human EGF receptor (EGF-R), which demonstrates expression of the EGF-R in brain tumor specimens. C, E. Ex vivo ^{111}In-autoradiography demonstrates that the brain tumor can be imaged with the EGF chimeric peptide that can undergo transport across the BBB in vivo via antiTfR mAb OX26. (C) There is no imaging of the brain tumor when the non TfR-targeted EGF peptide radiopharmaceutical is administered, because the EGF does not cross the BBB alone (E). Adapted with permission from the American Association for Cancer Research (Copyright © 1999): Kurihara A and Pardridge WM. Imaging brain tumors by targeting peptide radiopharmaceuticals through the blood-brain barrier. Cancer Res. 1999; 59 (24), 6159-63.

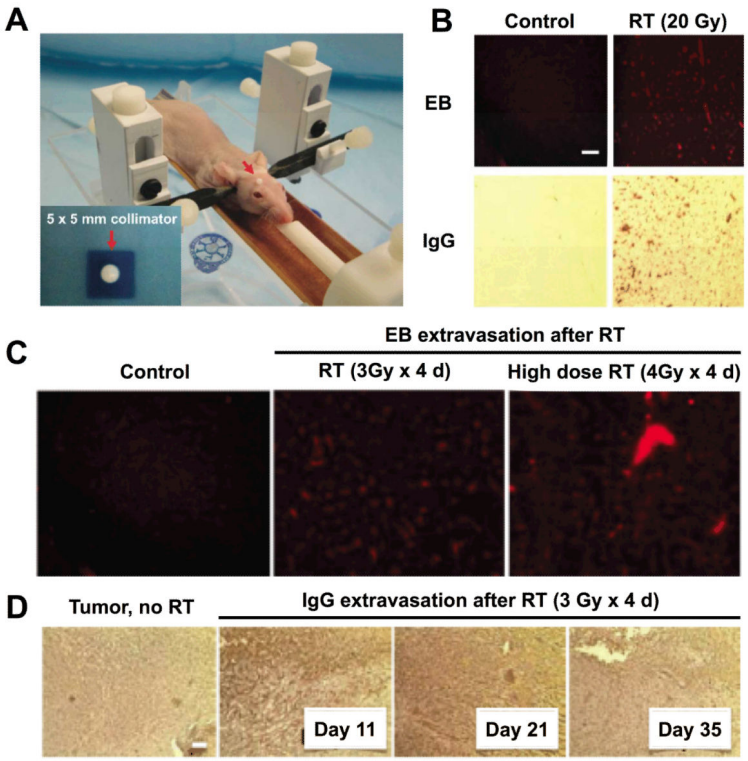

Figure 4. Targeted radiation therapy disrupts brain tumor blood-brain barrier and permits greater penetration of Evans Blue dye and IgG

A, Cranial RT is delivered using a novel mouse restrainer housed within the Small Animal Radiation Research Platform (SARRP). RT is focused using a 5 × 5 mm collimator (inset). B, Healthy nude mice, mock-irradiated ("Control", left column) or irradiated with 20 Gy in a single fraction to the right cerebral hemisphere ("RT", right column), show BBB disruption (BBBD) as visualized with Evans Blue dye (EB) extravasation 24 h post RT (top row), or via staining for IgG extravasation out of the systemic circulation (bottom row). C, Dose-response of BBBD by focused RT with normal mice that are mock-irradiated (left panel), irradiated with 3 Gy daily x 4 days (middle panel) or 4 Gy daily x 4 days (right panel). EB is injected and mice sacrificed 24 h after the final RT fraction, and dye uptake visualized with fluorescence imaging. D, RT induces Tumor-BBBD in GBM intracranial orthografts compared to mock-irradiated tumor-bearing controls. Mice with intracranial orthotopic GBM tumors are either mock-irradiated or irradiated with 3 Gy daily x 4 days. IgG extravasation out of the systemic circulation is evident out to 35 days after RT. Reprinted with permission from Baumann, BC et al. Oncotarget. Copyright © 2012 Impact Journals.

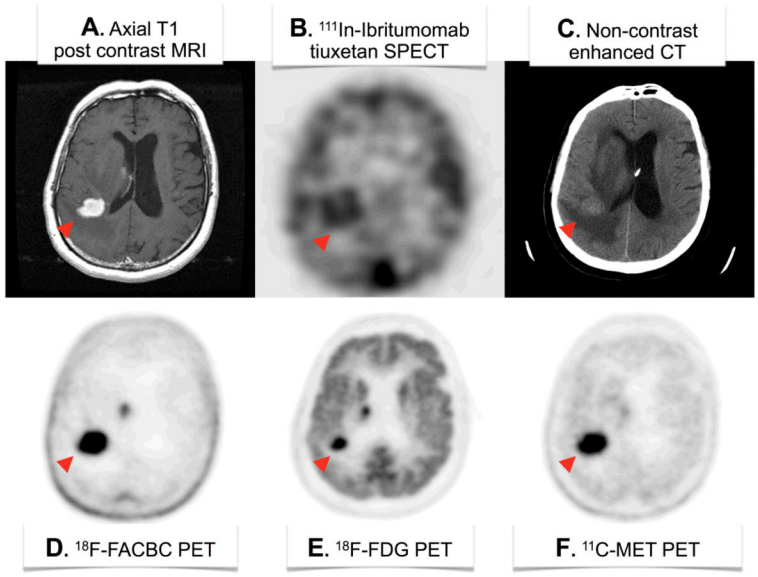

Figure 5.

Various brain scans in a patient with primary CNS lymphoma (red arrowhead). (A) Axial post-contrast MRI shows brisk enhancement indicative of BBBD. (B) ^{111}In-Ibritumomab tiuxetan SPECT shows binding of this anti-CD20 IgG mAb to the malignancy. (C) Non-contrast head CT reveals the anatomic mass and surrounding mass effect and edema. (D) ^{18}F-FACBC is a synthetic L-amino acid analog showing high amino acid metabolism in the disease and very low background utilization in normal brain parenchyma. (E) ^{18}F-FDG shows high glucose metabolism throughout the gray matter with focally increased uptake in sites of disease. (F) The positron-emitting natural amino acid ^{11}C-methionine also shows the high amino acid metabolism in the disease by PET, but with more uptake in normal brain parenchyma as compared to ^{18}F-FACBC. Images courtesy of Department of Radiology, Memorial Sloan-Kettering Cancer Center. Abbreviations: FDG, fluorodeoxyglucose; FACBC, 1-amino-3-fluorocyclobutane-1-carboxylic acid; MET, L-methionine.

Table 1

Antibody-Based Therapies in Clinical Trials for the Treatment of Malignant Glioma[a]

Antibody	Conjugate	Molecular Target	Other agents	Specific CNS Tumor	Trial Status
Bevacizumab		VEGF		Recurrent GBM	recruiting
TRC105		Endoglin			
Ramucirumab IMC-3G3		VEGFR-2 PDGFR		Recurrent GBM	active; not recruiting
TNT-1/B	^{131}I	Universal, intracellular nucleosomal determinants consisting of histone H1 and DNA		Progressive or recurrent GBM	completed
TNT-1/B	^{131}I	Histone H1 and DNA		GBM or AA	completed
Bevacizumab		VEGF	TMZ	GBM or gliosarcoma in older patients	recruiting
TRC105		Endoglin		Recurrent GBM	recruiting
chTNT-1/B	^{131}I	Histone H1 and DNA		Recurrent GBM	completed
Bevacizumab		VEGF	TMZ or irinotecan	Recurrent GBM	active; not recruiting
chTNT-1/B	^{131}I	VEGF		Recurrent GBM	completed
Bevacizumab		VEGF	TPI287	Recurrent GBM	recruiting
Bevacizumab		VEGF	Lomustine	Recurrent GBM	recruiting
Bevacizumab		VEGF	Vorinostat	Recurrent GBM	active; not recruiting
Bevacizumab		VEGF	Radiosurgery	GBM	recruiting
Bevacizumab		VEGF	RT	Recurrent GBM	not yet recruiting
Bevacizumab		VEGF	Hypofractionated RT and TMZ	Recurrent high grade GBM	active; not recruiting
Bevacizumab		VEGF	RT	Newly diagnosed GBM	active; not recruiting
Bevacizumab		VEGF	Trabanaib	Recurrent brain tumor	suspended
TRC105		Endoglin		Recurrent GBM	recruiting
Bevacizumab		VEGF	R04929097	Progressive or recurrent GBM	recruiting
Neuradiab		Tenascin C		Recurrent GBM	unknown
Bevacizumab		VEGF			
3F8	^{131}I	Ganglioside GD2		CNS Cancer or leptomeningeal cancer	recruiting
Bevacizumab		VEGF	Cediranib maleate	Metastasis or unresectable solid tumor, Lymphoma, GBM, gliosarcoma or AA	active; not recruiting
Bevacizumab		VEGF	Cliengitide	Recurrent GBM	not yet recruiting

Antibody	Conjugate	Molecular Target	Other agents	Specific CNS Tumor	Trial Status
3F8	^{131}I	Ganglioside GD2		Leptomeningeal cancer	completed
81C6	^{131}I	Tenascin C		Recurrent glioma	completed
Me1-14		Chondroitin proteoglycan sulfate		Metastatic melanoma or brain tumors	completed
81C6	^{131}I	Tenascin C		Primary brain tumors	completed
81C6	^{131}I	Tenascin C		Primary or metastatic brain tumor	completed
81C6	^{131}I	Tenascin C	RT	Primary brain tumors	completed
81C6	^{211}At	Tenascin C		Primary and metastatic brain tumor	completed
MAB-425	^{125}I	EGFR		High grade glioma	active; not recruiting
Bevacizumab		VEGF	Sorafenib	Recurrent GBM	completed
Bevacizumab		VEGF	TMZ, external beam RT	GBM and gliosarcoma	active; not recruiting
Daclizumab		CD25	TMZ	GBM and TMZ-induced lymphopenia	active; not recruiting
Bevacizumab		VEGF	Dasatinib	Recurrent or progressive high-grade glioma or GBM	recruiting
Bevacizumab		VEGF	Lomustine	GBM in first recurrence	not yet recruiting
Bevacizumab		VEGF	Erlotinib	GBM and gliosarcoma	active; not recruiting
Basiliximab		CD25	Chemotherapy, RT and vaccine	GBM that has been surgery resected	active; not recruiting
Nimotuzumab		EGFR		GBM	completed
Bevacizumab		VEGF	Lenalidomide, sorafenib, temsirolimus, 5-FU, leucovorin, oxaliplatin	Advanced cancer	recruiting
Bevacizumab		VEGF	Rindopepimut with GM-CSF adjuvant	EGFR-vIII-positive GBM	recruiting
MGA271		B7-H3		Refractory cancer	recruiting
Bevacizumab		VEGF		Progressive or recurrent glioma	active; not recruiting
Bevacizumab		VEGF	TMZ	Recurrent glioma	unknown
Bevacizumab		VEGF	BCNU	Relapsed or progressive high-grade glioma	active; not recruiting
Bevacizumab		VEGF	Irinotecan	Recurrent or refractory glioma	completed
Bevacizumab		VEGF	Irinotecan	Malignant glioma	completed
MOC31	PE	EpCAM		Antigen positive carcinomas	completed
Bevacizumab		VEGF		Colorectal, lung, breast cancer and GBM	completed
Panitumumab		EGFR	Irinotecan	Malignant glioma	Terminated

[a]From ClinicalTrials.gov; accessed March 2013.

Abbreviations: VEGF, Vascular endothelial growth factor; VEGFR-2, Vascular endothelial growth factor receptor 2; PDGF, Platelet-derived growth factor receptor; EGFR, Epidermal growth factor receptor; GBM, Glioblasotma multiforme; AA, Anaplastic Astrocytoma; TMZ, Temozolomide; RT, Radiation therapy; B7-H3, B7 homolog 3; EpCAM, epithelial cell adhesion molecule; CD25, α chain of the IL-2 receptor; 5-FU, 5-fluorouracil; GM-CSF, Granulocyte-macrophage colony-stimulating factor; BCNU, bischloroethylnitrosourea; PE, *Pseudomonas* exotoxin.