REVIEW

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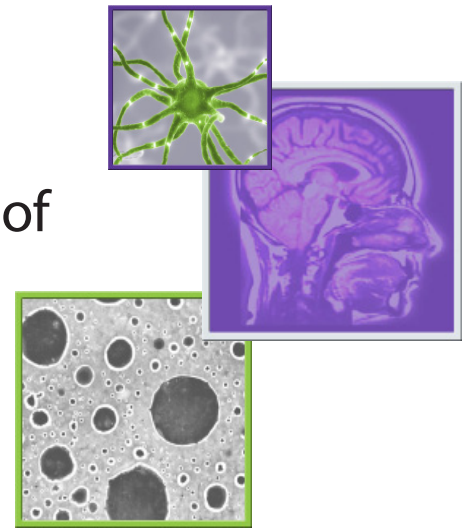
Strategies for overcoming the blood–brain barrier for the treatment of brain metastases



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Practice Points

- The blood–brain barrier (BBB) continues to pose a therapeutic challenge for the treatment of metastatic brain tumors, as demonstrated by the increasing incidence of brain metastases from HER2$^+$ breast cancer since the advent of trastuzumab therapy.
- Nearly all large-molecule therapeutic agents and 98% of small-molecule drugs are excluded from the CNS when the BBB is intact.
- Even if the BBB is disrupted in the center of a tumor, the BBB surrounding the leading invasive edge of the tumor is likely to be relatively intact.
- Transiently increasing BBB permeability for the purpose of improving chemotherapy delivery can be achieved using methods of osmotic or pharmacologic disruption.
- Inhibiting efflux transporters at the BBB in order to increase the exposure of cancer cells to therapeutic agents remains a tantalizing strategy, but results thus far have been modest, and data regarding efficacy for metastatic brain tumor patients are limited.
- Several efforts are underway to utilize endogenous receptors along the BBB to transport therapeutic compounds into the CNS.
- The effect of antiangiogenic therapy on chemotherapy delivery may depend on whether drug delivery is limited by permeability or blood flow.

SUMMARY The era of targeted therapy for cancer has been punctuated by some resounding successes, but with few exceptions, metastases to the brain remain frustratingly difficult to treat. It is increasingly apparent that old concerns regarding the ability of therapeutic agents to penetrate the blood–brain barrier have not been brushed aside by high-affinity small-molecule kinase inhibitors and monoclonal antibodies. Indeed, illustrative trends, such as the increasing incidence of brain metastases from HER2$^+$ breast cancer since the advent of trastuzumab therapy, have helped to solidify the concept of the CNS as a sanctuary site for cancer. With 200,000 patients diagnosed with brain metastases in the USA each year, the therapeutic challenge posed by the blood–brain barrier continues to be a big problem.

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Blood–brain barrier

There are 100 billion neurons in the human brain, served by 100 billion capillaries [1]. This incredibly intricate vascular network is much more than a simple barrier. It is a dynamic structure, coupled with and instantaneously responsive to the buzzing neural networks that make us who we are. It tightly regulates the volume and composition of extracellular fluid within the CNS, selectively transporting nutrients in. Yet it was its ability to keep unwanted substances out that led Lewandowsky to coin the term 'blood–brain barrier' (BBB) in 1900 [2].

The BBB is a structural and physiological entity composed of highly specialized endothelial cells, the pericytes that surround and modulate them, astrocytic end-feet and neuronal processes (Figure 1). The endothelial cells of brain capillaries are connected by tight junctions and adherens junctions that limit paracellular

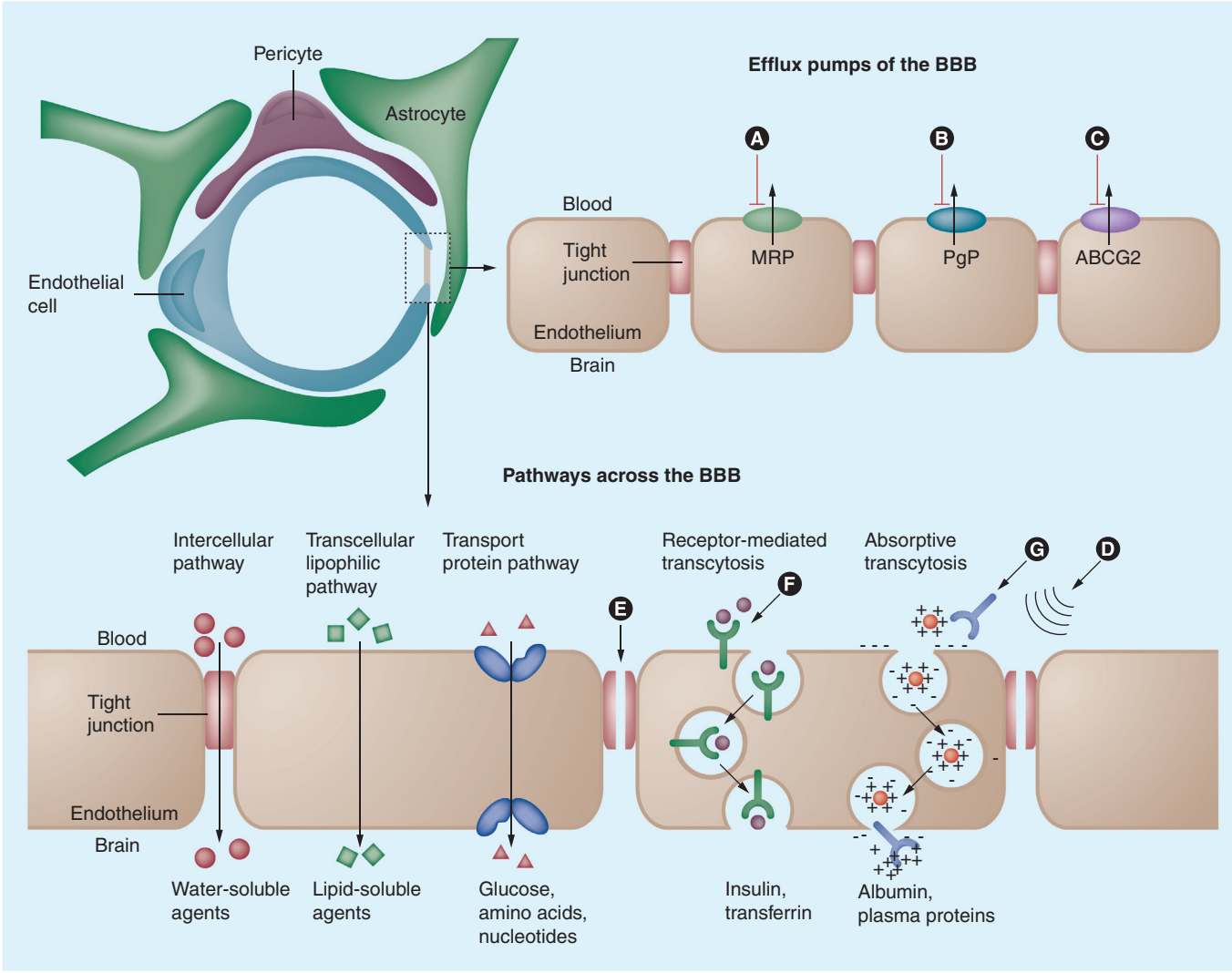

Figure 1. Approaches to enhance drug delivery to the brain. The main drug efflux transporters of brain capillary endothelial cells include MRPs, PgP and ABCG2. All of these transport proteins have been targeted for pharmacological inhibition. The action of **(A)** probenecid, sulfinpyrazone and MK-571; **(B)** verapamil, cyclosporin A, quinidine, valspodar, elacridar, biricodar, zosuquidar and tariquidar; and **(C)** GF120918 (elacridar) and fumitremorgin C. Tight junctions normally restrict the penetration of water-soluble compounds across the BBB, but they can be disrupted by mechanical and pharmacological methods via **(D)** ultrasound and **(E)** bradykinin analogs, respectively. **(F)** Receptor-mediated transcytosis of transferrin or insulin has been used to increase the transport of drugs across the BBB and **(G)** cationization (i.e., antibodies) can increase uptake of molecules by absorptive transcytosis.
BBB: Blood–brain barrier; MRP: Multidrug resistant protein; PgP: P-glycoprotein.
Reproduced with permission from [60].

transport, which is highly regulated and limited to substances that are essential for brain function. Compared with capillaries elsewhere, the endothelial cells of the BBB lack fenestrations, have low rates of pinocytotic and endosomal transport and are loaded with efflux transporters such as P-glycoprotein (PgP), BCRP, organic anion and cation transporters, and the family of multidrug resistance-associated proteins. These transporters effectively shuttle a diverse range of compounds out of the brain, including several chemotherapeutic agents. PgP alone binds over 200 known substrates.

The BBB is also an enzymatic barrier, with several enzymes – including CYP450, monoamine oxidase, catechol-*O*-methyltransferase, epoxide hydrolase, UDP-glucuronosyltransferase and glutathione *S*-transferase – that are all present on the lumenal surface of BBB endothelial cells, ready to metabolize potential intruders [3]. Electrical resistance at the BBB is also much higher than what is found in capillaries elsewhere [4].

Blood–tumor barrier

There is growing recognition that dysfunction of the BBB is a critical element in the pathogenesis of brain metastasis. Structural changes in the BBB can be detected with nascent collections of tumor cells as small as 0.2 mm^2 [5]. Tumor-associated capillaries exhibit endothelial hyperplasia and increased pinocytotic activity, with widened fenestrations in some areas and thickened basal lamina in others [6]. Some tumor cells are even capable of transdifferentiating into endothelial cells [7,8]. As the vascular architecture loses its organization, vessels become more tortuous, overperfusing some areas and underperfusing others [9]. Regions of hypoxia border widened, thickened blood vessels. Interstitial fluid pressure builds up, resulting in areas where nothing flows in or out, creating a swamp, despite the increased permeability of the vessels themselves. These distinctions from the normal BBB led to the use of the term 'blood–tumor barrier' (BTB).

In addition to the ultrastructural differences, the BTB also differs from the BBB on a molecular level. *PgP* expression is reduced in some brain tumors (e.g., lung, melanoma and untreated breast), while increased *BCRP* expression has been noted in others [3]. Some enzymes (e.g., nitric oxide synthase) are overexpressed along the BTB, while others (e.g., Ang I converting enzyme) are underexpressed. Non-neoplastic elements of the tumor microenvironment influence the BTB as well. Reactive astrocytes release matrix metalloproteinases, VEGF and heparanase – enzymes that further disrupt the integrity of the BTB [10].

Assessing the challenge

It has been estimated that 98% of small-molecule drugs and nearly all large-molecule therapeutic agents are excluded from the CNS when the BBB is intact [11]. Newer targeted therapies are not exempt [12]. For example, the cerebrospinal fluid:plasma ratio is only 6% for erlotinib and 0.33% for trastuzumab [13]. But are these numbers relevant? It used to be argued that the contrast enhancement displayed by brain metastases signified a level of BBB disruption sufficient to achieve therapeutic drug concentrations where it matters most – in the tumor. Dispelling this notion, Lockman *et al.* analyzed over 2000 brain metastases in two mouse models and found that while BBB permeability was indeed partially compromised in 89% of lesions, concentrations of ^{14}C-paclitaxel and ^{14}C-doxorubicin were still less than 15% of those found in other tissues or peripheral metastases [14]. Furthermore, even if the BBB is disrupted in the center of a tumor, the BBB surrounding the leading invasive edge of the tumor and nascent small collections of tumor cells (so-called 'micrometastases') is likely to be relatively intact. Indeed, the few studies assessing intratumoral drug concentrations that have been performed demonstrate that drug concentrations vary by as much as an order of magnitude depending on whether drug sampling is performed in the necrotic tumor core, active tumor periphery or adjacent normal brain tissue [6,15]. Therefore, while it is true that cerebrospinal fluid:plasma ratios are a poor proxy for intratumoral drug concentration, it can not be assumed that the problem of drug delivery to the CNS is obviated by a disrupted BBB.

To cross the BBB, a therapeutic agent should be smaller than 180 Da, unless it is ferried over by a specific transporter or receptor [11]. (Many conventional agents – such as vincristine, paclitaxel and etoposide – weigh over 400 Da.) The agent should be sufficiently liposoluble; water-soluble compounds generally must breach the BBB through the paracellular route somehow. Another factor that limits CNS penetration is plasma protein binding. Chlorambucil, for example, is 99% protein bound and only the free fraction of the drug is available to enter the CNS [16]. Efflux transporters must also be avoided. Although

classically described as limiting the efficacy of natural product chemotherapeutic agents such as vincristine and paclitaxel, efflux transporters also inhibit CNS penetration of several newer small-molecule tyrosine kinase inhibitors.

This review focuses on a few of the most prominent strategies for overcoming the obstacles posed by the BBB. Methods to transiently increase BBB permeability to provide a window for increased chemotherapy delivery will be discussed. The extensive history of efflux transporter inhibition will be summarized. Last, 'trojan horse' methods, in which therapeutic compounds gain entry into the CNS via receptors present on brain endothelial cells, will be covered. Intracavitary, intranasal, intrathecal, intraventricular and convection-enhanced delivery methods will not be addressed. Suffice to say, concerns regarding intratumoral drug penetration exist with these methods as well. The simple strategy of alternative dosing (e.g., high-dose intravenous [iv.] methotrexate for CNS lymphoma or the yet-to-be-proven strategy of weekly high-dose erlotinib for leptomeningeal carcinomatosis from *EGFR*-mutant lung cancer) will also not be addressed further, although its occasional efficacy is worth noting [17].

Increasing BBB permeability

Osmotic disruption

Osmotic disruption of the BBB involves the intra-arterial (ia.) administration of a hyperosmolar agent (most commonly mannitol), which draws water from endothelial cells into the bloodstream, resulting in osmotic shrinkage of the endothelial cells and increased paracellular permeability. This effect is transient, yet provides a window for increased chemotherapy penetration into the CNS. Numerous trials of osmotic BBB disruption followed by chemotherapy have been performed, dating back to 1979, yet the efficacy of this technique is still difficult to gauge [18]. Studies utilizing osmotic BBB disruption often enroll patients with a range of diagnoses at varying stages of disease, thus making even historical comparisons difficult. Radiation and chemotherapy may also affect osmotic BBB disruption to a variable degree. Given the chemoresistant nature of many brain tumors, it can also be difficult to tease apart whether treatment failures are due to failure of the osmotic BBB disruption technique or poor activity of the chemotherapeutic agent itself. The efficacy of iv. etoposide, for example, was not enhanced by mannitol in children with recurrent brain tumors, but the cause of failure is unclear [19]. Generally speaking, however, results reported with osmotic BBB disruption have been reasonable, even favorable (Table 1). One prospective study of ia. carboplatin with osmotic BBB disruption (plus iv. etoposide and cyclophosphamide) for patients with multiple brain metastases reported a median survival of 42.3 months for ovarian carcinoma (five patients, four received BBB disruption), 13.5 months for lung adenocarcinoma (nine patients, four received BBB disruption) and 8.1 months for breast carcinoma (four patients, three receivied BBB disruption) [20].

However, despite numerous trials, the practice of osmotic BBB disruption has not achieved widespread use. In part, this is due to the highly technical nature of the process. Patients must undergo a cerebral angiogram. Then the optimal rate of infusion must be determined to minimize backflow. After administration of the hyperosmolar agent, additional contrast is injected to confirm catheter position and rule out arterial injury. Finally, the chemotherapeutic agent is injected ia. Typically, the entire procedure is performed under general anesthesia. Even when all of these steps are taken, results are variable. Using technetium-99m-diethylenetriaminepentaacetic acid scintigraphy to assess BBB disruption, Singh *et al.* found minimal disruption in 23% of cases and no disruption in 12% [21].

Adverse effects are also a significant concern. In addition to the neurointerventional risks associated with catheter advancement, osmotic BBB disruption can exacerbate cerebral edema, a nontrivial concern in the brain tumor patient population. Transient increases in intracranial pressure, peaking at 16–23 cm of water 30-min postdisruption (amounting to a 1.5% increase in brain fluid content) are observed [18]. Owing to this risk, only one arterial territory can be treated at each procedure, which is often not ideal for patients with multiple brain metastases. Seizures, strokes, cardiovascular complications and encephalopathy (not always reversible) have all been noted in conjunction with osmotic BBB disruption. Owing to the demanding nature of the procedure, selecting appropriate patients is critical, although this also limits the generalizability of the data.

Osmotic BBB disruption is also nonselective. The entire contents of the bloodstream, including compounds that are typically excluded (e.g., albumin, which is toxic to astrocytes) are

Table 1. Results from trials of osmotic blood–brain barrier disruption that included patients with brain metastases.

Enrollment period	Study accrual	Chemotherapy	Treatment schedule	Tumor type	Patients (n)	Median OS (months)	Median TTP (months)	6-month PFS (%)	Best radiographic response	Comments	Ref.
1991–1993	34	ia. carboplatin ≤200 mg/m^2, iv. etoposide ≤200 mg/m^2	Two sequential infusions monthly	Breast SCLC Adenocarcinoma	3 1 1	– – –	– – –	– – –	One SD and two PD PD No follow-up	–	[61]
March 1994–November 1997	221	ia. carboplatin 200 mg/m^2 each dose, iv. cyclophosphamide 330 mg/m^2, iv. etoposide 200 mg/m^2	Days 1 and 2 of 4-week cycle	Metastases	13	–	–	–	Three CRs, two PRs, seven SD and one not evaluable	2204 of the 2464 treatments given on this study used BBBD	[62]
1981–2004	25	ia. carboplatin or MTX, cyclophosphamide, etoposide	–	Breast	25	10.5	4.13	32	Four CRs/PRs, 15 SD and six PD	–	[63]
November 1999–May 2005	38	ia. carboplatin 400 mg/m^2, iv. etoposide 400 mg/m^2, iv. cyclophosphamide 330–660 mg/m^2 Lymphoma: ia. MTX 5000 mg/m^2, iv. etoposide 150 mg/m^2, iv. cyclophosphamide 500 mg/m^2	Every 4 weeks	Lung Ovarian Breast Systemic lymphoma	18 5 4 8	13.5 42.3 8.1 16.3	– – – –	– – – –	One CR, ten PRs, five SD and two PD Four CRs, one PD One PR, three SD Two CRs, five PRs, one SD	19 out of 38 received BBBD None of the nine patients with SCLC were given BBBD	[20]

These trials also enrolled patients with primary brain tumors. Osmotic BBBD was performed in conjunction with chemotherapy administration in many but not all cases.

BBBD: Blood–brain barrier disruption; CR: Complete response; ia.: Intra-arterial; iv.: Intravenous; MTX: Methotrexate; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival; PR: Partial response; SCLC: Small-cell lung carcinoma; SD: Stable disease; TTP: Time to progression.

allowed into the CNS. Furthermore, the effect of the procedure is more pronounced in normal brain tissue (where it is not desired) than in the tumor (where it is). One study showed that osmotic BBB disruption increases drug delivery to normal brain tissue 50–100-fold, but only two- to three-fold in dense tumor [22].

Pharmacologic disruption

In an effort to increase BBB permeability more selectively, several investigators have taken a pharmacologic approach. The vasoactive peptide bradykinin increases BBB permeability by binding to bradykinin-2 (B2) receptors found on endothelial cells, resulting in increased nitric oxide signaling, which activates the cGMP signal transduction cascade. Ultimately, this leads to downregulation of ZO-1, occludin and claudin-5, and rearrangement of the actin cytoskeleton in endothelial cells, resulting in tight junctions opening and increased transcellular transport, as well as increased pinocytotic vesicular transport [23]. This effect is preferentially selective for the BTB – ia. bradykinin increases permeability two- to 12-fold in brain tumor capillaries, but does not increase permeability in the normal BBB except at high doses. This selective effect may be attributed to the increased expression of nitric oxide synthase in tumors or possibly due to the decreased expression of Ang I converting enzyme – the enzyme that degrades bradykinin – at the BTB [24]. A small trial of ia. RMP-7 – a B2 receptor agonist with a longer plasma half-life than bradykinin – with ia. carboplatin in patients with malignant glioma demonstrated durable responses of 5 years or longer in three out of six evaluable patients [25]. However, a multi-institutional, randomized, double-blind, placebo-controlled Phase II study of iv. RMP-7 in combination with ia. carboplatin for patients with recurrent malignant glioma failed to demonstrate a statistically significant difference in time to progression or survival between the two groups [26]. A trend toward improvement was noted (median time to progression: 9.7 weeks with RMP-7 vs 8.0 weeks without), so it is possible that a modest benefit may have been detected if the trial was designed differently. The lack of success of RMP-7 has been attributed to several possible causes. From a biological standpoint, expression of the B2 receptor among tumors is variable, and downregulation of the receptor may occur in response to treatment with RMP-7 [27]. Preclinical studies also suggest that the iv. dose administered may have been five-times too low to have a therapeutic effect [26]. As hypotension is dose limiting at high iv. doses, perhaps ia. administration of RMP-7 (as performed in earlier smaller trials) would have been more effective. The efficacy of RMP-7 for brain metastases has not been evaluated.

Downstream from bradykinin and nitric oxide, cGMP activity is regulated by phosphodiesterases, the enzyme class responsible for its degradation. Along the BBB, the phosphodiesterase-5 isoform is the most prevalent. Preclinical studies suggest that BTB permeability is transiently increased following oral administration of the selective phosphodiesterase-5 inhibitors sildenafil and vardenafil, which are both US FDA approved for the treatment of erectile dysfunction [28]. A twofold increase in fluorescently labeled trastuzumab was observed in a murine intracranial $HER2^+$ lung metastasis model following the administration of oral vardenafil [29]. This effect was mediated by an increase in caveolae-mediated endocytosis and macropinocytosis across the BTB and corresponded with a significant prolongation in survival. A pilot study to assess the ability of vardenafil to increase carboplatin delivery across the BTB is currently underway. In this study, brain tumor patients (including those with metastases) receive carboplatin immediately prior to surgical resection in order for intratumoral carboplatin levels to be assessed. Half of the patients will receive oral vardenafil before carboplatin infusion. Vardenafil activity at the BTB is also being assessed noninvasively in a pilot study utilizing dynamic contrast-enhanced MRI. Using pharmacokinetic modeling, parameters that reflect BBB permeability – such as the rate distribution constant K_{trans} – can be calculated. Ultimately, this technique has the potential to serve as a biomarker of vardenafil activity at the BTB.

Inhibiting efflux transporters

Back in the 1970s, it was noted that prolonged exposure of cancer cells to certain chemotherapeutic agents resulted in lower drug accumulation. The culprit, PgP, was characterized in 1976 by Juliano and Ling, with efforts to inhibit it beginning shortly thereafter [30]. The first generation of PgP inhibitors (verapamil, quinidine and cyclosporine – evaluated throughout the 1990s) had modest activity. A randomized Phase III trial with cyclosporine demonstrated

the benefit for patients with poor-risk acute myeloid leukaemia, and a randomized trial with verapamil for women with anthracycline-resistant metastatic breast cancer demonstrated an improved survival and radiographic response rate [31,32]. However, patients with brain metastases were excluded from this trial. Several other trials demonstrated no benefit. Off-target effects, including excess calcium channel blockade and immunosuppression, limited dose escalation.

Second generation inhibitors (valspodar and biricodar) were more potent but also more toxic, probably in part due to overlapping inhibition of CYP450 3A. Furthermore, drug metabolism and elimination through the kidneys and liver are physiological processes that are also dependent on PgP function. Two trials involving valspodar were closed early due to excess toxicity, one for non-small-cell lung carcinoma and one for untreated acute myeloid leukaemia [33]. A randomized Phase III trial of carboplatin and paclitaxel, with or without valspodar, for patients with advanced ovarian cancer (excluding brain metastases) demonstrated equivalent outcomes with greater toxicity in the valspodar-treated cohort (particularly ataxia) [34]. Biricodar, a dual inhibitor of PgP and MRP1, appeared to have modest activity in a Phase II trial of advanced ovarian cancer, but the drug is no longer being developed [35].

Third-generation inhibitors (elacridar, tariquidar and zosuquidar) were designed to be more selective in their effect on PgP, thereby minimizing the pharmacokinetic interactions with other drugs. However, two randomized Phase III clinical trials for patients with non-small-cell lung cancer combining tariquidar with paclitaxel/carboplatin or vinorelbine were terminated as a result of excess toxicity [36]. Tariquidar also showed little activity in a small, single-arm Phase II study for patients with chemotherapy-resistant advanced breast cancer and a randomized, placebo-controlled Phase II study of docetaxel, with or without zosuquidar, for women with metastatic or locally recurrent breast cancer demonstrated an acceptable safety profile, but no improvement in outcomes [37,38]. Elacridar is a dual inhibitor of PgP and BCRP. Preclinical studies with elacridar demonstrate increased CNS penetration of several tyrosine kinase inhibitors, including imatinib, dasatinib, gefitinib, sorafenib and sunitinib [39]. Clinical development has been hampered, however, by poor solubility and bioavailability.

Overall, clinical experience with efflux transporter inhibitors has been disappointing. Even the highest potency inhibitors are unable to abolish drug efflux activity completely. A greater concern, however, is toxicity. In the trials described above, patients treated with efflux transporter inhibitors often required chemotherapy dose reductions. Effects on nonchemotherapy drugs can also be problematic – the antidiarrheal opioid loperamide, for example, is normally excluded from the CNS by PgP; inhibiting PgP can, therefore, result in CNS opioid toxicity. A cynic might say that the basic strategy of inhibiting efflux transporters is flawed – by interfering with drug pharmacokinetics (often resulting in higher plasma concentrations and delayed clearance), efflux transporter inhibitors make appropriately dosing drugs an unpredictable task. However, because efflux transporters such as PgP play such a major role in limiting penetration of chemotherapy into brain tumors, the approach remains tantalizing. It is also worth noting that several of the aforementioned trials for efflux transporter inhibitors excluded patients with brain metastases; thus, the efficacy of this strategy for this population has not yet been determined. It may also be more fruitful to combine efflux transporter inhibitors with newer targeted therapies.

'Trojan horse' approaches

The BBB is not a barrier to all – in order to maintain homeostasis, essential nutrients, hormones and cofactors must be shuttled across the BBB. These pathways across the BBB can potentially be co-opted for therapeutic gain.

■ Absorptive transcytosis

The negatively charged endothelial cell membrane is capable of transporting positively charged plasma proteins across the BBB via absorptive transcytosis. To take advantage of this phenomenon, a therapeutic compound, such as an antibody, can be 'cationized' by the conversion of superficial carboxyl groups into primary amino groups. Theoretically, this may adversely affect the binding affinity of the antibody, but evidence suggests that it does not [40]. This technique is still in the developmental stage.

■ Small-molecule transporters

Essential small molecules such as glucose, amino acids, nucleosides and small peptides are shuttled across the BBB by specific transporters. The tripeptide glutathione is transported across the BBB

by a specific transporter. Glutathione is the body's major endogenous antioxidant. At the BBB, glutathione is also involved in both the nitric oxide signaling pathway and iron metabolism. When glutathione was added to polyethylene glycol (PEG)ylated liposomal doxorubicin (which by itself does not cross the BBB), significant activity was seen in glioma and breast cancer animal xenograft models [41]. A Phase I/IIa study of this compound (named 2B3-101) for patients with brain metastases or recurrent malignant glioma is currently enrolling patients in Europe.

■ Receptor-mediated transcytosis

Larger endogenous compounds are transported across the BBB via receptor-mediated transcytosis. Transferrin, insulin, leptin and low-density lipoprotein all enter the brain in this manner. Utilizing these receptors is an appealing therapeutic strategy for several reasons. The process of receptor-mediated transcytosis avoids efflux transporters; thus, drugs that would typically be substrates for efflux can be protected if they are carried across the BBB in this manner. Additionally, some of these receptors are upregulated in malignancy, probably in order to keep pace with the rapid proliferation of cancer cells. Targeting these receptors, therefore, provides an extra measure of selectivity.

One relatively simple method of inducing receptor-mediated transcytosis is to coat the therapeutic compound with polysorbate 80, which is also known as Tween® 80 (ICI Americas, Inc., NC, USA). Polysorbate 80 adsorbs ApoB and ApoE in the circulation – both ligands for receptor-mediated transcytosis – across the BBB. Using a rat glioblastoma model, Steiniger *et al.* demonstrated improved survival with polysorbate-coated nanoparticles loaded with doxorubicin [42]. Clinical studies of this method are pending.

Transferrin receptor

The transferrin receptor normally mediates the transcytosis of iron across the BBB. Several strategies have utilized this receptor to breach the BBB. This makes sense as transferrin receptor expression is upregulated in several malignancies, including glioma, breast cancer and lung adenocarcinoma, possibly because the ribonucleotide reductase enzyme involved in DNA synthesis requires iron as a cofactor [43]. Initial studies utilized transferrin itself as the ligand conjugated to a therapeutic payload, such as diphtheria toxin. Although radiographic responses were seen in early-phase trials for patients with recurrent malignant glioma, a Phase III trial was stopped early due to the lack of benefit [44]. Mathematical modeling of transferrin kinetics suggests that drug delivery may have been limited by rapid recycling of transferrin back to the bloodstream [45]. More recently, Yoon *et al.* developed a mutant variant of transferrin conjugated to diphtheria toxin that is not so rapidly recycled. Using this therapy, prolonged tumor regression in a murine flank tumor model of glioblastoma was seen [46].

Antibodies against the transferrin receptor conjugated to a therapeutic compound have also been developed. Indeed, this is a potential mechanism for targeting therapeutic nanoparticles to brain tumors. The biopolymer poly(β-L-malic acid) can be used as a scaffold to attach drugs and other active moieties. One such compound in development – dubbed polycefin – contains transferrin receptor antibodies, therapeutic antisense oligonucleotides, leucine ethyl ester units to facilitate endosomal escape and PEG for polymer stabilization [47]. The overall nanobioconjugate is approximately 20–30 nm in size, with a molecular weight of up to 680 kDa, and is nonimmunogenic, biodegradable and stable in the bloodstream. Activity against glioma and breast cancer has been reported in animal models with this conjugate [47,48].

Exploiting transferrin receptor-mediated transcytosis is an example of 'active targeting'. This contrasts with the approach that has actually had the most success for nanoparticle delivery to date – 'passive targeting' via the 'enhanced permeability and retention' effect. This term was coined by Matsumura and Maeda in 1986 to describe the phenomenon that drugs of a certain size (including macromolecules and nanoparticles) tend to accumulate in tumor tissue [49]. This happens because the process of dysregulated neoangiogenesis that occurs within tumors results in tortuous blood vessels with wide fenestrations, allowing these particles to accumulate in the extravascular space. The 'active targeting' approach, while intuitively appealing, has largely been unsuccessful to date [50]. One concern is that the targeting moieties on a nanoparticle may be shielded from their receptors by other moieties, such as PEG.

There are additional issues to consider with endothelial receptor antibody-based conjugates. One concern is that these compounds may be targeted by the lysosome for degradation, which

is potentially problematic for protein payloads such as diphtheria toxin. Another concern is that conventional antibodies may have too high an affinity for their targets. As a result, the antibody–drug conjugate may be unable to dissociate from the receptor and enter the brain, instead, remaining trapped in brain endothelial cells. One solution is simply to make lower affinity antibodies to the transferrin receptor [51].

It may also be possible to take advantage of receptor-mediated transcytosis for the purpose of gene therapy. Using an orthotopic mouse model of human glioblastoma, Staquicini *et al.* developed a chimeric adeno-associated virus/phage particle that displays an iron-mimic peptide capable of binding the transferrin receptor and crossing the BBB [52]. Nonviral gene therapy using a PEGylated immunoliposome with RNAi to EGF receptor in combination with antibodies to insulin and transferrin has been evaluated in preclinical studies [53].

LRP-1

LRP-1 is a ubiquitously expressed receptor that binds over 40 ligands, including apoE4 and amyloid precursor protein. GRN1005 (formerly ANG1005) is a conjugate of three paclitaxel molecules with a novel 19-amino acid peptide (named Angiopep-2) that binds to LRP-1, resulting in receptor-mediated endocytosis across the BBB. Once across the BBB, the peptide binds to LRP-1 present on tumor cells. In a murine model of metastatic breast cancer to the brain, uptake of radiolabeled GRN1005 exceeded radiolabeled paclitaxel by 86-fold [54]. Two Phase I trials for GRN1005 have been performed – one for recurrent malignant glioma and one for advanced solid tumors in which most patients had brain metastases. Good safety and tolerability were reported, with neutropenia being the dose-limiting toxicity. Four out of 20 patients with advanced solid tumors who were treated at the maximal tolerated dose achieved a partial response. An extracranial disease response in patients who had previously failed conventional taxane therapy was also noted [55]. Two Phase II studies of GRN1005 are currently enrolling patients – one for brain metastases from non-small-cell lung carcinoma, and one for breast cancer patients with brain metastases (with the addition of trastuzumab for patients with $HER2^+$ disease). Development of Angiopep-conjugated doxorubicin and etoposide is also underway.

Antiangiogenic therapy: friend or foe?

Targeting angiogenesis has become a mainstay of treatment for recurrent malignant glioma, as well as an important therapeutic strategy for brain metastases. By pruning nascent dysfunctional and disordered blood vessels, antiangiogenic therapy helps to restore the integrity of the BBB. The imaging that correlates with this phenomenon is diminished contrast enhancement – the conventional radiographic metric of antitumor activity – as well as decreased cerebral edema. By pruning abnormal vessels and decreasing vascular permeability (VEGF was previously referred to as 'vascular permeability factor'), interstitial pressure returns to baseline, thus (somewhat counterintuitively) increasing blood flow throughout the tumor. This phenomenon has been termed 'vascular normalization' and it is hypothesized that 'normalizing' blood flow to the tumor in this manner helps concomitant therapy penetrate the CNS [9].

Of course, efforts to increase BBB and BTB permeability do the opposite, albeit for a transient window and in conjunction with chemotherapy. Which approach works best? It probably depends on the situation. In circumstances where drug delivery is limited by vascular permeability, it makes sense to increase permeability, particularly if the window of increased permeability is timed to coincide with high first-pass serum concentrations or local bolus delivery. Nanoparticle delivery also appears to be permeability limited. As discussed above, nanoparticle accumulation within tumors relies on the enhanced permeability and retention effect, which is based on the increased permeability of tumor vessels relative to normal vessels. If tumor vessels are normalized, nanoparticle targeting may not occur. On the other hand, when drug delivery is limited by blood flow, antiangiogenic therapy may be more appropriate.

It is worth noting that steroid treatment, like antiangiogenic therapy, affects BBB integrity as well, albeit via different mechanisms. Dexamethasone upregulates efflux transporter expression and decreases paracellular permeability. The extent of this effect has not been well characterized, but one study using a rat glioma model demonstrated significantly lower intratumoral concentrations of methotrexate in animals that received dexamethasone prior to osmotic BBB disruption [56].

Conclusion & future perspective

Little by little, the bar for the treatment of brain metastases is being raised. Success stories, such as vemurafenib for the treatment of brain metastases from *BRAF* mutation-positive melanoma, are becoming more common [57]. Moving forward, clinical trials for patients with brain metastases should be designed in ways that allow us to learn from the results, even if they are negative. Hopefully, the days when resources and lives will be invested in trials that are unable to discern whether a treatment is inactive or if it simply did not get where it needed to go are over. Drugs should be routinely tested for their ability to penetrate the CNS and, when possible, early-phase surgical trials should be performed to assess intratumoral drug concentration.

In addition to the challenges posed by the BBB for delivery of chemotherapy, it is critical to understand the mechanisms of cancer cell entry through the BBB into the brain. We know that certain malignancies have a predilection for metastasizing to the brain. To borrow Paget's terminology, how exactly does the malignant 'seed' interact with the BBB 'soil' [58]? As we begin to understand these factors, we may be able to develop therapies that target these interactions, much like how RANK ligand inhibitors are being used to prevent bone metastases. We know, for example, that brain metastases from breast cancer overexpress COX-2 and ST6GALNAC5 (which enhances adhesion to brain capillaries) [59]. Perhaps inhibitors of these enzymes can play a role in chemoprevention.

As our knowledge of the underlying biology of neoplasia grows, we will continue to advance into the age of targeted therapies and personalized medicine. To successfully treat brain metastases, these advancements will need to go hand-in-hand with strategies to overcome the BBB.

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