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REVIEW ARTICLE

How Much is Enough? Impact of Efflux Transporters on Drug delivery Leading to Efficacy in the Treatment of Brain Tumors

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Abstract

The lack of effective chemotherapeutic agents for the treatment of brain tumors is a serious unmet medical need. This can be attributed, in part, to inadequate delivery through the blood-brain barrier (BBB) and the tumor-cell barrier, both of which have active efflux transporters that can restrict the transport of many potentially effective agents for both primary and metastatic brain tumors. This review briefly summarizes the components and function of the normal BBB with respect to drug penetration into the brain and the alterations in the BBB due to brain tumor that could influence drug delivery. Depending on what is rate-limiting a compound's distribution, the limited permeability across the BBB and the subsequent delivery into the tumor cell can be greatly influenced by efflux transporters and these are discussed in some detail. Given these complexities, it is necessary to quantify the extent of brain distribution of the active (unbound) drug to compare across compounds and to inform potential for use against brain tumors. In this regard, the metric, Kp,uu, a brain-to-plasma unbound partition coefficient, is examined and its current use is discussed. However, the extent of active drug delivery is not the only determinant of effective therapy. In addition to Kp,uu, drug potency is an important parameter that should be considered alongside drug delivery in drug discovery and development processes. In other words, to answer the question - *How much is enough?* - one must consider how much can be delivered with how much needs to be delivered.

Keywords blood-brain barrier · brain tumors · efflux transporters · Kp,uu · molecular-targeted therapeutic agents

Introduction

Brain and other central nervous system (CNS) cancers have high mortality rates in adults, and are the leading cause of death among cancer patients younger than 20 years [1]. Glioblastoma and meningioma are the most common malignant and benign CNS tumors, accounting for over half of the cases (50.1 % and 55.4 %, respectively) [2]. Glioblastoma (GBM) is a highly aggressive and infiltrative primary malignant brain tumor with a 5-year survival rate of 6.9 % [2]. Secondary, or metastatic brain tumors, where tumor cells originate outside the CNS before traveling to the brain, such as metastatic lung cancer, breast cancer and melanoma, are a significant concern in the management of cancer patients and occur more frequently than primary brain tumors.

Currently, there are limited therapeutic options for tumors in the brain and the standard-of-care for patients with newly diagnosed GBM is maximal surgical resection followed by concurrent radiation with cytotoxic temozolomide (TMZ) for 6 weeks, and then adjuvant TMZ chemotherapy for 6 months [3]. In addition to standard-of-care treatment, other therapeutic agents, such as carmustine, a cytotoxic in the form of implanted Gliadel® wafers, bevacizumab, an antiangiogenic agent, and lomustine, a cytotoxic agent, are also approved by the FDA for recurrent GBM. Despite advancements in therapy for peripheral, non-CNS tumors, the treatment options for brain metastases have not kept pace with those advancements, in part related to the challenge of drug delivery across the blood-brain barrier (BBB). Nevertheless, treatments such as surgery, systemic therapy (chemotherapy,

Dedicated to Professor David E. Smith on his retirement after more than 40 years of groundbreaking scholarship at the University of Michigan.

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immunotherapy, targeted agent therapy), stereotactic radiosurgery, and whole brain irradiation exist and need further refinement for the special circumstances of delivery and efficacy in the brain [4, 5].

Molecular-targeted chemotherapy is a critical component of brain tumor treatment [6]. However, despite enormous progress in the discovery of molecular targets and potential drug candidates for those targets, the success rate of targeted therapeutic agents for brain tumors is quite low. One clear reason for this low translation rate is limited drug delivery across the BBB, that has been observed in the failures of the dasatinib and Depatux-M. Dasatinib is a multitargeted tyrosine kinase inhibitor that has been evaluated in several phase II clinical trials for patients with GBM as monotherapy or in combination with other treatments (NCT00423735, NCT00892177, and NCT00869401). Unfortunately, dasatinib failed to demonstrate efficacy in GBM patients, possibly due to insufficient drug penetration into brain [7]. Preclinical studies have discovered that active efflux transport at the BBB limits delivery of dasatinib into brain [8, 9] and dasatinib significantly prolonged survival in glioma-bearing mice when efflux transport was genetically deleted [10]. Depatux-M (ABT-414), the EGFR-targeted antibody-drug conjugate (ADC) depatuxizumab mafodotin, showed encouraging results in preclinical and phase I and II clinical studies in combination with TMZ to treat GBM [11–13]. However, the results from an extensive phase III trial (NCT02573324) showed that the addition of Depatux-M to the standard treatment did not improve overall survival in GBM patients [14]. One potential explanation for this negative result is limited penetration of the BBB by Depatux-M, and heterogeneous distribution of Depatux-M into tumor tissues [14, 15]. One important, yet simple, lesson to learn from the failure of these two molecular-targeted therapeutic agents is that in order to achieve an efficacious treatment outcome, enough drug exposure at the site of action to elicit an antitumor effect is required, in other words, potential effective drug candidates should have enough BBB penetration to reach tumors in the brain. However, before rushing to look for drug candidates with "higher" penetration across the BBB, we need to answer a question – how much is "enough"?

This review will examine the effect of the BBB on drug delivery in healthy and brain tumor conditions, especially from the perspective of efflux transporters. While it is recognized that there are additional barriers in the CNS, such as the blood-CSF barrier [16–18], and the arachnoid barrier [19, 20], the current review focuses on the influence of the BBB. Following the discussion about the BBB, a brief overview on the quantification of the delivery of molecular-targeted brain tumor therapeutic agents to brain will be offered. The review will conclude with a perspective on the current, possibly misguided, practices in drug development for CNS targets, especially brain tumors, and future directions for improving drug discovery and development for the treatment of brain tumors.

Blood-brain Barrier: An Obstacle for Drug Delivery to the Brain

The BBB plays an indispensable role in preserving brain homeostasis and protecting the CNS from endogenous harmful substances and xenobiotics. The BBB is comprised of specialized endothelial cells (ECs) and functional barriers and the ECs are surrounded and supported by pericytes, astrocyte endfeet, and the basal lamina (Fig. 1) [21]. These cells, together with other essential cell types such as microglia and neurons, form the neurovascular unit (NVU) [22].

Adjacent ECs in the BBB are connected by junctional complexes, limiting paracellular transport. Substance exchange is further regulated by transport proteins expressed at the luminal and abluminal sides of the ECs. Junctional complexes are physical barriers at the BBB that separate brain tissue space from the systemic blood stream, limiting paracellular diffusion of substances from blood to brain parenchyma. Tight junctions (TJs) and adherens junctions (AJs) are two primary classes of junctional complexes, where AJs assist the formation of TJs, and the interaction between their components is critical to the function of the dynamic junctions [23]. TJs are a size- and charge-selective semipermeable barrier, primarily consisting of three classes of transmembrane proteins: claudins, occludins, and junction adhesion molecules, and also membrane-associated cytoplasmic proteins such as zonula occludens and cingulin that connect transmembrane proteins to actin [23–25]. These transmembrane proteins in ECs bind with the same type of proteins expressed on adjacent ECs (homophilic oligomerization) to form the seal of TJs, the lack of which can result in the loss of integrity of the BBB [23–25]. Similarly, AJs are formed through the homophilic interaction between the transmembrane proteins cadherin in neighboring brain ECs, contributing to ECs survival, blood vessel assembly, and TJs formation [23].

The sophisticated junctional complexes between ECs restrict paracellular transport across the BBB to prevent a majority of harmful substances from entering the brain; however, some lipophilic molecules can diffuse across the BBB using the transcellular pathway. Therefore, transporters expressed on the ECs are essential to selectively control substance permeability. These transporters include influx, for hydrophilic nutrients, and efflux, for lipophilic compounds, active transport systems. Influx transporters, such as cationic amino acid transporter 1 (CAT1) and glucose transporter 1 (GLUT1), localized on the luminal and/or abluminal sides of the ECs, supply nutrients and other essential molecules

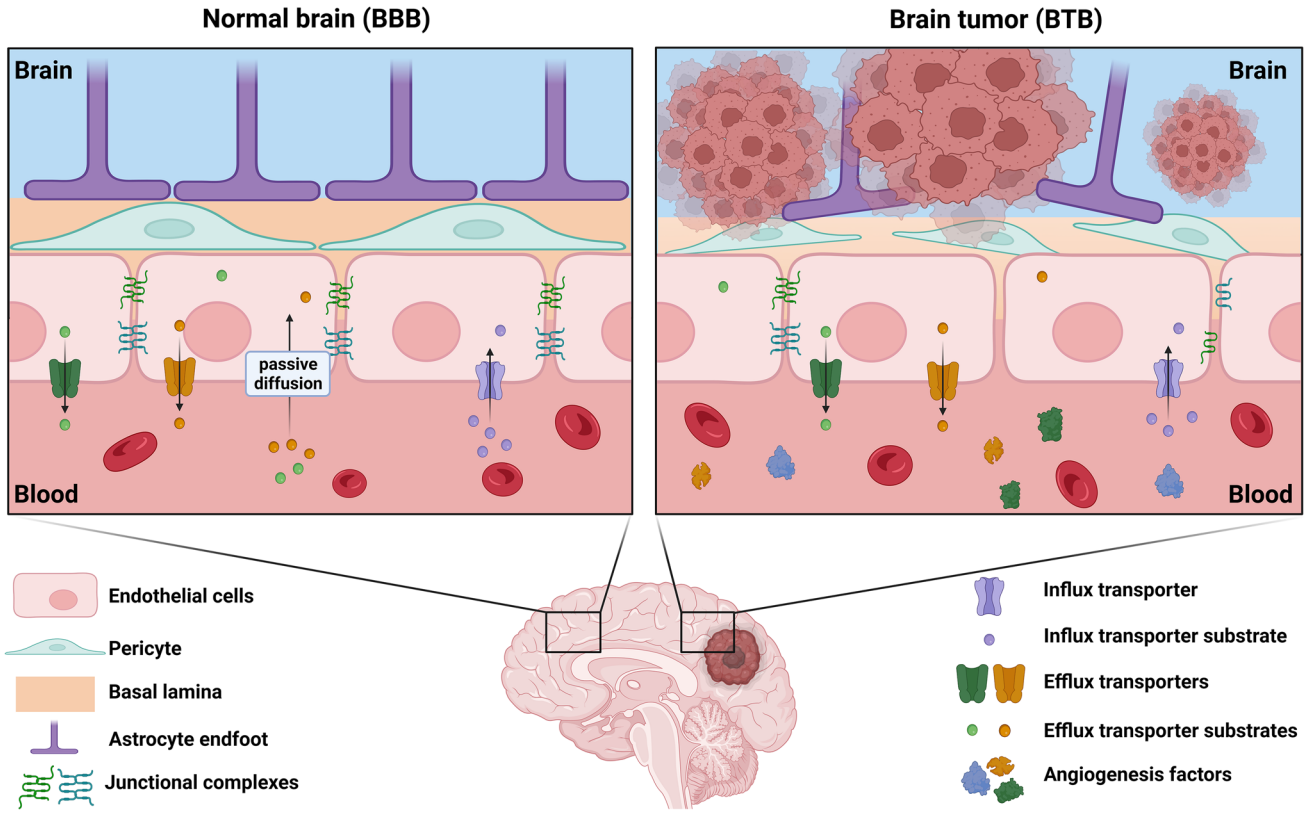

Fig. 1 Structure of the blood-brain barrier (BBB) in the normal brain and the blood-tumor barrier (BTB) in brain tumor regions. *Note*: Figure was created with BioRender.com.

to the brain [26]. In contrast, efflux transporters, as the biochemical barriers of the BBB, transfer substrates out of the brain, either from brain parenchyma to ECs and/or from ECs to blood (Fig. 1). More detailed discussions on efflux transporters will be provided later in this review.

Junctional complexes and efflux transporters at the BBB strictly control substance entrance to protect the brain, however, at the same time, it also presents a significant obstacle for therapeutic agents entering the brain to treat brain diseases, such as brain tumors. A majority of small molecule anticancer agents enter the brain parenchyma by passive diffusion across brain ECs membranes driven by a concentration gradient. However, this passive diffusion can be hindered by the biochemical barriers of the BBB, namely, active efflux transporters including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug-resistance proteins (MRPs). These efflux proteins are of great concern when using efflux substrates as a therapeutic for brain tumors [27]. In order to improve drug delivery to the brain, many strategies have been investigated in both preclinical and clinical studies, including CNS-targeted delivery strategies using such methods as focused ultrasound, osmotic opening, convection-enhanced delivery, trojan-horse carriers, and molecular modifications that enhance drug permeability [28, 29].

Changes in the Blood-brain Barrier Induced by Brain Tumors

The integrity and function of the BBB are altered under disease conditions, e.g., brain tumors. Brain tumor vasculature is different from that in healthy brain tissue. Increased nutrition and oxygen demand during tumorigenesis induces angiogenesis through stimulating the expression of pro-angiogenic growth factors, including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF), resulting in a dense microvasculature in the tumor [30, 31]. However, because of deregulated angiogenic factors and hypoxic environment in tumor, the tumor microvasculature is often disordered and functionally immature with blood vessels of variable lumen diameters, changes in perfusion, and higher permeability, known as pathological angiogenesis [30].

The integrity of the BBB in regions of primary and metastatic brain tumors may be heterogeneously altered due to spatial differences in expression of TJs proteins like claudin-5, claudin-3 and occludin, breakdown of the basal lamina, aberrant pericyte coverage, and loss of astrocytic endfeet. These changes, taken as a whole, lead to what is often termed the blood-tumor barrier (BTB) (Fig. 1) [32].

The permeability of the BTB is generally higher than that of the BBB, and the causative mechanisms have been explored; including increased desmin$^+$ subpopulation of pericytes, decreased CD13$^+$ subpopulation of pericytes, and decreased laminin α2 in astrocytic basal lamina [33], upregulated expression of aquaporin-4 (AQP4) that facilitate the flow of edema fluid in edematous brain tumors [34], and increased secretion of cytokines and chemokines like IL-6, CC-chemokine ligand 2 from sphingosine 1-phosphate receptor 3 (S1PR3) that is overexpressed in brain metastases [35].

The breakdown of the BBB is a well appreciated feature of many brain tumors. In clinic, conventional magnetic resonance imaging (MRI) remains the standard-of-care imaging method for brain tumor visualization [36]. Furthermore, contrast-enhanced MRI through intravenous administration of small hydrophilic gadolinium-based contrast agents could increase tissue contrast (increased T1 signal intensity) based on a leaky BBB in the brain tumor condition, where contrast agents leaking out of the blood vessels into the surrounding interstitial tissues, resulting in image enhancement of tumor regions (Fig. 2) [1, 37]. However, given the selectivity and sensitivity of this imaging method, the capacity of contrast-enhanced T1-weighted MRI to show the full extent of the tumor, differentiate tumor from nonspecific tissues, and determine tumor progression after treatment, is limited, so other alternative imaging methods, e.g., positron-emission-tomography (PET), are emerging to improve the identification of brain tumors [38, 39]. What's more, advanced imaging techniques like diffusion-weighted imaging (DWI), susceptibility-weighted imaging (SWI), perfusion-weighted imaging (PWI) and MR spectroscopy (MRS) enable evaluation of tumor microenvironment, depiction of internal vascular architecture, and assessment of tumor metabolic profiles [36].

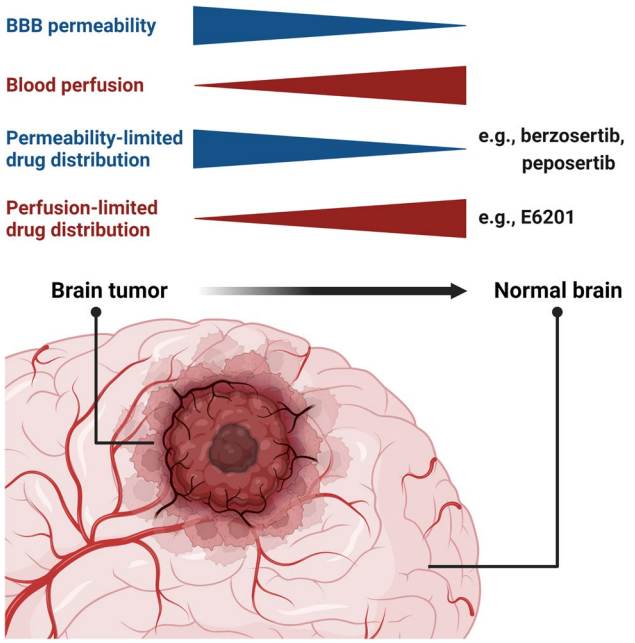

Fig. 3. Heterogeneous BBB permeability and corresponding drug distribution in brain with tumors. *Note*: Figure was created with BioRender.com

It is critical to recognize that regions delineated by contrast-enhancement do not fully capture the tumor mass [40]. While contrast-enhancement is indicative of a more leaky BBB, changes of BBB permeability in brain tumors are heterogeneous (Fig. 3). Lockman *et al*. determined the permeability of the BBB in brain metastases of breast cancer, and found that BBB permeability is compromised in most of tumor lesions varying in magnitude both within and between metastases [41]. The inter-tumor heterogeneity of BBB disruption across brain tumor subtypes results in a limited

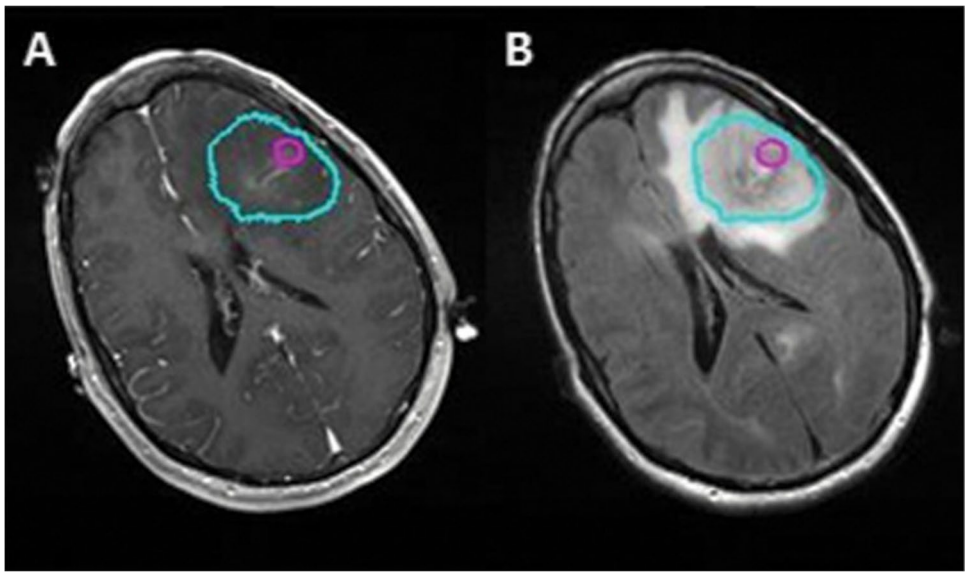

Fig. 2. Magnetic resonance imaging (MRI) imaging of a patient with GBM burden (from reference [37]). Sequential imaging with (**A**) contrast-enhanced T1-weighted MRI, (**B**) T2-weighted fluid attenuation inversion recovery (FLAIR).

response to clinical and preclinical studies. Erlotinib and gefitinib, EGFR inhibitors with significant *in vitro* potency, showed modest efficacy against non-small cell lung cancer brain metastases. However, both drugs failed to extend survival of GBM patients, which could be potentially due to different BBB disruption in GBM as compared to brain metastases, or different expression of efflux transporters in diverse tumor types and associated capillary ECs [42–44]. Depatux-M, an EGFR targeted ADC, was evaluated in two patient-derived xenograft (PDX) GBM models that had different extravasation of fibrinogen, a large blood-borne protein, and expression of claudin-5 in brain tumor capillaries, indicating different BBB permeability, and had significantly different efficacy [15] with the ADC most efficacious when the BBB was more "leaky". As a clear example of how the BBB disruption by a tumor is highly heterogeneous, even between subtypes of a tumor type, the Phoenix group showed that tumor-associated vasculature in different pediatric brain tumor subtypes of medulloblastoma was disrupted to a greater extent in WNT-medulloblastoma subtypes, while is relatively intact and functional in SHH-medulloblastoma [45]. Moreover, it has been reported that mouse models of diffuse midline glioma (DMG) exhibit a more normal BBB than pediatric high-grade gliomas (pHGG) [45, 46].

Despite the heterogeneous changes of the BBB permeability within and between tumor lesions by the mechanisms described above, overall, brain tumors retain a sufficiently intact BBB in different spatial regions to prevent drug delivery, leading to the failure of therapy [37, 41]. Tumor regions can be effectively treated only when adequate drug is delivered to the target tumor cells. Recently, an increasing body of evidence has been published demonstrating that the lack of efficacy in brain tumors could be attributed to heterogeneous drug distribution resulting from variable BBB permeability. Lapatinib showed only partial efficacy against breast cancer brain metastases in preclinical and clinical studies, that could be explained by the results of quantitative drug measurement in the tumor tissue, that is, lapatinib concentrations vary among brain metastases due to heterogeneous permeability, even though on average higher than surrounding normal brain tissues [47]. Talele *et al*. have observed heterogeneous spatial distribution of berzosertib, peposertib, and AZD1390 in orthotopic GBM models, where the drugs distributed more in intracranial tumor core than the rim, followed by that in surrounding normal brain tissues [48–50]. Similar observations of heterogeneous spatial distribution in orthotopic tumors are also reported for ispinesib, AB095-MMAF and Depatux-M [15, 51]. However, depending on the mechanisms leading to distribution of a drug, the rank order of delivery is also variable (Fig. 3). For example, in contrast with the above mentioned compounds, E6201, a novel mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitor, has excellent brain penetration, and showed higher drug distribution in the normal brain regions than in the tumor regions, in intracranial melanoma brain metastases mouse models [52, 53]. Recent advances in quantitative imaging techniques such as combination of matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) facilitate increased spatial resolution and have been used to assess spatial distribution of drugs targeted to other pathological conditions in the brain [54]. This powerful tool can provide critical insights about heterogeneity in BBB breakdown and in spatially variable drug distribution.

Efflux Transporters Limit Drug Delivery to Brain Tumors

Major Efflux Transporters in Brain – Abc and Slc Transporters

As discussed above, the efflux transport systems at the BBB protects the brain from the influence of harmful substances in the systemic circulation, but also prevents the anticancer therapeutic agents in the blood from entering the brain and reaching the site of action, i.e., the brain tumor lesion. Multiple transporters, not only expressed on the ECs at the BBB but also on the tumor cells, are involved in this selective delivery process, and this section will provide an overview on some dominant transporters that are involved in drug delivery to brain tumors.

ATP-binding cassette (ABC) transporters actively transport their substrates against a concentration gradient by energy provided by ATP. P-glycoprotein (P-gp, ABCB1) is the first ABC transporter that was initially identified in drug-resistant Chinese hamster ovary cell membranes. The "P" was designated for its influence on drug permeability [55]. The function of P-gp in brain tumor treatment has been demonstrated to result in multidrug resistance in tumor cells [56] and prevention of anticancer drugs penetration from blood to the brain across the brain capillary ECs [57, 58]. P-gp was detected in ECs of brain capillaries in 1989 [56] and its localization was determined on the luminal side of the ECs at the BBB by immunohistochemical staining [57–59]. Some studies also reported that P-gp is expressed in astrocytes and the abluminal side of ECs [60–62], however this has been subject to some controversy. Nevertheless, it is commonly believed that P-gp is predominantly expressed on the luminal endothelial membrane at the BBB. Besides the ECs, P-gp has also been reported to be expressed on the epithelial cells in choroid plexus [19, 63].

The multidrug resistance-associated protein (MRPs, ABCC) family has multiple members, of which MRP1 was the first ABC transporter involved in multidrug resistance identified in a multidrug-resistant human lung cancer cell

line [64, 65]. In spite of some overlap in the drug resistance profile with P-gp, MRP1 has preference to transport a broad range of lipophilic anionic anticancer drugs [65]. In addition to MRP1, several other MRP homologues have been identified in brain capillary ECs at the BBB, such as MRP3, MRP4, MRP5 and MRP6, where MRP1 and MRP5 are predominantly localized on the luminal ECs membrane, and MRP4 has similar distribution on both luminal and abluminal sides at the BBB [66, 67]. MRP7, MRP8, and MRP9 have been also discovered to express in the brain, but details have not been reported [65]. Recently, MRP2 has been found in the human ECs of the BBB [19].

After the discovery of P-gp and MRPs, breast cancer resistance protein (BCRP, ABCG2) was identified in a human breast cancer subline that exhibits multidrug resistance in the absence of P-gp and MRP1 overexpression [68]. BCRP is primarily expressed on the luminal side on the ECs at the BBB, exhibiting efflux function for drug transport from the ECs back into the blood stream [69–71]. Many molecular-targeted therapeutic small molecules are dual substrates of both P-gp and BCRP. These two crucial efflux transporters at the BBB have been shown to cooperate in limiting drug delivery into the brain: when either P-gp or BCRP is absent, the other transporter would functionally compensate for drug efflux. This compensation does not occur through changes in protein expression [72]; if both transporters are absent, brain penetration of dual substrates would be significantly improved [49, 53, 73–77].

In addition to the active efflux by ABC transporters, solute carrier (SLC) transporter-mediated translocation can also contribute to the efflux of therapeutic agents from brain to blood. The organic anion transporter 3 (OAT3, *SLC22A8*), expressed on the abluminal side of brain capillary ECs membranes, has been demonstrated to participate in the efflux of organic anions such as benzylpenicillin and para-aminohippuric acid (PAH) [78, 79]. The organic anion transporting polypeptide 2 (OATP2), located on both luminal and abluminal sides of ECs at the BBB, is a bidirectional transporter, that is involved in the transport of 17β-estradiol-d-17β-glucuronide (E217βG) and dehydroepiandrosterone sulfate (DHEAS) out of the brain in rat models [80–82].

Expression of Efflux Systems in Normal Brain and Brain Tumors

Identification of efflux transporters and corresponding substrate profiles will help to understand the mechanisms of drug delivery to the brain, and will be beneficial to drug design, development, and application. A quantification of transporter expression at the BBB can provide guidance for translational drug development and precision medicine (Table I). Agarwal *et al.* has quantified the expressions of P-gp and Bcrp in mouse brain capillary ECs and found that the expression level of P-gp is approximately 4.6-fold and 7.5-fold higher than that of Bcrp and Mrp4, respectively [72]. Kubo *et al.* reported Bcrp as a luminal-dominant transporter in porcine brain capillaries through proteomic analysis by using P-gp as a luminal marker [83]. In human brain microvessels, P-gp and BCRP are dominant ABC transporters, and BCRP protein expression is higher than that of P-gp (almost two fold), while MRP4 has much less expression [84, 85]. Interspecies difference of efflux transporters expression has been observed in different groups. The expression of BCRP is slightly higher in the human than in rodent BBB with less than a 2-fold difference, whereas a much higher expression of P-gp is observed in rats and mice compared to humans [85–87]. Bao *et al.* reported a 6.5-fold higher P-gp expression in rats (22.09 *vs.* 3.38, fmol/μg protein) and a 2.5-fold higher in mice (8.57 *vs.* 3.38) than that in humans [87]. Uchida *et al*. demonstrated that the absolute expression of P-gp in rats is 14.3 fmol/μg protein while in humans is 3.91 fmol/μg protein [86]; in mice is 14.1 fmol/ug protein compared to 6.06 fmol/ug protein in humans [85]. The expression of efflux transporters also differs between brain and spinal cord, the two primary organs in the CNS. Uchida *et al*. have observed lower expression levels of P-gp and BCRP in spinal cord capillaries than brain capillaries in humans [86]. However, the expression of P-gp and BCRP seen in human CNS tissue regions are similar, including frontal cortex, temporal cortex, white matter and thoracic spinal cord [86].

Efflux transporters expressed on brain capillary ECs are a significant barrier to restrict brain penetration of their substrates. The BBB can be altered under pathological conditions, presenting as the BTB in brain tumors, in part through the changes of the efflux transporters. The BTB shares efflux transporter types with the BBB but differs in expression profile. Compared with normal brain, the expression levels of efflux transporters in brain tumors are more complex, and different labs have different or even contradictive opinions (Table I). Bao *et al.* demonstrated significantly lower protein expression P-gp and BCRP in glioblastoma microvessels compared to microvessels isolated from human normal brain [87], while Schaffenrath *et al*. detected downregulated gene expression of P-gp, upregulated genes of MRP3 and MRP5, and no change in BCRP, in GBM vasculature [88]. The alteration of efflux transporters also depends on the type of the brain tumors, for which Schaffenrath *et al*. found upregulated MRP1 and MRP3, downregulated MRP4, and no change in P-gp and BCRP in adenocarcinoma brain metastasis, suggesting a different direction of gene regulation in the vasculature [88]. Chaves *et al.* observed no statistically different expression levels of P-gp, Bcrp and Mrp1 in normal rat brain compared with diffuse intrinsic pontine

Table I Protein Expression of Efflux Transporters in Healthy Rodent Brain and Human Brain, and in Human with GBM

Efflux transporter	Protein expression (fmol/μg protein)			
	Mouse brain (strain)	Rat brain	Human brain	Human GBM
P-gp (*ABCB1*)	16.3 ± 0.8 (FVB)[a]	21.95 (19.63 - 25.93) (Fischer 344; cortex)[d]	3.98 ± 0.88 (cortex)[b]	0.14 (BLQ - 2.87)[d]
	14.1 ± 2.1 (ddy; cerebellum)[c]	22.22 (17.16 - 29.73) (Fischer 344; non-cortex)[d]	6.06 ± 1.69 (cortex)[c]	↓[f]
	8.57 (BLQ - 12.82) (Balb/c)[d]	14.3 ± 0.4 (cerebrum)[e]	3.38 (1.00 - 7.42) (cortex)[d]	
			3.91 ± 1.38 (frontal cortex)[e]	
			2.72 ± 1.04 (temporal and parietal cortex)[e]	
			2.10 ± 1.54 (white matter)[e]	
BCRP (*ABCG2*)	3.53 ± 0.21 (FVB)[a]	4.59 (2.50 - 4.91) (Fischer 344; cortex)[d]	6.15 ± 1.41 (cortex)[b]	1.69 (BLQ - 12.11)[d]
	4.41 ± 0.69 (ddy; cerebellum)[c]	5.57 (BLQ - 7.43) (Fischer 344; non-cortex)[d]	8.14 ± 2.26 (cortex)[c]	no change[f]
	3.84 (0.29 - 9.16) (Balb/c)[d]	6.24 ± 0.06 (cerebrum)[e]	6.21 (2.23 - 16.53) (cortex)[d]	
			3.39 ± 0.74 (frontal cortex)[e]	
			2.31 ± 0.70 (temporal and parietal cortex)[e]	
			1.84 ± 0.96 (white matter)[e]	
MRP1 (*ABCC1*)	N.D.[a]	0.818 ± 0.125 (cerebrum)[e]	BLQ[c, e]	no change[f]
MRP2 (*ABCC2*)	N.D.[a]		BLQ[c]	
MRP3 (*ABCC3*)	N.D.[a]		BLQ[c]	↑[f]
MRP4 (*ABCC4*)	2.18 ± 0.13 (FVB)[a]	BLQ[d]	0.31 ± 0.11 (cortex)[b]	BLQ[d]
	1.59 ± 0.22 (ddy; cerebellum)[c]	1.63 ± 0.03 (cerebrum)[e]	0.195 ± 0.069 (cortex)[c]	no change[f]
	BLQ[d]		BLQ[d, e]	
MRP5 (*ABCC5*)	N.D.[a]		BLQ[c]	↑[f]
MRP6 (*ABCC6*)	N.D.[a]		BLQ[c]	
MRP7 (*ABCC9*)	N.D.[a]		BLQ[c]	no change[f]
MRP9 (*ABCC12*)			BLQ[c]	

N.D., not detected; BLQ, below the limit of quantification

[a]From isolated brain capillary endothelial - Agarwal *et al*. (mean ± SEM) [72]; b From freshly isolated brain microvessels - Shawahna *et al*. (mean ± SD) [84]; c From brain microvessels - Uchida *et al*. (mean ± SD) [85]; d From isolated microvessels - Bao *et al*. (median (mean ± SD) [87]; e From isolated capillaries from minced tissue - Uchida *et al*. (mean ± SD for human brain; mean ± SEM for rat brain) [86]; f From isolated endothelial cells - Schaffenrath *et al*. (compared with normal cortex) [88]

glioma-bearing brain [89]. However, Aronica *et al.* showed increased BCRP expression in brain tumor tissues, including GBMs [71]. Therefore, in the general milieu of brain tumors, the efflux transporters expression should be evaluated case by case. In addition to the transporters at the BTB, efflux transporters expressed on tumor cells act as the 2nd barrier that can restrict adequate anticancer therapeutic agents from reaching the site of action, i.e., brain tumor cells (Fig. 4). Besides the cell lines in which these efflux transporters were identified, P-gp, BCRP and MRPs have been reported to express on a variety of glioma cells and positively correlated with the pathological grade or drug resistance of glioma in several studies [90–92]. Also, the expression of efflux transporters is associated with the process of tumor proliferation [93].

Can Clinical Inhibition of Efflux Improve Drug Delivery to Brain Tumors?

It has been widely shown that efflux transporters in brain tumors can limit the drug delivery of anticancer therapeutic agents, and thus several strategies have been developed to improve the drug delivery of efflux substrates to the brain. Pharmacological inhibition of efflux transporters presents a potential approach to overcome efflux barriers. However, the clinical usage of efflux transporter inhibitors lacks success due to a number of reasons: 1) complexity of adjuvant therapy, 2) possible CNS toxicity of other agents [94], 3) systemic toxicity of chemotherapy, 4) need inhibitor to reach the 2nd barrier on tumor cells if it exists, 5) selectivity of the inhibitor (matching the affinity for inhibition with that for

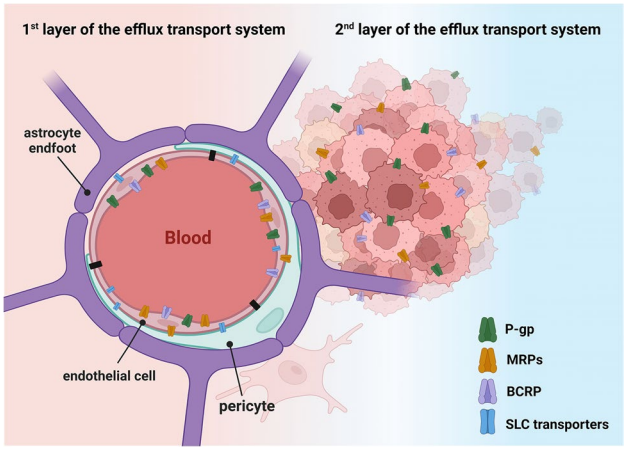

Fig. 4 Two layers of efflux transporter system in brain tumors. *Note*: Figure was created with BioRender.com.

efflux for multiple transporters). Nevertheless, some patents have been filed for the combination of efflux transport inhibitors with chemotherapeutic agents (U.S. Pat. No. 6703400, 20090170880, 20140235631). A more feasible strategy for development is using medicinal chemistry approaches to engineer molecules with decreased affinity for efflux. Other strategies, such as modification of the BBB, targeted drug delivery, and bypassing the BBB, have been developed to overcome efflux and improve drug delivery to the brain [28, 29].

Kp,uu – A Useful Parameter to Assess Drug Delivery to the Brain

Efficient discovery and development of drugs for brain tumors requires two important properties to be evaluated: 1) drug potency, and 2) drug exposure at the active site (tumor) in the brain. Among them, drug exposure, dependent on the concentration and time course of drug in the tumor, is often quantified by the brain-to-plasma partition coefficient (Kp), the ratio of the area under the (conentration) curve (AUC) of brain concentration normalized to the AUC of the plasma concentration, or the ratio of the brain concentration to the plasma concentration at steady-state (Fig. 5). This concept has been elaborated by contemplating free drug hypothesis that only unbound molecules will cross the BBB, and the tumor cell membrane, and exert a pharmacological effect at the target sites. Indeed, the driving force for the brain

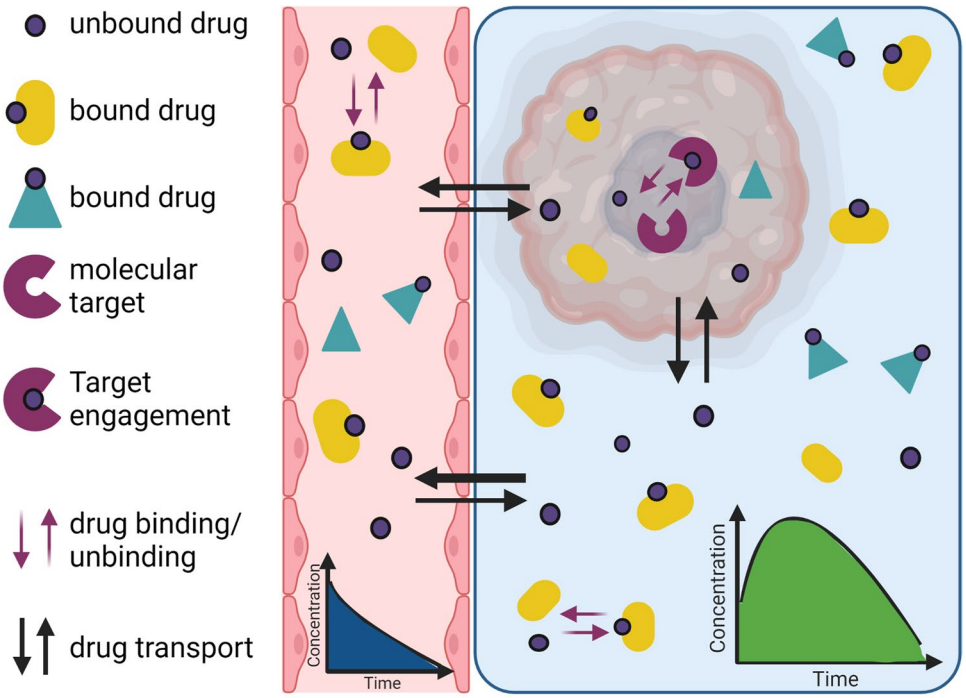

Fig. 5 A schematic illustration of multiple equilibrium processes that determine the relative drug exposure to the brain, i.e., the Kp and Kp,uu. *Note*: Figure was created with BioRender.com.

$$\mathrm{fu} = \frac{[unbound\ drug]}{[unbound\ drug]+[bound\ drug]} \qquad \mathrm{Kp} = \frac{AUC_{brain}}{AUC_{plasma}}$$

$$\mathrm{Kp,uu} = \frac{fu_{\mathrm{brain}} * AUC_{brain}}{fu_{\mathrm{plasma}} * AUC_{plasma}} = \mathrm{Kp} * \frac{fu_{\mathrm{brain}}}{fu_{\mathrm{plasma}}}$$

distribution of therapeutic agents after systemic administration is the unbound concentration in plasma (or blood), rather than the total concentration which is the sum of the bound and unbound drug concentrations (Fig. 5) [95–97]. Moreover, given that the target concentration is determined by *in vitro* efficacy studies, where only unbound drug interacts with therapeutic targets, the potential for *in vivo* efficacy should be based on the free drug concentrations at the target sites. In this sense, Kp,uu, instead of Kp, has been adopted to help describe the relative exposure of pharmacologically available unbound drug in the brain.

Conceptually, the Kp,uu can be described as the AUC ratio of free drug concentration in the brain to that in plasma or the ratio of free drug concentration of brain to that of plasma at steady-state. In practice, the Kp,uu values can be determined by the free drug concentrations in plasma and brain interstitial fluid at the steady state with microdialysis sampling technique or by incorporating drug unbound fractions in plasma and in brain to Kp (Fig. 5) [95]. Drug unbound fractions in plasma and brain are often different, therefore, the Kp,uu values could be lower or higher than Kp values, depending on the extent of binding to plasma and brain components. For example, the MEK inhibitor E6201 has a lower unbound fraction in the brain (0.14%) than in plasma (2.6%), leading to a 19-fold lower Kp,uu compared to Kp (Kp=2.66 *vs.* Kp,uu=0.14) [53]. Other drugs shown in Table II, such as berzosertib, ponatinib, and AZD3759, also suggest that drug delivery to the brain could be overestimated or underestimated if it is evaluated without regard to unbound drug concentrations. Inaccurate quantification of drug delivery can lead to a puzzling correlation between drug exposure and efficacy, potentially misleading during candidate drug screening. Currently, Kp,uu has been regarded as a "game changing parameter" in the pharmaceutical industry and is widely accepted in the decision making process in the selection of candidates for further development and prediction of an effective therapeutic dose [98].

In the following section of this review, Kp,uu will be employed as a drug delivery parameter in the design of brain tumor treatments, and a more specific discussion will be provided regarding the correlation between drug potency, exposure and efficacy of molecular-targeted anti-cancer agents for brain tumor treatments in the drug discovery and development process.

Molecular-Targeted Anti-cancer Agents for Brain Tumor Treatment

The development of molecular-targeted therapeutic agents for brain tumors is evolving as a result of the emerging discovery and understanding of critical molecular targets in the progression of tumors, i.e., proliferation, survival, and apoptosis. However, a majority of anticancer agents have failed in providing effective therapy for brain tumors in preclinical and/or clinical studies, in part due to the hindrance of the BBB to drug delivery. Table II shows the "potential" for brain delivery, i.e., Kp,uu, of some molecular-targeted agents that have been investigated for brain tumors, and the corresponding efflux transporter liability. A closer look at the efficacy, or lack thereof, of these molecular-targeted agents provides us with clues about the relationship between drug delivery and efficacy, and helps us to further answer the critical question – "how much is enough", as illustrated in Fig. 6.

DNA alkylating agents, a well-studied group of anticancer drugs that constitute many frontline chemotherapeutic agents, inflict cytotoxic DNA damage through transferring alkyl groups on nucleobases during DNA replication [112]. Temozolomide (TMZ) is a DNA methylation agent, approved by FDA for treating GBM patients in 2005, and is currently the sole chemotherapeutic agent in the standard-of-care for newly diagnosed GBM. Because of its favorable physicochemical properties – small size (MW = 194.15 g/mol) and hydrophilic/lipophilic balance (logP = -1.153), TMZ penetrates the BBB relatively well, at least well "enough" to improve the median survival of GBM patients by 2.5 months (14.6 *vs.* 12.1 months) compared to the placebo treatment [113]. The van Tellingen group investigated the BBB penetration of TMZ in wild-type and efflux transporter (P-gp and BCRP) deficient mouse models, and found that both genetic deletion and pharmacological inhibition of P-gp and BCRP could modestly increase the brain penetration of TMZ [99]. The Kp value of TMZ after single intravenous administration of 50 mg/kg in wild-type mice was 0.56 ($AUC_{0\text{-}7h}$ ratio), and the Kp increased to 0.89 when P-gp and BCRP were genetically knocked out (Table II) [99]. The drug potency of TMZ in glioma cells, measured by median IC50 ranging from 123.9 μM ~ 438.3 μM in various glioma cell lines, quantitatively is not as high as other agents listed in the Table III [114]. Therefore, in the case of TMZ, the noted clinical efficacy can be attributed, in part, to its penetration across the BBB, placing the compound in quadrant 2 (Q2) in Fig. 6.

A series of DNA repair pathways are activated when DNA is damaged after exposure to genotoxic stress such as ionizing radiation and/or chemotherapeutic agents (e.g., TMZ), and are collectively referred to as the DNA damage response (DDR). The DDR process constitutes various proteins to detect DNA damage, transduce the signal of DNA damage, and promote DNA repair. This repair protects tumor cells against death from DNA damage, hence, functional DDR in tumor cells provides a mechanism for radiation- and chemotherapy- resistance [118]. Inhibition of DDR regulator proteins such as ataxia telangiectasia mutated kinase (ATM), ataxia telangiectasia and Rad3 related protein kinase

Table II Brain Distribution and Efflux Transporter Liability of some Molecular-targeted Therapeutic Agents for the Treatment of Brain Tumors

Molecular target	Compound	Species	Kp	fu,plasma	fu,brain	Kp,uu	P-gp substrate	BCRP substrate	Ref.
DNA alkylation	TMZ	mouse	0.56	NR	NR	NR	Yes	Yes	[99]
ATR	Berzosertib	mouse	0.64	0.081	0.0014	0.011	Yes	Yes	[73]
ATM	AZD1390	mouse	0.29	0.203	0.075	0.1	Yes	No	[100]
ATM	AZD1390	mouse	NR	NR	NR	0.04	Yes or no#	Yes or no#	[50]
ATM	AZD1390	Cynomolgus monkey	5.8*	0.175	0.01	0.33	NR	NR	[50]
ATM	WSD-0628	mouse	NR	0.0285	0.0269	0.3	No	No	[101]
DNA-PK	Peposertib	mouse	0.09	0.114	0.041	0.03	Yes	Yes	[49]
PARP	Pamiparib	rat	0.18	NR	NR	0.158	No	No	[102]
p53/MDM2	SAR405838	mouse	0.0275	0.00059	0.00015	0.007	Yes	No	[103]
p53/MDM2	BI-907828	mouse	0.009	0.0015	0.0003	0.002	Yes	No	[104]
EGFR	AEE788	mouse	0.066	0.068	0.029	0.029	Yes or no#	Yes or no#	[105]
EGFR	Afatinib	mouse	0.254	0.08	0.014	0.046	Yes	Yes	[105]
EGFR	AZD3759	mouse	1.7	0.058	0.101	2.96	No	No	[105]
EGFR	Dacomitinib	mouse	0.612	0.008	0.007	0.493	Yes or no#	Yes or no#	[105]
EGFR	Erlotinib	mouse	0.062	0.045	0.096	0.134	Yes	Yes	[105]
EGFR	Gefitinib	mouse	0.358	0.041	0.012	0.103	Yes	Yes	[105]
EGFR	Osimertinib	mouse	0.988	0.005	0.001	0.289	Yes	Yes	[105]
EGFR	Vandetanib	mouse	0.635	0.055	0.012	0.138	Yes	Yes	[105]
PDGFR-α	Ponatinib	mouse	0.82	0.0023	0.0003	0.11	Yes	Yes	[106]
PI3K	GNE-317	mouse	1.1	NR	NR	0.398	NR	NR	[107]
PI3K	Pictilisib	mouse	0.0127	NR	NR	0.0035	Yes	Yes	[107]
PI3K	GDC-0084	mouse	1.37	0.29	0.067	0.41	No	No	[108]
panRAF	CCT196969	mouse	0.006	0.001	0.005	0.03	Yes or no#	Yes or no#	[109]
panRAF	LY3009120	mouse	0.05	0.0182	0.0093	0.02	Yes or no#	Yes or no#	[109]
panRAF	MLN2480	mouse	0.2	0.0421	0.0098	0.05	Yes or no#	Yes or no#	[109]
MEK	E6201	mouse	2.66	0.026	0.0014	0.14	No	No	[75]
MEK	Trametinib	mouse	0.15	0.0021	0.0021	0.15	Yes or no#	Yes or no#	[75]
MEK	Cobimetinib	mouse	0.32	0.014	0.0012	0.027	Yes or no#	Yes or no#	[75]
KIF11	Ispinesib	mouse	0.23	0.06	0.005	0.02	Yes	Yes	
Aurora A kinase	Alisertib	mouse	0.03	0.042	0.063	0.0044	Yes	No	[110]
CDK4/6	Abemaciclib	mouse	1.2	0.054	0.0079	0.17	Yes	Yes	[111]
CDK4/6	Palbociclib	mouse	0.1	0.23	0.02	0.01	Yes	Yes	[111]

*yielded by PET data; #substrate of P-gp and/or BCRP; NR, not reported

Kp: drug partition coefficient in the brain; fu,plasma: drug unbound fraction in plasma; fu,brain: drug unbound fraction in brain; Kp,uu: unbound drug partition coefficient in the brain

(ATR), poly (ADP-ribose) polymerases (PARP), and DNA-dependent protein kinase (DNA-PK), in combination with radiation and/or chemotherapeutic agents, has great potential to improve the tumor treatment efficacy [118, 119].

The brain delivery of inhibitors of the above DDR pathways constitute excellent examples of the various efflux mechanisms that can influence the brain penetration and therefore, the therapeutic efficacy. Berzosertib is a potent and selective inhibitor of the ATR kinase that is activated by single-stranded DNA breaks [73]. The effective concentration of berzosertib *in vitro* in GBM cells ranges from 100 to 300 nM when in combination with TMZ, demonstrating a potent synergy [73]. However, due to limited brain delivery restricted by P-gp and BCRP, and higher binding to brain tissue (Table II), berzosertib did not improve the efficacy of TMZ in orthotopic GBM models [73]. Pamiparib, a selective PARP inhibitor, represents a different situation when considering the delivery-potency-efficacy triad. Pamiparib is effective in tumors harboring homologous recombination deficiency and tumor suppressor protein BRCA mutations, with an EC50 ranging from 30 to 300 μM in combination with TMZ in various GBM cell lines, demonstrating a moderate TMZ-sensitivity [102]. Importantly, given this potency,

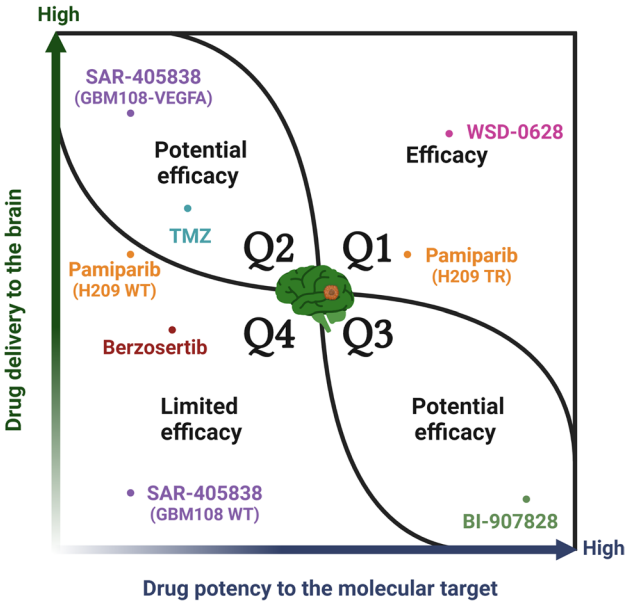

Fig. 6 Classification of molecular targeted therapeutic agents for brain tumors with respect to the contribution of drug delivery and potency to efficacy. *Note*: Figure was created with BioRender.com.

pamiparib exhibits a Kp,uu of 0.158 in rats, implying a possible "enough" with respect to BBB penetration (delivery), which is reflected by a significant extension in survival (efficacy) after combination treatment of pamiparib and TMZ in small cell lung cancer brain metastasis models (H209 TMZ-resistant) [102]. In Fig. 6, pamiparib locates in Q1 in H209 TMZ-resistant models, however, when it is combined with TMZ to treat H209 wild type brain metastases it has significantly decreased drug potency, causing a shift to Q4 in Fig. 6 in spite of equivalent delivery [102].

When DNA double-strand breaks occur, activated ATM plays a central role in DDR to arrest the cell cycle and activate downstream checkpoint kinases for recognizing DNA lesions and initiating DNA repair prior to DNA replication [119]. WSD-0628, an ATM kinase inhibitor, potentially sensitizes tumor cells to radiation, and has demonstrated remarkable cytotoxicity at 10-30 nM in GBM and melanoma brain metastasis PDX models [101]. WSD-0628 was shown to have little or no P-gp/BCRP substrate liability in *in vitro* studies, and has a relatively high Kp,uu of 0.3 in mice [101]. Therefore, the significant survival benefits from the combination of WSD-0628 with radiation in orthotopic GBM models can be attributed to both the remarkable radio-sensitizing potency and robust drug delivery to the brain (Kp,uu). These attributes make it locate in Q1 of Fig. 6, showing great potential for translation to clinical trials. Another ATM inhibitor, AZD1390, is also a potent radio-sensitizer, shows different BBB penetration ability in different species [50, 100]. Durant *et al.* reported that AZD1390 is brain-penetrant with a higher Kp,uu of 0.33 in cynomolgus monkey brain according to the positron emission tomography (PET) imaging results [100]. However, Talele *et al*. demonstrated that brain distribution of AZD1390 in the mouse is primarily restricted by P-gp efflux, resulting a Kp,uu of 0.10 in mouse brain [50], which is also observed in Durant's study that Kp,uu of AZD1390 in mouse is 0.04 and increased to 0.77 when co-administration with elacridar, a dual inhibitor of both P-gp and BCRP [100]. This BBB penetration difference between species may be caused by different expression of P-gp in mouse and cynomolgus monkey, different binding properties of AZD1390 among species, and also possibly from technical differences between different quantification methods (i.e., PET combined with modeling for cynomolgus monkey, and calculations of directly measured AUC ratios for rodents) of brain penetration.

Tumor suppressor protein p53, a multifunctional transcription factor that can be activated by DDR regulators, e.g., ATM, Chk2, and ATR, plays a central role in the downstream response to DNA damage through stimulating the transcription of essential genes that are involved in DNA repair, cell-cycle arrest, angiogenesis, senescence, and apoptosis [120]. Murine double minute 2 (MDM2) is an important negative regulator of p53 to stabilize the function of p53 [120]. The inhibition of MDM2 to restore the function of p53 offers an opportunity for the treatment of brain tumors. SAR405838 is a highly selective MDM2 inhibitor that has shown significant *in vitro* potency in GBM PDX models (Table III). Consistent with the *in vitro* efficacy, SAR405838 significantly suppressed the tumor growth of GBM108 PDX heterotopic (flank) models, however, the identical dosing regimen did not show efficacy in GBM108 PDX orthotopic models due to poor penetration of SAR405838 across the BBB (Table II) [103, 115]. SAR405838 is actively transported by P-gp out of the brain with a Kp,uu as low as 0.007. In contrast, BI-907828, another, even more highly potent MDM2 inhibitor, even though with a similarly poor brain penetration (Kp,uu=0.002), demonstrated a profound efficacy in GBM108 PDX orthotopic models [104]. This remarkably different efficacy of SAR405838 and BI-907828 can be explained by the comparatively high potency of BI-907828 – a free IC50 of 12 pM in the GBM108 PDX models (Table III). In another words, even though the drug delivery of BI-907828 into brain is extremely limited (0.2%), the small amount of BI-907828 that enters brain is enough to suppress the molecular target and subsequently show significant *in vivo* efficacy. According to the delivery and potency properties, BI-907828 is sorted into Q3 because of its higher potency to inhibit MDM2, while SAR405838 is sorted into Q4. Instead of simply tuning up drug potency through optimizing compound structure for target selectivity, enhancing drug delivery across the BBB might offer an

Table III Drug Potency of some Molecular-targeted Therapeutic Agents for the Treatment of Brain Tumors

Molecular target	Compound	Potency	Tumor type	Cell lines	Ref.
DNA alkylation	TMZ	IC50=220 μM	GBM	patient-derived cell line	[114]
DNA alkylation	TMZ	IC50=230.0 μM in U87 cells	GBM	U87	[114]
ATR	Berzosertib	effective range: 100–300 nM in combination with TMZ	GBM	U87, U251, GBM22	[73]
ATM	WSD-0628	effective range: 10-30 nM in combination with radiation	GBM, melanoma brain metastasis, SV-40 transformed astrocyte line	U251, M12, SVG-A	[101]
DNA-PK	Peposertib	effective range: ≥ 300 nM in combination with radiaton	melanoma brain metastases	M12	[49]
PARP	Pamiparib	EC50 range: 32-300 μM in combination with TMZ	GBM	SNB-19, SF-295, T98G, SF-539, U-118MG, U251, LN-229, U87-MG	[102]
p53/MDM2	SAR405838	effective concentration: ≥ 100 nM	GBM	GBM108	[115]
p53/MDM2	BI-907828	IC50=0.53, 5.33, 0.86 nM; free IC50=0.012, 0.119, 0.019 nM	GBM	GBM108, GBM10, GBM14	[104]
PDGFR-α	Ponatinib	IC50=1.08 μM; free IC50=0.032 μM	GBM	GBM6	[106]
panRAF	CCT196969	IC50=0.19, 0.53, 1.58 μM; free IC50=0.04, 0.11, 0.33 μM	melanoma brain metastases	M12, M27, M15	[109]
panRAF	LY3009120	IC50=2, 1, 3 nM; free IC50=0.6, 0.3, 0.8 nM	melanoma brain metastases	M12, M27, M16	[109]
panRAF	MLN2480	IC50=3.59, 3.83, 7.71 μM; free IC50=1.22, 1.3, 2.62 μM	melanoma brain metastases	M12, M27, M17	[109]
MEK	E6201	IC50=43.7 nM; free IC50=1.14 nM	melanoma	SK-MEL-28	[75]
KIF11	Ispinesib	EC50=0.5-14.4 nM	GBM	GBM1A	
Aurora A kinase	Alisertib	IC50=1-100 μM	diffuse midline glioma	MC-PED8, MC-PED17, SF9427	[116]
Aurora A kinase	Alisertib	IC50=30-95 nM	GBM	GBM6, GBM10, GBM12, GBM39	[117]

additional approach to improve efficacy. This could also be achieved by the development of targeted delivery system or device-assisted modification of the BBB. As a proof of concept for improving delivery in preclinical models, the *in vivo* orthotopic efficacy of SAR405838 was significantly enhanced in GBM108-VEGFA tumors, in which the intactness of the BBB was genetically disrupted [115], shifting SAR405838 from Q4 to Q2.

In addition to DNA alkylating agents and DDR inhibitors, there are additional molecular-targeted agents listed in Table II and III that have been shown to interfere with other important tumor progression processes, such as angiogenesis inhibitors [105, 106], PI3K–AKT–mTOR pathway inhibitors [107, 108], RAS–RAF–MEK–ERK signaling pathway inhibitors [75, 109], mitotic spindle-targeting agents [110, 116, 117], and cell cycle regulator inhibitors [111]. The drugs listed in these two tables show the relative magnitudes of both efflux (Table II) and potency (Table III), leading to an appreciation of how both characteristics are critical in drug development.

Conclusion and Perspective

Efflux transporters at the BBB and BTB are critical obstacles for drug delivery to their intracellular targets, and have undoubtedly contributed to many failures of translation to the clinic for brain tumor therapy. In this review, we explored this lack of efficacy from the perspective of limited drug delivery by the BBB, especially the impact of active efflux transporters at the BBB and BTB in the disease condition of brain tumors. The Kp,uu of anticancer agents for brain tumors that have shown efficacy in preclinical studies varies from 0.002 to 0.3 (Table II). This range, across two orders of magnitude, emphasizes the question that was posed at the beginning of this review: how much is enough? In another words, is there a numerical cutoff value for Kp,uu for potentially effective treatment of brain tumors? The short answer is NO.

An efficient drug development process for CNS diseases must determine the extent of drug exposure to the brain or other CNS tissues. Compounds with poor brain penetration are often precluded from further investigation and

development. However, this practice can be misleading. High throughput requirements may make the simplicity of a threshold for delivery attractive, however, in reality we need to consider potency at the same time. Both drug potency and delivery are essential to effective drug treatment. An ideal therapeutic agent for a brain tumor should have "good" potency to a specific molecular target and "enough" delivery to the site of action. However, a therapeutic agent has the potential to be effective *in vivo* if it is potent enough and/or penetrable enough to the brain, even though one or the other property is not so satisfying. Therefore, determining the relationship between drug potency, delivery, and efficacy in the treatment of brain tumors has important implications for drug discovery and development. Figure 6 provides a dynamic understanding that drug potency and delivery are both important and should be considered in unison during drug discovery and development.

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Declarations

Conflict of Interest None

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin US. 2023;73:17–48.
2. Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, *et al*. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015–2019. Neuro Oncol. 2022;24:v1-95.
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med US. 2005;352:987–96.
4. Vogelbaum MA, Brown PD, Messersmith H, Brastianos PK, Burri S, Cahill D, *et al*. Treatment for Brain Metastases: ASCO-SNO-ASTRO Guideline. J Clin Oncol. 2022;40:492–516.
5. Nabors LB, Portnow J, Ahluwalia M, Baehring J, Brem H, Brem S, *et al*. Central Nervous System Cancers, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw US. 2020;18:1537–70.
6. Agarwal S, Sane R, Oberoi R, Ohlfest JR, Elmquist WF. Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. Expert Rev Mol Med. 2011;13:e17.
7. Lassman AB, Pugh SL, Gilbert MR, Aldape KD, Geinoz S, Beumer JH, *et al*. Phase 2 trial of dasatinib in target-selected patients with recurrent glioblastoma (RTOG 0627). Neuro Oncol. 2015;17:992–8.
8. Lagas JS, van Waterschoot RAB, van Tilburg VACJ, Hillebrand MJ, Lankheet N, Rosing H, *et al*. Brain accumulation of dasatinib is restricted by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by elacridar treatment. Clin Cancer Res an Off J Am Assoc Cancer Res US. 2009;15:2344–51.
9. Chen Y, Agarwal S, Shaik NM, Chen C, Yang Z, Elmquist WF. P-glycoprotein and Breast Cancer Resistance Protein Influence Brain Distribution of Dasatinib. J Pharmacol Exp Ther. 2009;330:956–63.
10. Mittapalli RK, Chung AH, Parrish KE, Crabtree D, Halvorson KG, Hu G, *et al*. ABCG2 and ABCB1 Limit the Efficacy of Dasatinib in a PDGF-B-Driven Brainstem Glioma Model. Mol Cancer Ther. 2016;15:819–29.
11. Phillips AC, Boghaert ER, Vaidya KS, Mitten MJ, Norvell S, Falls HD, *et al*. ABT-414, an Antibody-Drug Conjugate Targeting a Tumor-Selective EGFR Epitope. Mol Cancer Ther. 2016;15:661–9.
12. Lassman AB, Van Den Bent MJ, Gan HK, Reardon DA, Kumthekar P, Butowski NA, *et al*. Efficacy analysis of ABT-414 with or without temozolomide (TMZ) in patients (pts) with EGFR-amplified, recurrent glioblastoma (rGBM) from a multicenter, international phase I clinical trial. J Clin Oncol. 2017;35:2003.
13. Van Den Bent M, Eoli M, Sepulveda JM, Smits M, Walenkamp A, Frenel JS, *et al*. INTELLANCE 2/EORTC 1410 randomized phase II study of Depatux-M alone and with temozolomide *vs* temozolomide or lomustine in recurrent EGFR amplified glioblastoma. Neuro Oncol. 2020;22:684–93.
14. Lassman AB, Pugh SL, Wang TJC, Aldape K, Gan HK, Preusser M, *et al*. Depatuxizumab mafodotin in EGFR-amplified newly diagnosed glioblastoma: A phase III randomized clinical trial. Neuro Oncol. 2023;25:339–50.
15. Marin B-M, Porath KA, Jain S, Kim M, Conage-Pough JE, Oh J-H, *et al*. Heterogeneous delivery across the blood-brain barrier limits the efficacy of an EGFR-targeting antibody drug conjugate in glioblastoma. Neuro Oncol England. 2021;23:2042–53.
16. Nau R, Sörgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev US. 2010;23:858–83.
17. Hosoya K, Tachikawa M. Roles of organic anion/cation transporters at the blood-brain and blood-cerebrospinal fluid barriers involving uremic toxins. Clin Exp Nephrol Japan. 2011;15:478–85.
18. Morris ME, Rodriguez-Cruz V, Felmlee MA. SLC and ABC Transporters: Expression, Localization, and Species Differences at the Blood-Brain and the Blood-Cerebrospinal Fluid Barriers. AAPS J US. 2017;19:1317–31.
19. Huttunen KM, Terasaki T, Urtti A, Montaser AB, Uchida Y. Pharmacoproteomics of Brain Barrier Transporters and Substrate Design for the Brain Targeted Drug Delivery. Pharm Res. 2022;39:1363–92.
20. Takeuchi H, Suzuki M, Goto R, Tezuka K, Fuchs H, Ishiguro N, *et al*. Regional Differences in the Absolute Abundance of Transporters, Receptors and Tight Junction Molecules at the Blood-Arachnoid Barrier and Blood-Spinal Cord Barrier among Cervical, Thoracic and Lumbar Spines in Dogs. Pharm Res US. 2022;39:1393–413.
21. Abbott NJ. Blood–brain barrier structure and function and the challenges for CNS drug delivery. J Inherit Metab Dis. 2013;36:437–49.
22. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. Neuron. 2017;96:17–42.
23. Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. J Cell Biol. 2015;209:493–506.
24. Luissint A-C, Artus C, Glacial F, Ganeshamoorthy K, Couraud P-O. Tight junctions at the blood brain barrier: physiological

architecture and disease-associated dysregulation. Fluids Barriers CNS. 2012;9:23.
25. Kadry H, Noorani B, Cucullo L. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids Barriers CNS. 2020;17:69.
26. Ohtsuki S, Terasaki T. Contribution of Carrier-Mediated Transport Systems to the Blood-Brain Barrier as a Supporting and Protecting Interface for the Brain; Importance for CNS Drug Discovery and Development. Pharm Res. 2007;24:1745–58.
27. Arvanitis CD, Ferraro GB, Jain RK. The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat Rev Cancer. 2020;20:26–41.
28. Griffith JI, Rathi S, Zhang W, Zhang W, Drewes LR, Sarkaria JN, *et al*. Addressing BBB Heterogeneity: A New Paradigm for Drug Delivery to Brain Tumors. Pharmaceutics. 2020;12:1205.
29. Rathi S, Griffith JI, Zhang W, Zhang W, Oh JH, Talele S, *et al*. The influence of the blood–brain barrier in the treatment of brain tumours. J Intern Med. 2022;292:3–30.
30. Ahir BK, Engelhard HH, Lakka SS. Tumor Development and Angiogenesis in Adult Brain Tumor: Glioblastoma. Mol Neurobiol. 2020;57:2461–78.
31. Zhang W, Talele S, Sarkaria JN, Elmquist WF. Changes in the vasculature of human brain tumors: Implications for treatment. Neuro Oncol. 2021;23:1995–7.
32. Arvanitis CD, Ferraro GB, Jain RK. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. Nat Rev Cancer. 2020;20:26–41.
33. Lyle LT, Lockman PR, Adkins CE, Mohammad AS, Sechrest E, Hua E, *et al*. Alterations in Pericyte Subpopulations are Associated with Elevated Blood-Tumor Barrier Permeability in Experimental Brain Metastasis of Breast Cancer. Clin Cancer Res. 2016;22:5287.
34. Saadoun S, Papadopoulos MC, Davies DC, Krishna S, Bell BA. Aquaporin-4 expression is increased in oedematous human brain tumours. J Neurol Neurosurg Psychiatry. 2002;72:262–5.
35. Gril B, Paranjape AN, Woditschka S, Hua E, Dolan EL, Hanson J, *et al*. Reactive astrocytic S1P3 signaling modulates the blood–tumor barrier in brain metastases. Nat Commun 2018;9:2705.
36. Villanueva-Meyer JE, Mabray MC, Cha S. Current Clinical Brain Tumor Imaging. Neurosurgery. 2017;81:397–415.
37. Sarkaria JN, Hu LS, Parney IF, Pafundi DH, Brinkmann DH, Laack NN, *et al*. Is the blood-brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. Neuro Oncol. 2018;20:184–91.
38. Langen K-J, Galldiks N, Hattingen E, Shah NJ. Advances in neuro-oncology imaging. Nat Rev Neurol. 2017;13:279–89.
39. Galldiks N, Law I, Pope WB, Arbizu J, Langen K-J. The use of amino acid PET and conventional MRI for monitoring of brain tumor therapy. NeuroImage Clin. 2017;13:386–94.
40. Griffith JI, Sarkaria JN, Elmquist WF. Efflux Limits Tumor Drug Delivery Despite Disrupted BBB. Trends Pharmacol Sci. 2021;42:426–8.
41. Lockman PR, Mittapalli RK, Taskar KS, Rudraraju V, Gril B, Bohn KA, *et al*. Heterogeneous blood-tumor barrier permeability determines drug efficacy in experimental brain metastases of breast cancer. Clin Cancer Res. 2010;16:5664–78.
42. Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, *et al*. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. J Clin Oncol. 2008;26:5603–9.
43. Van Den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MCM, Kros JM, Carpentier AF, *et al*. Randomized phase II trial of erlotinib *versus* temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. J Clin Oncol. 2009;27:1268–74.
44. Kim JE, Lee DH, Choi Y, Yoon DH, Kim SW, Suh C, *et al*. Epidermal growth factor receptor tyrosine kinase inhibitors as a first-line therapy for never-smokers with adenocarcinoma of the lung having asymptomatic synchronous brain metastasis. Lung Cancer. 2009;65:351–4.
45. Phoenix TN, Patmore DM, Boop S, Boulos N, Jacus MO, Patel YT, *et al*. Medulloblastoma Genotype Dictates Blood Brain Barrier Phenotype. Cancer Cell. 2016;29:508–22.
46. Wei X, Meel MH, Breur M, Bugiani M, Hulleman E, Phoenix TN. Defining tumor-associated vascular heterogeneity in pediatric high-grade and diffuse midline gliomas. Acta Neuropathol Commun. Acta Neuropathol Commun; 2021;9:142.
47. Taskar KS, Rudraraju V, Mittapalli RK, Samala R, Thorsheim HR, Lockman J, *et al*. Lapatinib distribution in HER2 overexpressing experimental brain metastases of breast cancer. Pharm Res. 2012;29:770–81.
48. Talele S, Zhang W, Burgenske DM, Kim M, Mohammad AS, Dragojevic S, *et al*. Brain Distribution of Berzosertib: An Ataxia Telangiectasia and Rad3-Related Protein Inhibitor for the Treatment of Glioblastoma. J Pharmacol Exp Ther. 2021;379:343–57.
49. Talele S, Zhang W, Oh JH, Burgenske DM, Mladek AC, Dragojevic S, *et al*. Central Nervous System Delivery of the Catalytic Subunit of DNA-Dependent Protein Kinase Inhibitor Peposertib as Radiosensitizer for Brain Metastases. J Pharmacol Exp Ther. 2022;381:217–28.
50. Talele S, Zhang W, Chen J, Gupta SK, Burgenske DM, Sarkaria JN, *et al*. Central Nervous System Distribution of the Ataxia-Telangiectasia Mutated Kinase Inhibitor AZD1390: Implications for the Treatment of Brain Tumors. J Pharmacol Exp Ther. 2022;383:91–102.
51. Gampa G, Kenchappa RS, Mohammad AS, Parrish KE, Kim M, Crish JF, *et al*. Enhancing Brain Retention of a KIF11 Inhibitor Significantly Improves its Efficacy in a Mouse Model of Glioblastoma. Sci Rep. 2020;10:6524.
52. Gampa G. Improving the Delivery of Novel Molecularly-targeted Therapies for the Treatment of Primary and Metastatic Brain Tumors. ProQuest Diss. Theses. University of Minnesota; 2019.
53. Gampa G, Kim M, Cook-Rostie N, Laramy JK, Sarkaria JN, Paradiso L, *et al*. Brain Distribution of a Novel MEK Inhibitor E6201: Implications in the Treatment of Melanoma Brain Metastases. Drug Metab Dispos. 2018;46:658–66.
54. Ntshangase S, Mdanda S, Naicker T, Kruger HG, Baijnath S, Govender T. Spatial distribution of elvitegravir and tenofovir in rat brain tissue: Application of matrix-assisted laser desorption/ionization mass spectrometry imaging and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2019;33:1643–51.
55. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta. 1976;455:152–62.
56. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, *et al*. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci USA. 1989;86:695–8.
57. Tsuji A, Terasaki T, Takabatake Y, Tenda Y, Tamai I, Yamashima T, *et al*. P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. Life Sci. 1992;51:1427–37.
58. Tatsuta T, Naito M, Oh-hara T, Sugawara I, Tsuruo T. Functional involvement of P-glycoprotein in blood-brain barrier. J Biol Chem. 1992;267:20383–91.
59. Sugawara I, Hamada H, Tsuruo T, Mori S. Specialized localization of P-glycoprotein recognized by MRK 16 monoclonal antibody in endothelial cells of the brain and the spinal cord. Jpn J Cancer Res. 1990;81:727–30.
60. Schlachetzki F, Pardridge WM. P-glycoprotein and caveolin-1α in endothelium and astrocytes of primate brain. Neuroreport. 2003;14:2041–6.

61. Bendayan R, Ronaldson PT, Gingras D, Bendayan M. In *situ* localization of P-glycoprotein (ABCB1) in human and rat brain. J Histochem Cytochem. 2006;54:1159–67.
62. Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia. 1995;36:1–6.
63. Rao VV, Dahlheimer JL, Bardgett ME, Snyder AZ, Finch RA, Sartorelli AC, *et al*. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. Proc Natl Acad Sci USA. 1999;96:3900–5.
64. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, *et al*. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science. 1992;258:1650–4.
65. Sodani K, Patel A, Kathawala RJ, Chen Z-S. Multidrug resistance associated proteins in multidrug resistance. Chin J Cancer. 2012;31:58–72.
66. Zhang Y, Han H, Elmquist WF, Miller DW. Expression of various multidrug resistance-associated protein (MRP) homologues in brain microvessel endothelial cells. Brain Res. 2000;876:148–53.
67. Zhang Y, Schuetz JD, Elmquist WF, Miller DW. Plasma Membrane Localization of Multidrug Resistance-Associated Protein Homologs in Brain Capillary Endothelial Cells. J Pharmacol Exp Ther. 2004;311:449 LP – 55.
68. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, *et al*. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci. 1998;95:15665–70.
69. Cooray HC, Blackmore CG, Maskell L, Barrand MA. Localisation of breast cancer resistance protein in microvessel endothelium of human brain. Neuroreport. 2002;13:2059–63.
70. Eisenblätter T, Hüwel S, Galla H-J. Characterisation of the brain multidrug resistance protein (BMDP/ABCG2/BCRP) expressed at the blood–brain barrier. Brain Res. 2003;971:221–31.
71. Aronica E, Gorter JA, Redeker S, Van Vliet EA, Ramkema M, Scheffer GL, *et al*. Localization of Breast Cancer Resistance Protein (BCRP) in Microvessel Endothelium of Human Control and Epileptic Brain. Epilepsia. 2005;46:849–57.
72. Agarwal S, Uchida Y, Mittapalli RK, Sane R, Terasaki T, Elmquist WF. Quantitative proteomics of transporter expression in brain capillary endothelial cells isolated from P-glycoprotein (P-gp), breast cancer resistance protein (Bcrp), and P-gp/Bcrp knockout mice. Drug Metab Dispos. 2012;40:1164–9.
73. Talele S, Zhang W, Burgenske DM, Kim M, Mohammad AS, Dragojevic S, *et al*. Brain Distribution of Berzosertib: An Ataxia Telangiectasia and Rad3-Related Protein Inhibitor for the Treatment of Glioblastoma. J Pharmacol Exp Ther. 2021;379:343 LP – 357.
74. Agarwal S, Sane R, Ohlfest JR, Elmquist WF. The role of the breast cancer resistance protein (ABCG2) in the distribution of sorafenib to the brain. J Pharmacol Exp Ther. 2011;336:223–33.
75. Gampa G, Kim M, Cook-Rostie N, Laramy JK, Sarkaria JN, Paradiso L, *et al*. Brain Distribution of a Novel MEK Inhibitor E6201: Implications in the Treatment of Melanoma Brain Metastases. Drug Metab Dispos United States. 2018;46:658–66.
76. Laramy JK, Kim M, Parrish KE, Sarkaria JN, Elmquist WF. Pharmacokinetic Assessment of Cooperative Efflux of the Multitargeted Kinase Inhibitor Ponatinib Across the Blood-Brain Barrier. J Pharmacol Exp Ther United States. 2018;365:249–61.
77. Parrish KE, Pokorny J, Mittapalli RK, Bakken K, Sarkaria JN, Elmquist WF. Efflux Transporters at the Blood-Brain Barrier Limit Delivery and Efficacy of Cyclin-Dependent Kinase 4/6 Inhibitor Palbociclib (PD-0332991) in an Orthotopic Brain Tumor Model. J Pharmacol Exp Ther. 2015;355:264.
78. Mori S, Takanaga H, Ohtsuki S, Deguchi T, Kang Y-S, Hosoya K-I, *et al*. Rat organic anion transporter 3 (rOAT3) is responsible for brain-to-blood efflux of homovanillic acid at the abluminal membrane of brain capillary endothelial cells. J Cereb Blood Flow Metab. 2003;23:432–40.
79. Kikuchi R, Kusuhara H, Sugiyama D, Sugiyama Y. Contribution of Organic Anion Transporter 3 (Slc22a8) to the Elimination of p-Aminohippuric Acid and Benzylpenicillin across the Blood-Brain Barrier. J Pharmacol Exp Ther. 2003;306:51–8.
80. Asaba H, Hosoya K, Takanaga H, Ohtsuki S, Tamura E, Takizawa T, *et al*. Blood—Brain Barrier Is Involved in the Efflux Transport of a Neuroactive Steroid, Dehydroepiandrosterone Sulfate, via Organic Anion Transporting Polypeptide 2. J Neurochem. 2000;75:1907–16.
81. Sugiyama D, Kusuhara H, Shitara Y, Abe T, Meier PJ, Sekine T, *et al*. Characterization of the Efflux Transport of 17β-Estradiol-d-17β-glucuronide from the Brain across the Blood-Brain Barrier. J Pharmacol Exp Ther. 2001;298:316–22.
82. Fricker G, Miller DS. Modulation of Drug Transporters at the Blood-Brain Barrier. Pharmacology. 2004;70:169–76.
83. Kubo Y, Ohtsuki S, Uchida Y, Terasaki T. Quantitative Determination of Luminal and Abluminal Membrane Distributions of Transporters in Porcine Brain Capillaries by Plasma Membrane Fractionation and Quantitative Targeted Proteomics. J Pharm Sci. 2015;104:3060–8.
84. Shawahna R, Uchida Y, Declèves X, Ohtsuki S, Yousif S, Dauchy S, *et al*. Transcriptomic and Quantitative Proteomic Analysis of Transporters and Drug Metabolizing Enzymes in Freshly Isolated Human Brain Microvessels. Mol Pharm. 2011;8:1332–41.
85. Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, *et al*. Quantitative targeted absolute proteomics of human blood–brain barrier transporters and receptors. J Neurochem. 2011;117:333–45.
86. Uchida Y, Yagi Y, Takao M, Tano M, Umetsu M, Hirano S, *et al*. Comparison of Absolute Protein Abundances of Transporters and Receptors among Blood-Brain Barriers at Different Cerebral Regions and the Blood-Spinal Cord Barrier in Humans and Rats. Mol Pharm American Chemical Society. 2020;17:2006–20.
87. Bao X, Wu J, Xie Y, Kim S, Michelhaugh S, Jiang J, *et al*. Protein Expression and Functional Relevance of Efflux and Uptake Drug Transporters at the Blood-Brain Barrier of Human Brain and Glioblastoma. Clin Pharmacol Ther Nature Publishing Group. 2020;107:1116–27.
88. Schaffenrath J, Wyss T, He L, Rushing EJ, Delorenzi M, Vasella F, *et al*. Blood-brain barrier alterations in human brain tumors revealed by genome-wide transcriptomic profiling. Neuro Oncol. 2021;23:2095–106.
89. Chaves C, Declèves X, Taghi M, Menet MC, Lacombe J, Varlet P, *et al*. Characterization of the blood–brain barrier integrity and the brain transport of SN-38 in an orthotopic xenograft rat model of diffuse intrinsic pontine glioma. Pharmaceutics. 2020;12:399.
90. Mohri M, Nitta H, Yamashita J. Expression of multidrug resistance-associated protein (MRP) in human gliomas. J. Neurooncol. 2000;49:105–15.
91. Jin Y, Bin ZQ, Qiang H, Liang C, Hua C, Jun D, *et al*. ABCG2 is related with the grade of glioma and resistance to mitoxantone, a chemotherapeutic drug for glioma. J Cancer Res Clin Oncol. 2009;135:1369–76.
92. Nakai E, Park K, Yawata T, Chihara T, Kumazawa A, Nakabayashi H, *et al*. Enhanced MDR1 Expression and Chemoresistance of Cancer Stem Cells Derived from Glioblastoma. Cancer Invest. 2009;27:901–8.
93. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side Population Is Enriched in Tumorigenic, Stem-Like Cancer Cells, whereas ABCG2+ and ABCG2− Cancer Cells Are Similarly Tumorigenic. Cancer Res. 2005;65:6207–19.
94. Kalvass JC, Polli JW, Bourdet DL, Feng B, Huang S-M, Liu X, *et al*. Why Clinical Modulation of Efflux Transport at the Human

Blood-Brain Barrier Is Unlikely: The ITC Evidence-Based Position. Clin Pharmacol Ther. 2013;94:80–94.
95. Hammarlund-Udenaes M, Fridén M, Syvänen S, Gupta A. On the rate and extent of drug delivery to the brain. Pharm Res. 2008;25:1737–50.
96. Gampa G, Talele S, Kim M, Mohammad A, Griffith J, Elmquist WF. Chapter 9 - Influence of transporters in treating cancers in the CNS. In: Sosnik A, Bendayan RBT-DEP in CRPFMR and C to PIS in C, editors. Cancer Sensitizing Agents for Chemotherapy. Academic; 2020. p. 277–301.
97. Loryan I, Hammarlund-Udenaes M, Syvänen S. Brain Distribution of Drugs: Pharmacokinetic Considerations. Handb Exp Pharmacol. 2022;273:121–50.
98. Loryan I, Reichel A, Feng B, Bundgaard C, Shaffer C, Kalvass C, *et al*. Unbound Brain-to-Plasma Partition Coefficient, Kp, uu, brain-a Game Changing Parameter for CNS Drug Discovery and Development. Pharm Res. 2022;39:1321–41.
99. de Gooijer MC, de Vries NA, Buckle T, Buil LCM, Beijnen JH, Boogerd W, *et al*. Improved Brain Penetration and Antitumor Efficacy of Temozolomide by Inhibition of ABCB1 and ABCG2. Neoplasia US. 2018;20:710–20.
100. Durant ST, Zheng L, Wang Y, Chen K, Zhang L, Zhang T, *et al*. The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. Sci Adv. 2018;4:eaat1719.
101. Tuma AM, Zhong W, Liu L, Burgenske DM, Carlson BL, Bakken KK, *et al*. Abstract 3305: WSD-0628, a novel brain penetrant ATM inhibitor, radiosensitizes GBM and melanoma patient derived xenografts. Cancer Res. 2022;82:3305.
102. Xiong Y, Guo Y, Liu Y, Wang H, Gong W, Liu Y, *et al*. Pamiparib is a potent and selective PARP inhibitor with unique potential for the treatment of brain tumor. Neoplasia. 2020;22:431–40.
103. Kim M, Laramy JK, Gampa G, Parrish KE, Brundage R, Sarkaria JN, *et al*. Brain Distributional Kinetics of a Novel MDM2 Inhibitor SAR405838: Implications for Use in Brain Tumor Therapy. Drug Metab Dispos Drug Metab Dispos. 2019;47:1403–14.
104. Zhang W, Vaubel, RA, Oh J-H, Mladek AC, Talele S, Zhang W, Waller KL, Burgenske DM, Sarkaria JN, Elmquist, WF. Delivery *versus* potency in treating brain tumors: BI-907828, a MDM2-p53 antagonist with limited BBB penetration but significant *in vivo* efficacy in glioblastoma. 2023;(In revision).
105. Kim M, Laramy JK, Mohammad AS, Talele S, Fisher J, Sarkaria JN, *et al*. Brain Distribution of a Panel of Epidermal Growth Factor Receptor Inhibitors Using Cassette Dosing in Wild-Type and Abcb1/Abcg2-Deficient Mice. Drug Metab Dispos. 2019;47:393–404.
106. Laramy JK, Kim M, Gupta SK, Parrish KE, Zhang S, Bakken KK, *et al*. Heterogeneous binding and CNS distribution of the multi-targeted kinase inhibitor ponatinib restrict orthotopic efficacy in a patient-derived xenograft model of glioblastoma. J Pharmacol Exp Ther. 2017;363:136–47.
107. Salphati L, Shahidi-Latham S, Quiason C, Barck K, Nishimura M, Alicke B, *et al*. Distribution of the Phosphatidylinositol 3-Kinase Inhibitors Pictilisib (GDC-0941) and GNE-317 in U87 and GS2 Intracranial Glioblastoma Models—Assessment by Matrix-Assisted Laser Desorption Ionization Imaging. Drug Metab Dispos. 2014;42:1110–6.
108. Salphati L, Alicke B, Heffron TP, Shahidi-Latham S, Nishimura M, Cao T, *et al*. Brain Distribution and Efficacy of the Brain Penetrant PI3K Inhibitor GDC-0084 in Orthotopic Mouse Models of Human Glioblastoma. Drug Metab Dispos US. 2016;44:1881–9.
109. Gampa G, Kim M, Mohammad AS, Parrish KE, Mladek AC, Sarkaria JN, *et al*. Brain Distribution and Active Efflux of Three panRAF Inhibitors: Considerations in the Treatment of Melanoma Brain Metastases. J Pharmacol Exp Ther. 2019;368:446–61.
110. Oh J-H, Power EA, Zhang W, Daniels DJ, Elmquist WF. Murine Central Nervous System and Bone Marrow Distribution of the Aurora A Kinase Inhibitor Alisertib: Pharmacokinetics and Exposure at the Sites of Efficacy and Toxicity. J Pharmacol Exp Ther. 2022;383:44–55.
111. Raub TJ, Wishart GN, Kulanthaivel P, Staton BA, Ajamie RT, Sawada GA, *et al*. Brain Exposure of Two Selective Dual CDK4 and CDK6 Inhibitors and the Antitumor Activity of CDK4 and CDK6 Inhibition in Combination with Temozolomide in an Intracranial Glioblastoma Xenograft. Drug Metab Dispos US. 2015;43:1360–71.
112. Chaney SG, Sancar A. DNA Repair: Enzymatic Mechanisms and Relevance to Drug Response. JNCI J Natl Cancer Inst. 1996;88:1346–60.
113. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, *et al*. Effects of radiotherapy with concomitant and adjuvant temozolomide *versus* radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10:459–66.
114. Poon MTC, Bruce M, Simpson JE, Hannan CJ, Brennan PM. Temozolomide sensitivity of malignant glioma cell lines - a systematic review assessing consistencies between *in vitro* studies. BMC Cancer England. 2021;21:1240.
115. Kim M, Ma DJ, Calligaris D, Zhang S, Feathers RW, Vaubel RA, *et al*. Efficacy of the MDM2 inhibitor SAR405838 in glioblastoma is limited by poor distribution across the blood–brain barrier. Mol Cancer Ther. 2018;17:1893–901.
116. Power EA, Rechberger JS, Zhang L, Oh J-H, Anderson JB, Nesvick CL, *et al*. Overcoming translational barriers in H3K27-altered diffuse midline glioma: Increasing the drug-tumor residence time. Neuro-Oncol Adv. 2023;5:vdad033.
117. Kurokawa C, Geekiyanage H, Allen C, Iankov I, Schroeder M, Carlson B, *et al*. Alisertib demonstrates significant antitumor activity in bevacizumab resistant, patient derived orthotopic models of glioblastoma. J Neurooncol US. 2017;131:41–8.
118. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461:1071–8.
119. Bindra RS, Chalmers AJ, Evans S, Dewhirst M. GBM radiosensitizers: dead in the water … or just the beginning? J Neurooncol Springer US. 2017;134:513–21.
120. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408:307–10.