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# Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain

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**Glioblastoma multiforme, because of its invasive nature, can be considered a disease of the entire brain. Despite recent advances in surgery, radiotherapy and chemotherapy, current treatment regimens have only a marginal impact on patient survival. A crucial challenge is to deliver drugs effectively to invasive glioma cells residing in a sanctuary within the central nervous system. The blood–brain barrier (BBB) restricts the delivery of many small and large molecules into the brain. Drug delivery to the brain is further restricted by active efflux transporters present at the BBB. Current clinical assessment of drug delivery and hence efficacy is based on the measured drug levels in the bulk tumour mass that is usually removed by surgery. Mounting evidence suggests that the inevitable relapse and lethality of glioblastoma multiforme is due to a failure to effectively treat invasive glioma cells. These invasive cells hide in areas of the brain that are shielded by an intact BBB, where they continue to grow and give rise to the recurrent tumour. Effective delivery of chemotherapeutics to the invasive glioma cells is therefore critical, and long-term efficacy will depend on the ability of a molecularly targeted agent to penetrate an intact and functional BBB throughout the entire brain. This review highlights the various aspects of the BBB, and also the brain–tumour-cell barrier (a barrier due to expression of efflux transporters in tumour cells), that together can significantly influence drug response. It then discusses the challenge of glioma as a disease of the whole brain, which lends emphasis to the need to deliver drugs effectively across the BBB to reach both the central tumour and the invasive glioma cells.**

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The past two decades have witnessed major advances in molecular and cellular biology that have substantially improved our understanding of human malignancies. Unfortunately, this period has also seen a significant rise in the incidence of malignant brain tumours along with only a modest increase in the survival rates associated with them, which are often poor (Ref. 1). Out of the approximately 22 020 new cases of primary malignant brain tumours that were estimated to be diagnosed in the USA in 2010, 80% were expected to be malignant gliomas (Refs 2, 3). Gliomas represent a group of highly malignant and lethal tumours of the brain that, despite all therapeutic advances, have an extremely poor prognosis. The median survival of patients with glioblastoma multiforme, the most common and most malignant subtype of glioma, is only 12–18 months (Ref. 4). The current standard of care in glioblastoma multiforme is treatment with the DNA-alkylating agent temozolomide combined with radiation, a treatment that has been proven to prolong patient survival by a few months (Ref. 4). Many new molecularly targeted agents that were developed to inhibit signalling pathways critical for glioma growth and proliferation have failed to elicit any clinical benefit (Ref. 5).

Compared with treatment of other types of tumours, targeting tumours of the central nervous system (CNS) is particularly challenging owing to the location of the tumour in a pharmacological and immunological sanctuary within the CNS. The blood–brain barrier (BBB) presents a major obstacle to systemic chemotherapy and is capable of significantly limiting drug response (Ref. 6). Drug efflux transporters at the BBB restrict the passage of drugs into the brain and thus shield the tumour cells from exposure to cytotoxic chemotherapy. In addition to the BBB, the presence of similar drug efflux pumps within tumour cells [the brain–tumour-cell barrier (BTB)] further protects them from chemotherapy. Systemically administered drugs thus have to cross these two sequential barriers to reach their intended molecular target.

This review focuses on the special challenge that these barriers pose to molecularly targeted and cytotoxic chemotherapeutic drugs. The aim is to provide an overview of the various molecular targets and target-directed chemotherapy for glioma. We review the most important ATP-driven transporters at the BBB and in tumour cells and their role in limiting the delivery and hence efficacy of systemic chemotherapy. Finally, we summarise how treatment of an infiltrative tumour like glioblastoma multiforme requires targeting the invasive tumour cells that often reside in areas away from the primary tumour – cells that are not removed by surgery and are shielded by multiple barriers, and therefore continue to grow and give rise to the recurrent tumour (Ref. 7).

## Malignant glioma

Malignant glioma represents one of the greatest challenges faced by the neuro-oncology community. Gliomas are tumours that are thought to arise from glial progenitor and glial cells, and include astrocytoma, glioblastoma, oligodendroglioma, ependymoma, mixed glioma and a few other, rare histologies (Ref. 2). These tumours account for 32% of all primary brain tumours and, as stated above, 80% of all malignant primary brain tumours diagnosed in the USA (Ref. 2). The World Health Organization classifies gliomas into four grades based on their histological features and malignancy. Grade I (pilocytic astrocytoma) and grade II (diffuse astrocytoma) tumours are slow growing and the least malignant forms of glioma, whereas grade III tumours (anaplastic astrocytoma) are more malignant and associated with poorer prognosis (Ref. 8). Grade IV is assigned to the most malignant and mitotically active tumours associated with extremely poor survival rates. Glioblastoma multiforme is a grade IV glioma and is characterised by uncontrolled cellular proliferation, diffuse infiltration, necrosis, angiogenesis and resistance to apoptosis. The name 'multiforme' signifies the vast intratumoural heterogeneity seen in the disease. Glioblastoma multiforme is the most common subtype of glioma, accounting for ~50% of gliomas, and glioblastoma multiforme and astrocytoma together account for ~75% of gliomas. Survival rates for patients with malignant gliomas are the worst among all brain tumours: less than 5% of glioblastoma multiforme patients survive for 5 years postdiagnosis (Refs 1, 2).

The majority of glioblastomas are primary tumours that develop de novo in the brain without any evidence of a precursor tumour; a relatively smaller fraction (~10%) are secondary



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tumours that start as low-grade astrocytomas but subsequently progress to high-grade gliomas (Refs 9, 10). Progress in our understanding of the molecular pathogenesis of malignant gliomas has made it possible to distinguish between these two types of glioblastoma multiforme based on the genetic aberrations and deregulated growth factor pathways presented by the tumour. Primary glioblastomas are characterised by amplification of the epidermal growth factor receptor (EGFR) and its mutant EGFR vIII, loss of heterozygosity of chromosome 10q, amplification/overexpression of the *MDM2* gene (mouse double minute 2), deletion of the *PTEN* gene (phosphatase and tensin homologue), and alterations in the RB1 (retinoblastoma 1) and p53 (TP53) signalling pathways (Refs 9, 10). Secondary glioblastoma multiformes are characterised mainly by overexpression of the platelet-derived growth factor receptor (PDGFR) and genetic mutations in the p53 and RB1 signalling pathways (Refs 9, 10). Despite the genetic differences, no differences in sensitivity to conventional chemotherapy between primary and secondary glioblastoma multiformes have been reported. The molecular and genetic aberrations in glioma have been extensively studied and show remarkable heterogeneity even within an individual tumour (Refs 11, 12). The enormous intratumoural variability combined with the complexity of the deregulated signalling pathways might be one of the reasons why most target-directed therapeutics are ineffective against the disease.

Despite aggressive treatment, essentially all malignant gliomas recur (Ref. 13), eventually leading to death. The median survival of a glioblastoma patient after recurrence is approximately 5–7 months (Ref. 5). Surgery remains one of the most effective treatments and almost all patients undergo surgery, unless the location of the tumour makes any degree of surgical debulking impossible (Ref. 14). Studies have shown a correlation between the extent of surgical debulking and increased patient survival (Refs 15, 16). Unfortunately, the grim reality is that regardless of the extent of resection, tumour recurrence and death are almost always inevitable. Radiotherapy is another treatment option for glioblastoma multiforme that has been proven to increase survival in patients after surgery (Ref. 4). Chemotherapy is rapidly assuming an increasingly important role in the treatment of malignant gliomas. Although many earlier studies failed to show any benefit with adjuvant chemotherapy, the finding that temozolomide in combination with radiotherapy increases patient survival dramatically changed chemotherapeutic treatment of glioma (Ref. 4). Temozolomide is now the standard of care in glioma, with almost every patient receiving the drug. However, reports of resistance to temozolomide have intensified the search for more effective target-directed therapies. A recent study showed that treatment with bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor A (VEGFA), in combination with radiotherapy was well tolerated and resulted in better overall survival (Ref. 17). It is thought that such antiangiogenic therapy can potentiate the effects of radiation mainly by normalising tumour blood vessels and enhancing oxygen delivery (Ref. 18). Consequently, several ongoing clinical trials are evaluating the effects of concurrent chemotherapy with radiotherapy in glioma.

A potentially significant advancement in the treatment of gliomas is the development of molecularly targeted small-molecule anticancer agents. There has been considerable progress in understanding the molecular pathogenesis of glioma and in identifying key oncogenic pathways that can be targeted using these small-molecule inhibitors. This has led to the development of several small-molecule agents that inhibit such deregulated signalling pathways in glioma. The recent success of such small-molecule inhibitors in other cancers has propelled the rapid development of similar therapies for the treatment of malignant gliomas.

## Molecularly targeted therapy

Molecular abnormalities in signal transduction pathways are characteristic features of many brain tumours, including glioma, and result in uncontrolled tumour cell proliferation, survival and apoptotic resistance. The growth factor pathways that are commonly altered in malignant glioma are epidermal growth factor (EGF) (Refs 19, 20, 21), platelet-derived growth factor (PDGF) (Refs 22, 23, 24) and vascular endothelial growth factor (VEGF) (Refs 24, 25, 26) pathways. Deregulation in receptors of these pathways (EGFR, PDGFR and VEGFR) results in



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constitutive activation of downstream effectors that regulate gene transcription, ultimately leading to the phenotype in malignant glioma (Fig. 1). Thus, an attractive approach to inhibit the aberrant signalling pathways in glioma is to use small-molecule tyrosine kinase inhibitors (TKIs) that inhibit the activity of upstream receptors of these pathways.

## Targeting EGFR and PDGFR

Aberrant signalling through the EGFR pathway is one of the most common genetic alterations seen in glioma (Refs 19, 27), and therefore several therapeutic strategies have used small-molecule TKIs to target EGFR in glioma. Gefitinib (Iressa, Astra Zeneca) and erlotinib (Tarceva, OSI Pharmaceuticals) were some of the first TKIs to show potent inhibitory effects on EGFR, prolonging survival in preclinical models of brain tumours (Refs 28, 29, 30, 31). However, neither of these two promising drugs showed any significant survival benefit in glioblastoma multiforme patients (Refs 32, 33, 34, 35, 36, 37, 38).

PDGFR is another attractive therapeutic target in glioma because it is commonly overexpressed in glioma and is thought to contribute to the aggressive phenotype of the tumour (Refs 22, 23). Imatinib (Gleevec, Novartis), a potent inhibitor of the tyrosine kinases BCR–ABL, c-Kit (KIT) and PDGFR, was the first selective TKI to

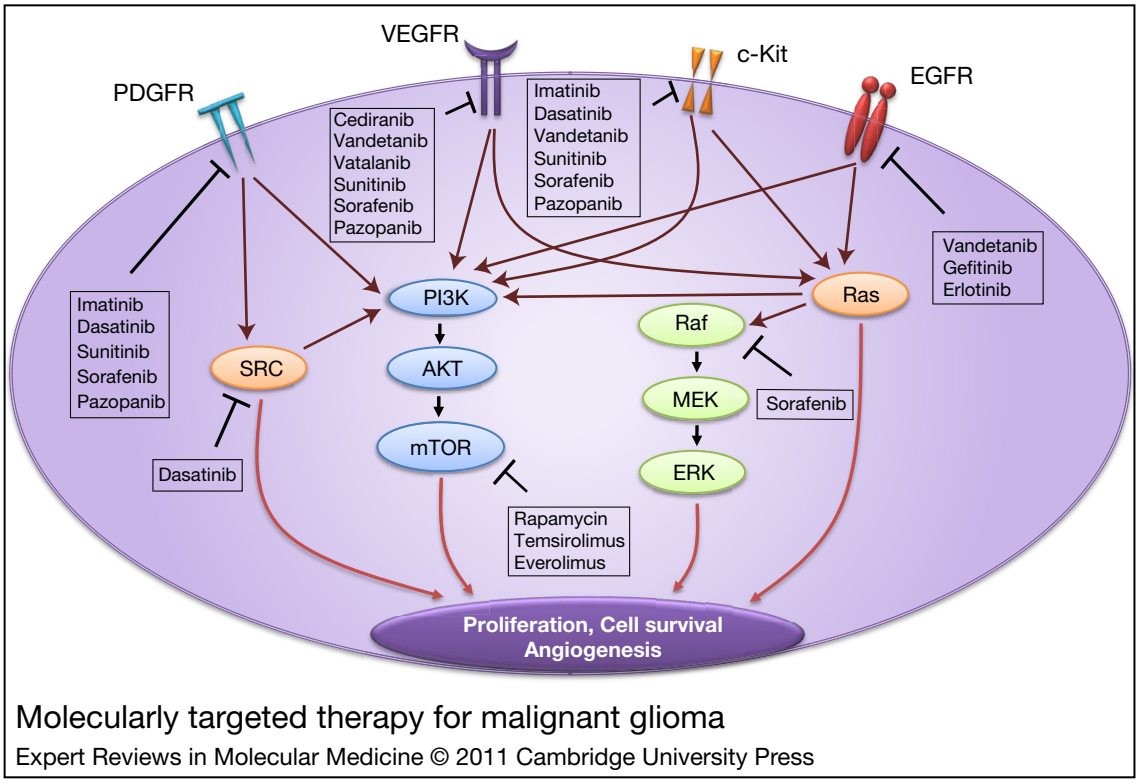


**Figure 1. Molecularly targeted therapy for malignant glioma.** Several signalling pathways are aberrantly activated in glioma, the most common being signalling through the receptors EGFR, PDGFR, VEGFR and c-Kit (Ref. 24). These pathways can be deregulated as a result of one or more mechanisms such as auto-activation, aberrant expression, mutations, and decreased activity of phosphatases that turn off the signal. Signalling through these pathways can be shut down by targeted therapies that inhibit these receptors, thereby preventing the downstream effects that ultimately lead to growth and proliferation of the tumour. Such molecularly targeted therapeutic agents are listed in the figure near the targets that they inhibit. Abbreviations: AKT, AKT8 virus oncogene cellular homologue; c-Kit, v-Kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homologue; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase [mitogen-activated protein kinase (MAPK)]; MEK, MAPK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PDGFR, platelet-derived growth factor receptor; SRC, rous sarcoma oncogene cellular homologue; VEGFR, vascular endothelial growth factor receptor.



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be approved for the treatment of cancer (Refs 39, 40). Imatinib showed encouraging antiglioma activity in preclinical studies, raising hopes in the clinical trials that followed (Refs 41, 42, 43). However, the preclinical success did not translate into significant clinical benefit, with Phase II trials reporting insignificant antitumour effects in glioma patients (Refs 44, 45). Dasatinib (Sprycel, Bristol-Myers Squibb) is another PDGFR inhibitor with an additional inhibitory effect on the Src family of kinases. It has also been shown that dasatinib can inhibit the growth and migration of glioma cells and induce cellular apoptosis, again warranting clinical investigation in glioma (Ref. 46); however, there is no published literature on the clinical efficacy of dasatinib in glioma (Table 1).

## Targeting VEGFR

Angiogenesis, the process of vascular proliferation due to the formation of new blood vessels, is a histopathological hallmark of malignant glioma. The angiogenic effect is mediated primarily through the VEGFR pathway, which is frequently upregulated in glioblastoma multiforme, making it a prime target for growth inhibition and therapeutic efficacy (Refs 25, 26). Numerous small-molecule VEGFR inhibitors, such as cediranib, sunitinib, sorafenib, vatalanib and vandetanib, have shown promising results in preclinical glioma models.

Cediranib (Recentin, AstraZeneca), a pan inhibitor of the VEGFR tyrosine kinase, is one of the most exciting prospects for antiangiogenic therapy in glioma. It has demonstrated significant effects in mouse glioma models, decreasing oedema by vascular normalisation in the tumour and leading to improvement in survival (Ref. 56). These preclinical effects have been mirrored in the clinic, where cediranib treatment results in normalisation of tumour vessels, decreased vessel permeability and alleviation of vasogenic oedema (Ref. 47). Encouraging new data from a recently concluded Phase II trial suggest that cediranib therapy results in significant radiographic response and increases progression-free survival (Ref. 48).

Sunitinib (Sutent, Pfizer) and sorafenib (Nexavar, Bayer) are two multitargeted TKIs that show both antiproliferative and antiangiogenic activity by simultaneously targeting VEGFR and PDGFR (Refs 57, 58). Separate studies have shown that both these compounds can increase survival in mouse glioma models at doses achievable in the clinic (Refs 59, 60). Several clinical trials are currently evaluating the efficacy of these two agents in human malignant glioma. Vandetanib (Zactima, AstraZeneca) is a novel small-molecule inhibitor that simultaneously targets VEGFR and EGFR (Ref. 61). It has demonstrated potent antiglioma effects in clinically relevant glioblastoma multiforme models, suppressing tumour cell proliferation and angiogenesis while inducing apoptosis by inhibition of EGFR (Ref. 62). There are many ongoing clinical studies that are evaluating the efficacy and toxicity of vandetanib in glioma patients.

## Targeting PI3K–AKT–mTOR

Other important molecularly targeted agents include inhibitors of the PI3K–AKT–mTOR pathway [comprising phosphoinositide 3-kinase, the serine/threonine protein kinase AKT and the mammalian target of rapamycin (mTOR/MTOR)] (Fig. 1), which is thought to be highly activated in human glioblastomas, modulating key translational processes (Ref. 63). Rapamycin (sirolimus) and its analogues temsirolimus (CCI779) and everolimus (RAD001) are the three mTOR inhibitors that have undergone extensive preclinical and clinical evaluation for therapy in glioma. Clinical trials with mTOR inhibitors as a single agent in glioma have been largely unsuccessful, with no therapeutic benefits reported (Refs 35, 49, 54). However, several trials are currently evaluating mTOR inhibitors in combination with other TKIs with an aim to shut down multiple signalling cascades feeding the tumour.

## Improving the efficacy of molecularly targeted agents

Most promising molecularly targeted agents have failed to provide any survival benefit in malignant gliomas (Table 1). Given the dismal prognosis of patients with glioma, the quest to find newer effective therapeutic options has gained precedence over the need to find the reasons behind the failure of these agents, although the two goals are closely linked. Some of the reasons suggested for this lack of efficacy have been related to the genetic heterogeneity of gliomas and the complexity of signalling pathways, such as negative feedback



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**Table 1. Molecularly targeted agents for tumours of the central nervous system**

| Compound | Molecular target | Results from clinical trials for glioblastoma multiforme | Number of clincal trials[a] |
|---|---|---|---|
| Cediranib | VEGFRs 1, 2 and 3 | Median OS in 16 patients of 211 days (Ref. 47)<br>6-month PFS in 25.8% and PR in 56.7% of patients (Ref. 48) | 6 |
| Dasatinib | BCR–ABL, c-Kit, PDGFR, SRC | No published results | 4 |
| Erlotinib | EGFR | 6-month PFS in 3.1% of patients (Ref. 35)<br>Median OS 19.3 months (Ref. 36)<br>6-month PFS in 3% and 12-month OS in 57% of patients (Ref. 37)<br>Median PFS 2.8 months, median OS 8.6 months (Ref. 38) | 20 |
| Everolimus | mTOR | Stable disease in 36% and PR in 14% of patients (Ref. 49) | 11 |
| Gefitinib | EGFR | Median EFS 8.1 weeks, median OS 39.4 weeks (Ref. 34)<br>Median time to progression 8.4 weeks, 6-month PFS in 14.3% of patients, median OS 24.6 weeks (Ref. 33) | 4 |
| Imatinib | BCR–ABL, c-Kit, PDGFR | 6-month PFS in 3% of patients (Ref. 44)<br>6-month PFS in 16% of patients (Ref. 45)<br>6-month PFS in 27% of patients, median PFS 14.4 weeks (Ref. 50)<br>6-month PFS in 24% of patients (Ref. 51) | 7 |
| Lapatinib | EGFR2 | No published results | 3 |
| Pazopanib | c-Kit, PDGFR, VEGFRs 1, 2 and 3 | No published results | 1 |
| Sirolimus | mTOR | 6-month PFS in 3.1% of patients (Ref. 35) | 4 |
| Sorafenib | c-Kit, PDGFR, Raf | Median PFS 6 months, median OS 16 months (Ref. 52) | 7 |
| Sunitinib | VEGFRs 2 and 3, c-Kit, FLT3, PDGFR | Median TTP 1.5 months, OS 3 months (Ref. 53) | 8 |
| Temsirolimus | mTOR | 6-month PFS in 7.8% of patients, median OS 4.4 months (Ref. 54) | 9 |
| Vandetanib | EGFR, VEGFR | No published results | 10 |
| Vatalanib | c-Kit, PDGFR, VEGFRs 1, 2 and 3 | PR in 29% of patients (Ref. 55) | 2 |

[a]The number was determined from the clinical trials that were listed as either completed or ongoing for therapy in glioma at http://www.clinicaltrials.gov on 1 December 2010.
Abbreviations: EFS, event-free survival; FLT3, fms-related tyrosine kinase 3; OS, overall survival; PFS, progression-free survival; PR, partial response; TTP, time to progression. For full names of other molecular targets, see main text.



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mechanisms and upregulation of alternative pathways. However, all these hypotheses rely on the a priori assumption that there is adequate drug delivery to the target. The lack of drug delivery to the target is an often overlooked yet perfectly plausible explanation for a lack of efficacy. Would this delivery failure be detected in preclinical models that were used to justify the clinical trials? This would probably not be the case if the preclinical model used was not established in the brain (e.g. flank model) or if the assessment involved well-circumscribed brain tumours with a leaky BBB amid no appreciable infiltrative growth to provide a pharmacological sanctuary (discussed further below). The latter well-circumscribed phenotype is the growth pattern of the majority of standard implanted models that are typically used for preclinical validation in the process of drug development (Ref. 64).

So the question germane to the efficacy of molecularly targeted agents in glioma is: are these drugs delivered to the tumour-infiltrated normal brain present after surgical removal of the bulk tumour mass at levels that are adequate to disrupt the function of their targets? Treatment of a brain tumour requires the drug to bypass several barriers and gain access to what is considered a 'sanctuary' in the CNS. The CNS is protected by a highly developed and well-regulated interface that separates it from the peripheral circulation and maintains homeostasis in the brain (Ref. 65). This interface also prevents most drugs and chemicals from entering the brain, thereby rendering them ineffective. Once inside the brain, the drug faces additional barriers that further limit its delivery to the ultimate target. It is critical to recognise that the intracellular targets in question are in the invasive glioma cell – that is, cells left behind after resection. A discussion of these barriers that limit drug delivery to tumour, and hence the drug efficacy, is the essence of this review.

## Barriers restricting drug delivery to the brain and brain tumour

### The blood–brain barrier

The BBB is a natural defence mechanism in the CNS that separates the brain from the peripheral circulation. The barrier is formed by a dense network of blood capillaries supplying the brain, wherein the endothelial cells are joined together by tight junctions such that most drugs and chemicals cannot readily cross into the brain parenchyma. The BBB thus shields the brain from exposure to circulating toxins and potentially harmful chemicals by preventing them from entering the brain. Besides the presence of tight junctions, a relative paucity of fenestrae and pinocytotic vesicles within the brain capillary endothelial cells along with the presence of the surrounding extracellular matrix, pericytes and astrocyte foot processes further restrict brain uptake (Ref. 65). As a result of the tight junctions in the BBB, circulating molecules gain access to the brain only by (1) passive diffusion of small nonpolar molecules through the BBB or (2) active transport (Ref. 66).

Numerous studies have endeavoured to correlate brain penetration and CNS activity of compounds to their physicochemical properties. These studies used different approaches for predicting BBB permeability and reported that compounds that have activity within the CNS have high lipophilicity ($\log P = \sim 4$), few hydrogen-bond donors (two to seven), low polar surface area (~40 Å) and low molecular weight (~400 Da) (Refs 67, 68, 69, 70). It is not surprising that all these properties impart greater membrane permeability to the drug molecule, resulting in enhanced transport to the brain (Ref. 70). However, several molecules with these favourable properties have been found to have modest permeability into the brain, which is a result of active efflux transporters that further make the BBB impermeable (Refs 67, 71). The BBB is fortified by the presence of numerous drug transport proteins, many of which transport drugs out of the brain. It has been shown that ATP-dependent transporters can severely restrict the brain penetration of therapeutic agents – even those molecules with favourable physicochemical properties that were predicted to cross the BBB with relative ease (Refs 67, 71). Most of these transporters belong to two superfamilies: the ATP-binding cassette (ABC) and solute carrier families. P-glycoprotein (P-gp, ABCB1), breast-cancer-resistance protein (BCRP, ABCG2) and multidrug-resistance-associated proteins (MRPs, ABCCs) are important members of the ABC family. We limit our discussion in this review to P-gp, BCRP and MRPs. The reader is directed to several excellent reviews that cover other drug efflux transporters



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in greater detail than is possible within the scope of this article (Refs 72, 73, 74, 75, 76, 77, 78).

### *P-glycoprotein*

P-gp, the product of the *ABCB1* gene (previously known as multidrug resistance 1 gene, MDR1), is by far the most extensively studied member of the ABC superfamily of transporters. It was originally discovered by Juliano et al. in 1976 while studying the mechanisms behind the resistance in tumour cell lines (Ref. 79). The group noticed that cell membranes of the resistant cells expressed a 170 kDa surface glycoprotein capable of altering the permeability of drugs, and designated it as 'permeability glycoprotein' or 'P glycoprotein'. A decade later, in 1986, the gene encoding the protein was discovered (Ref. 80) and the complete primary structure of P-gp was determined (Ref. 81). The existence of P-gp at the BBB was first reported in 1989 when Cordon-Cardo et al. detected P-gp expression in brain capillary endothelial cells and proposed that it had a role in regulating the entry of drug molecules into the CNS (Ref. 81). Shortly thereafter, Theibaut and colleagues reported the expression of P-gp at the rat BBB (Ref. 82), which was followed by numerous studies showing the presence of P-gp in the brain capillaries of other species such as mice, rats, cows and pigs (Refs 83, 84, 85). However, it was a seminal study by Beaulieu et al. that reported the localisation of P-gp on the luminal side of the capillary endothelial cells and bolstered theories that the transporter is involved in preventing drugs from entering the brain and in the development of multidrug resistance in cancer (Ref. 86).

The most compelling early evidence of the protective role of P-gp at the BBB was a chance discovery when mice deficient in the *Abcb1a* (*Mdr1a*) gene (P-gp-knockout mice) were found to be 100-fold more sensitive to the neurotoxin ivermectin compared with the normal wild-type mice (Ref. 87). The study revealed elevated levels of ivermectin in the brains of P-gp-knockout mice, which confirmed that P-gp protects the CNS by preventing drugs and chemicals from crossing the BBB. P-gp has since been implicated in restricting CNS penetration of hundreds of drugs, including several chemotherapeutic agents in clinical practice. The development of *Abcb1a/1b*$^{-/-}$ double knockout mice (Ref. 88) and *Abcb1a/1b*$^{-/-}$*Abcg2*$^{-/-}$ triple knockout mice (Ref. 89) has provided researchers with powerful tools to examine the influence of P-gp in the transport of drugs to the brain. Studies exploring the interaction of chemotherapeutic agents with P-gp have used these in vivo models to illustrate how potent anticancer drugs and many molecularly targeted TKIs are avid P-gp substrates and how this limits their distribution to the CNS (Table 2).

### *Multidrug-resistance-associated proteins*

The discovery of P-gp as an efflux transporter capable of transporting drugs out of tumour cells led to an increased interest among researchers to find other proteins involved in drug transport and resistance. In 1987, Cole and co-workers noticed that an adriamycin-selected lung cancer cell line was resistant to drugs such as colchicine, vinca alkaloids and anthracycline analogues (Ref. 105). These cells were known not to overexpress P-gp, leading researchers to believe that the observed resistance might be due to a transporter-mediated mechanism that was similar to P-gp. Molecular analysis revealed the presence of a cDNA encoding a 190 kDa protein that was later confirmed to be present in several multidrug resistance cell lines that did not express P-gp. This protein, which was named multidrug-resistance-associated protein (Ref. 106), was the first of 12 members of a subfamily of ABC transporters now designated as subfamily-C (ABCC). Cloning of MRP in 1992 resulted in renewed enthusiasm for drug-resistance investigations, which were now focused on identifying additional transporters capable of transporting drugs out of cells.

There is now evidence that nine of the 12 ABCC family members (MRPs 1–9) mediate some form of xenobiotic or drug resistance (Ref. 97). The discovery of MRPs also resulted in several studies investigating the localisation and role of these transporters at the BBB. However, studies on the expression of MRP transporters at the BBB have been controversial and often contradictory. In 1998, Huai-Yun et al. demonstrated the functional expression of MRP1 in bovine brain microvessel endothelial cells and suggested that the most likely localisation of MRP1 at the BBB should be apical (Ref. 98). In 2004, Zhang et al. described the localisation of various MRPs in bovine brain microvessel endothelial cells, showing that MRP1 and MRP5, which are predominantly localised



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**Table 2. Selected ABC transporters at the blood–brain barrier (BBB) and the brain–tumour-cell barrier and their substrate chemotherapeutic agents**

| Transporter | Gene | Localisation at the BBB | Presence in glioma cells | Selected substrate chemotherapeutic agents |
|---|---|---|---|---|
| P-glycoprotein (P-gp) | *ABCB1* (*MDR1*) | Luminal (Ref. 84) | Yes (Refs 90, 91, 92) | Vincristine, vinblastine, paclitaxel, docitaxel, doxorubicin, daunorubicin, mitoxantrone, etoposide, teniposide, methotrexate, topotecan, imatinib, dasatinib, lapatinib, gefitinib, sorafenib, erlotinib, tandutinib |
| Breast-cancer-resistance protein (BCRP) | *ABCG2* (*MXR*) | Luminal (Ref. 93) | Yes (Refs 94, 95, 96) | Doxorubicin, daunorubicin, mitoxantrone, methotrexate, topotecan, SN-38 (active metabolite of irinotecan), gimatecan, imatinib, dasatinib, lapatinib, gefitinib, sorafenib, erlotinib, tandutinib |
| Multidrug-resistance-associated protein 1 (MRP1) | *ABCC1* (*MRP1*) | Luminal, apical (Refs 97, 98) | Yes (Refs 99, 100, 101) | Etoposide, teniposide, vincristine, vinblastine, paclitaxel, docitaxel, doxorubicin, daunorubicin, mitoxantrone, topotecan, irinotecan, methotrexate |
| Multidrug-resistance-associated protein 2 (MRP2) | *ABCC2* (*MRP2*) | Luminal (Ref. 111) | ? | Cisplatin, etoposide, vincristine, vinblastine, doxorubicin, daunorubicin, topotecan, irinotecan, methotrexate, paclitaxel, docitaxel |
| Multidrug-resistance-associated protein 3 (MRP3) | *ABCC3* (*MRP3*) | ? | Yes (Refs 100, 101, 103) | Etoposide, teniposide, vincristine, methotrexate |
| Multidrug-resistance-associated protein 4 (MRP4) | *ABCC4* (*MRP4*) | Luminal, apical (Refs 97, 98) | Yes (Refs 100, 104) | Methotrexate, topotecan, 6-mercaptopurine, thioguanine, cisplatin |
| Multidrug-resistance-associated protein 5 (MRP5) | *ABCC5* (*MRP5*) | Luminal, apical (Refs 97, 98) | Yes (Refs 100, 101, 104) | 6-Mercaptopurine, thioguanine, gemcitabine |

basolaterally in various tissues, were highly expressed on the apical side, whereas MRP2 was not detected (Ref. 102). The group also reported equal localisation of MRP4 on the apical and basolateral plasma membranes in these cells. Nies and co-workers quantitatively studied the expression and localisation of MRPs in several regions of the adult human brain and showed the presence of MRPs 1, 4 and 5 on the luminal side of the BBB, consistent with the findings in the bovine brain (Ref. 107). In contrast to these earlier studies that report the absence of MRP2



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at the BBB, some studies have shown MRP2 expression at the luminal membranes of the human (Ref. 108), rat and pig BBB (Ref. 109).

Although equivocal, expression of MRPs at the BBB has thus now been described in several studies; however, the exact localisation and role of MRPs at the BBB are still debated. There have been reports that demonstrate the influence of MRPs at the BBB, wherein absence or inhibition of the transporter(s) results in enhanced brain penetration of substrate drugs (Refs 110, 111, 112, 113, 114). Recently, it was shown that transport of topotecan to the brain was enhanced when MRP4 was absent in the MRP4-knockout mice (Ref. 115). These studies strongly suggest that some of the MRPs act as an active drug efflux transporter at the BBB. However, further investigation is necessary to completely understand the function of these transporters at the BBB. The availability of newer tools such as knockout mice deficient in one or more of the MRPs can provide answers to remaining questions about the protective role of MRPs at the BBB.

### *Breast-cancer-resistance protein*

BCRP is another member of the ABC superfamily of transporters that confers drug resistance in cancer by virtue of its ability to translocate drugs out of cells. BCRP was originally identified independently and almost simultaneously by three different groups studying non-P-gp- and non-MRP-mediated drug resistance in cancer cell lines (Refs 93, 116, 117). In 1999, Doyle and colleagues observed an ATP-dependent reduction in the intracellular accumulation of anthracycline anticancer drugs in MCF-7 breast cancer cells and were not able to ascribe this to overexpression of known multidrug-resistance transporters, P-gp or MRP. RNA fingerprinting identified overexpression of a mRNA that encoded a 655 amino acid protein in the resistant cells, a protein that they designated as the breast-cancer-resistance protein (Ref. 116). A similar study investigating the occurrence of mitoxantrone resistance in cancer cell lines isolated a novel cDNA that encoded an ATP-dependent transporter that was named mitoxantrone-resistance protein (MXR) (Ref. 117). Around the same time, Allikmets and co-workers identified a novel gene that was highly expressed in the human placenta. They showed that the gene encoded an ABC transporter protein, which they termed the ABCP (ABC transporter in the placenta) (Ref. 93). When the sequences of genes from these three studies were eventually compared, they were recognised as essentially identical and belonging to a subfamily of ABC transporters not previously associated with drug resistance in humans (Ref. 118). Subsequently, the Human Genome Nomenclature Committee assigned this gene the name *ABCG2*. Following the cloning of BCRP, its role in the efflux of drugs from multidrug-resistant cells has been widely studied, and there are several reports on BCRP-mediated resistance to chemotherapeutic agents (Table 2).

The putative role of BCRP in the barrier function at the BBB has been controversial. Several studies have reported that BCRP is localised on the luminal side of the capillary endothelial cells in human (Ref. 119) and rat (Ref. 120) brains. Others have reported the enriched presence of BCRP in the brain capillaries of mice (Ref. 121) and pigs (Ref. 122). However, this presence of BCRP at the BBB has not been unequivocally correlated to the low brain penetration of all BCRP substrates. Lee et al. conducted in situ brain perfusion studies using dehydroepiandrosterone sulfate and mitoxantrone, two drugs that are efficiently transported by BCRP, and reported no enhancement in brain penetration of the two compounds in *Abcg2*$^{-/-}$ mice (Ref. 123). Similarly, another study showed that in vitro interaction of BCRP with substrate compounds rarely translates to visible effects at the BBB in vivo (Ref. 124). The authors from both studies concluded that BCRP has a minor role in the efflux of drugs at the BBB. By contrast, there have been several studies that demonstrate the role of BCRP in the efflux of drugs at the BBB. Cisternino and colleagues showed that BCRP-mediated efflux of prazosin and mitoxantrone at the BBB limits permeability of the brain to these prototypical substrates (Ref. 121). Likewise, Enokizono et al. showed that brain partitioning of drugs increased significantly when BCRP was absent in *Abcg2*$^{-/-}$ mice (Ref. 125). Breedveld et al. showed that brain penetration of imatinib was restricted by BCRP (Ref. 126), and we recently reported that sorafenib transport to the brain was significantly increased in *Abcg2*$^{-/-}$ mice (Ref. 127). There has been a recent increase in the number of studies investigating the role of BCRP-mediated active efflux in the transport of



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drugs out of the brain. This surge has been driven by reports suggesting a possible cooperative role of P-gp and BCRP in keeping drugs out of the brain (Refs 127, 128, 129, 130, 131, 132, 133, 134). Several studies have shown that there is a dramatic increase in the brain penetration of dual P-gp and BCRP substrates when these two transporters are absent simultaneously in *Abcb1*$^{-/-}$*Abcg2*$^{-/-}$ mice. First seen with topotecan (Ref. 128), this phenomenon has now been reported for several other compounds, including important TKIs such as lapatinib (Ref. 129), dasatinib (Refs 130, 131), gefitinib (Ref. 132), erlotinib (Ref. 133) and sorafenib (Refs 127, 134). These findings, along with reports that there is extensive overlap in the expression pattern and substrate specificity of BCRP and P-gp (Ref. 135), suggest that P-gp and BCRP work together at the BBB to limit the brain penetration of dual substrates.

In summary, the BBB is a major bottleneck that limits drug delivery to the brain; a significant fraction of large and small molecules do not effectively cross the BBB (Ref. 6). It is clear that drug efflux transporters, a key component of this barrier, can significantly restrict the passage of drugs into the brain, even those with favourable physicochemical properties to cross biological membranes. The fact that there are several drug transporters at the BBB, some of which might be working together, further complicates the problem. Effective targeting of tumours in the brain will require novel strategies to inhibit these gatekeepers so that promising drug candidates are not rendered ineffective because of their inability to enter the brain.

### *Is the BBB compromised in glioma?*

Recently, the role of the BBB in limiting treatment efficacy in glioma has been questioned based on studies that report high concentrations of chemotherapeutic agents in tumour resections (Ref. 136). These reports suggest that the BBB does not influence delivery in glioma. This has caused confusion in the clinical assessment of drug delivery when using drug concentrations in the tumour core (the resected tissue) as a guide for the adequacy of drug delivery. It is true that the BBB can be disrupted at or near the tumour because the central core of the tumour is highly angiogenic, containing new and leaky blood vessels (Ref. 137). Although drug delivery might be greatly enhanced in such areas of the tumour, surgery almost completely removes the central core of the tumour in the brain (contrast-enhancing area). Therefore, concentrations in these areas do not represent those in the brain areas that are not removed by surgery. Moreover, the BBB is intact at the growing edge of the tumour and early in the development of the vascular niche of invasive glioma cells (Ref. 138). The disruption of brain vasculature is directly related to tumour size and distance from the central core (Ref. 137). Invasive glioma cells that are not removed by surgery reside in areas of diffuse glioma invasion, which can be centimetres away from the main tumour (Ref. 139) and have an intact BBB capable of restricting drug levels. Given the diffusely infiltrating growth of glioma, it is not surprising that the tumour eventually recurs from areas of the tumour rim that are not resected (Ref. 13), where drug delivery is impaired because of the BBB.

Effective delivery of chemotherapeutics to the invasive glioma cells is therefore critical, and long-term efficacy will depend on the ability of a molecularly targeted agent to penetrate an intact and functional BBB throughout the entire brain. This idea of glioma as a disease of the whole brain lends particular credence to the need to use systemic circulation to effectively deliver drug across the BBB to encompass the central tumour, the growing edge of the tumour and invasive glioma cells. We present this problem in Figure 2, where a hypothetical schematic of a brain tumour can be seen with a gradient of drug concentration around the tumour (Fig. 2a). The tumour core (the area with a disrupted BBB) can have high drug levels; however, areas immediately surrounding the core can receive significantly less drug owing to an intact BBB. The tumour core is usually removed after surgery, but glioma cells invade areas of restricted drug delivery away from the tumour (Fig. 2b). The goal of effective chemotherapy should be to effectively deliver drug in areas that can harbour the invasive glioma cells and not just the tumour core, the part of the tumour removed by surgery (Fig. 2c). This idea has been supported in a study by Fine et al., where the authors measured paclitaxel concentrations in resected tissue specimens from brain tumour patients and showed that concentrations in the normal brain surrounding the tumour were tenfold lower than those in the



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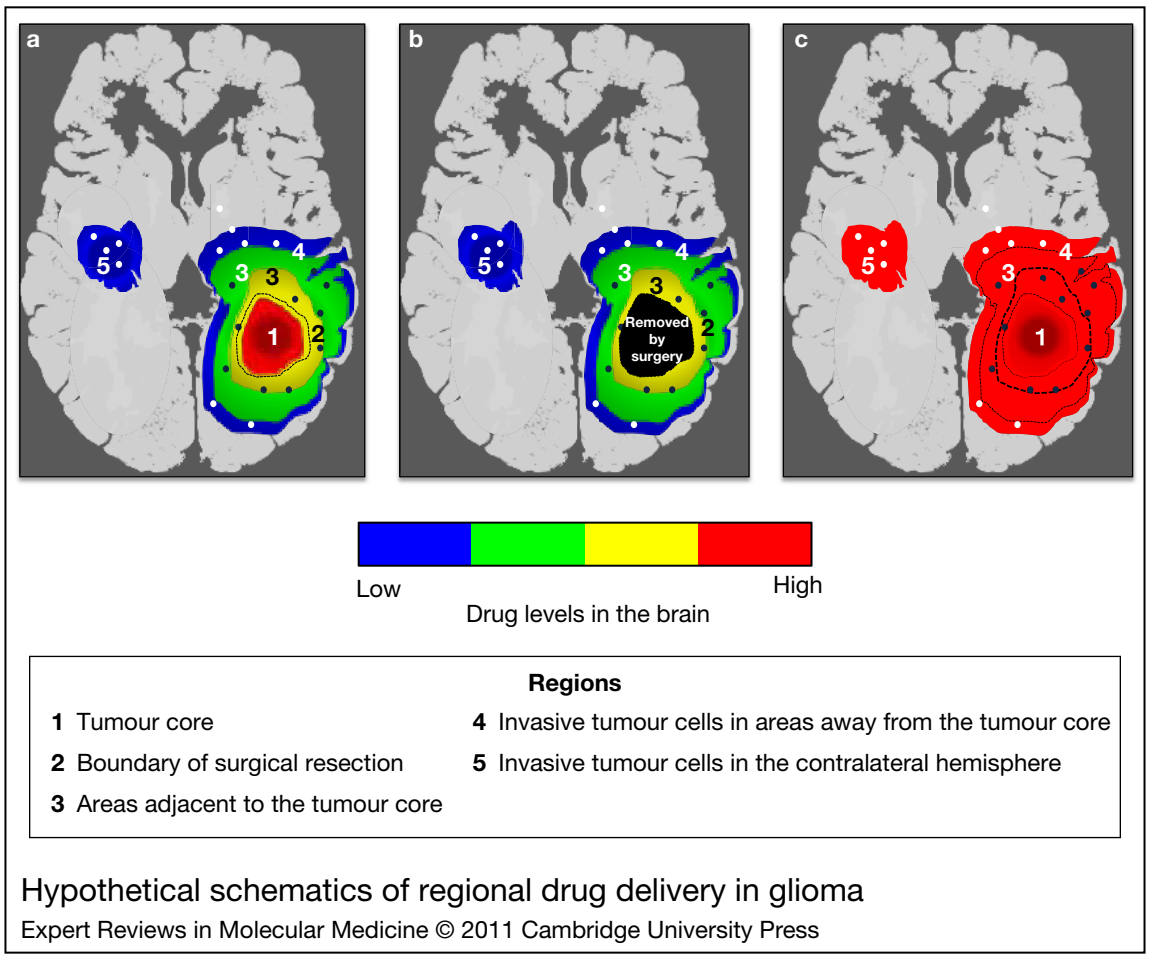


**Figure 2. Hypothetical schematics of regional drug delivery in glioma.** Schematics of a brain tumour are shown, with a simulated gradient of drug concentration around the site of the tumour. (a) The tumour core, the area with a disrupted blood–brain barrier (BBB), can have high drug levels (region '1'); however, areas immediately surrounding the core (region '3') can receive significantly less drug owing to incomplete BBB breakdown. (b) The tumour core is usually removed after surgery (up to boundary '2'); however, glioma cells that have invaded areas away from the central tumour are not removed. In these areas, including those far away from the tumour (region '4') and even in the normal hemisphere (region '5'), glioma cells can continue to grow and give rise to the recurrent tumour. (c) The goal of chemotherapy should be to effectively deliver drug in areas that can harbour the invasive glioma cells and not just the tumour core, the part of the tumour removed by surgery.

tumour core (Ref. 90). Furthermore, Pitz et al. recently summarised clinical studies reporting anticancer drug concentrations in brain tumours and suggested that drug concentrations in contrast-enhancing areas of the tumour (tumour core) were relatively higher than those in non-contrast-enhancing areas (tumour periphery and normal brain) (Ref. 91).

## The brain–tumour-cell barrier

In addition to the BBB, the BTB is another barrier that the drug has to cross to reach its intracellular target. The tumour cell membrane, which forms this barrier, regulates the transport of nutrients, growth factors, drugs and other substances into and out of the cell. A considerable amount of work has been done studying the expression, regulation and activity of ABC transporters in cells from various tumours, including glioblastoma multiforme. There is increasing evidence suggesting that drug efflux transporters on the tumour cells decrease intracellular drug uptake, resulting in the multidrug-resistant phenotype often observed in glioma cells.



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P-gp is by far the most extensively studied efflux transporter in glial tumours, and its presence has been confirmed by several studies. Fattori and co-workers used immunohistochemistry to show that P-gp was heterogeneously expressed in about 82% of glioblastomas (Ref. 92). Similarly, several other studies have reported enhanced expression of P-gp in tissue specimens from human gliomas (Refs 99, 100). However, there have also been several conflicting reports indicating the absence of P-gp in glioblastoma multiforme cells. These studies suggest that expression of P-gp in human glioma specimens is relatively low and rare (Ref. 140). Decleves et al. showed that P-gp was not expressed in human glioma cells at either the transcript or the protein level (Ref. 141). These widely differing results on the expression of P-gp have been attributed in part to the assay technique used for the detection of P-gp (Ref. 94). Nevertheless, the recent reports mentioned above confirm the presence of P-gp in glioma cells and its effect on accumulation of anticancer drugs in these cells.

In contrast to P-gp, very few studies have investigated the expression of BCRP in tumour cells from glioma. Despite its original isolation from drug-resistant breast cancer cell lines, the expression of BCRP in many solid tumours has been found to be negligible (Ref. 95). However, new evidence implicates this transporter with a special side population of tumour cells that are believed to have stem-cell-like properties (Refs 96, 142). These precursor cells, responsible for driving tumour growth and proliferation, are thought to be drug resistant because of efflux by BCRP. In a mouse model of glioma, Bleau et al. recently demonstrated enhanced tumourigenicity of BCRP-enriched stem-like cells (Ref. 104). This suggests a similar role of BCRP wherein the transporter confers resistance in glioma cells by virtue of its ability to pump drugs out of the cell.

Other than P-gp and BCRP, MRPs have also been found to be expressed in glioblastoma cells (Ref. 101). Transporters of this family have been reported to be expressed at levels that are in some cases greater than that of P-gp (Ref. 140). Histochemical analysis of glioma specimens has revealed the presence of significant amounts of MRPs 1, 3, 4 and 5 (Refs 103, 141, 143). The influence of MRPs on chemoresistance in glioma has also been reported: nonspecific inhibition of MRPs enhanced the cytotoxic effects of anticancer agents in glioma cell lines (Ref. 144). In a recent study, Kuan and co-workers reported elevated expression of MRP3 in human glioblastoma multiforme in contrast to negligible presence in normal brain (Ref. 145), and suggested the potential use of MRP3 as a prognostic marker and molecular target for glioblastoma multiforme.

In summary, expression of ABC transporters in human glioma cells and their role in acquired drug resistance have been reported by several studies in the past few years. The findings have often been ambiguous and conflicting. Although the genetic heterogeneity of the tumour in glioma can account for some of the variability in the reports, more research is clearly needed to elucidate the role of these transporters in tumour cells. Nonetheless, it is clear that the BTB can be a significant second barrier that has the ability to hamper drug delivery to the intracellular target.

## BBB and BTB: complex barriers that limit delivery of TKIs to glioma

The impact of the BBB and BTB on drug delivery to the target site can be significant, especially when the drug is a substrate for transporters present at both the barriers (Fig. 3). The recent surge in the development of molecularly targeted TKIs for CNS tumours has led to several investigations on their interaction with important efflux transporters. The availability of tools in the form of transgenic mouse models and transporter-overexpressing cell lines has made it possible to study drug–transporter interactions with the aim to modulate these and enhance drug transport to the target tissue.

Given that P-gp and BCRP are the two important transporters that limit drug delivery to the brain and tumour cells, most studies have investigated the interaction of TKIs with these two efflux pumps. Imatinib was the first TKI that was reported to be a substrate for drug-effluxing transporters, when it was discovered that distribution of imatinib to the brain was restricted by P-gp-mediated efflux (Ref. 146). This was followed by a number of studies that reported that imatinib was effluxed by both P-gp and BCRP at the BBB (Refs 126, 147). The finding that imatinib does not effectively cross the BBB was crucial in explaining its lack of efficacy against brain relapses in chronic myeloid leukaemia (Ref. 148). Similarly, Polli et al. showed that the EGFR inhibitor lapatinib was a substrate



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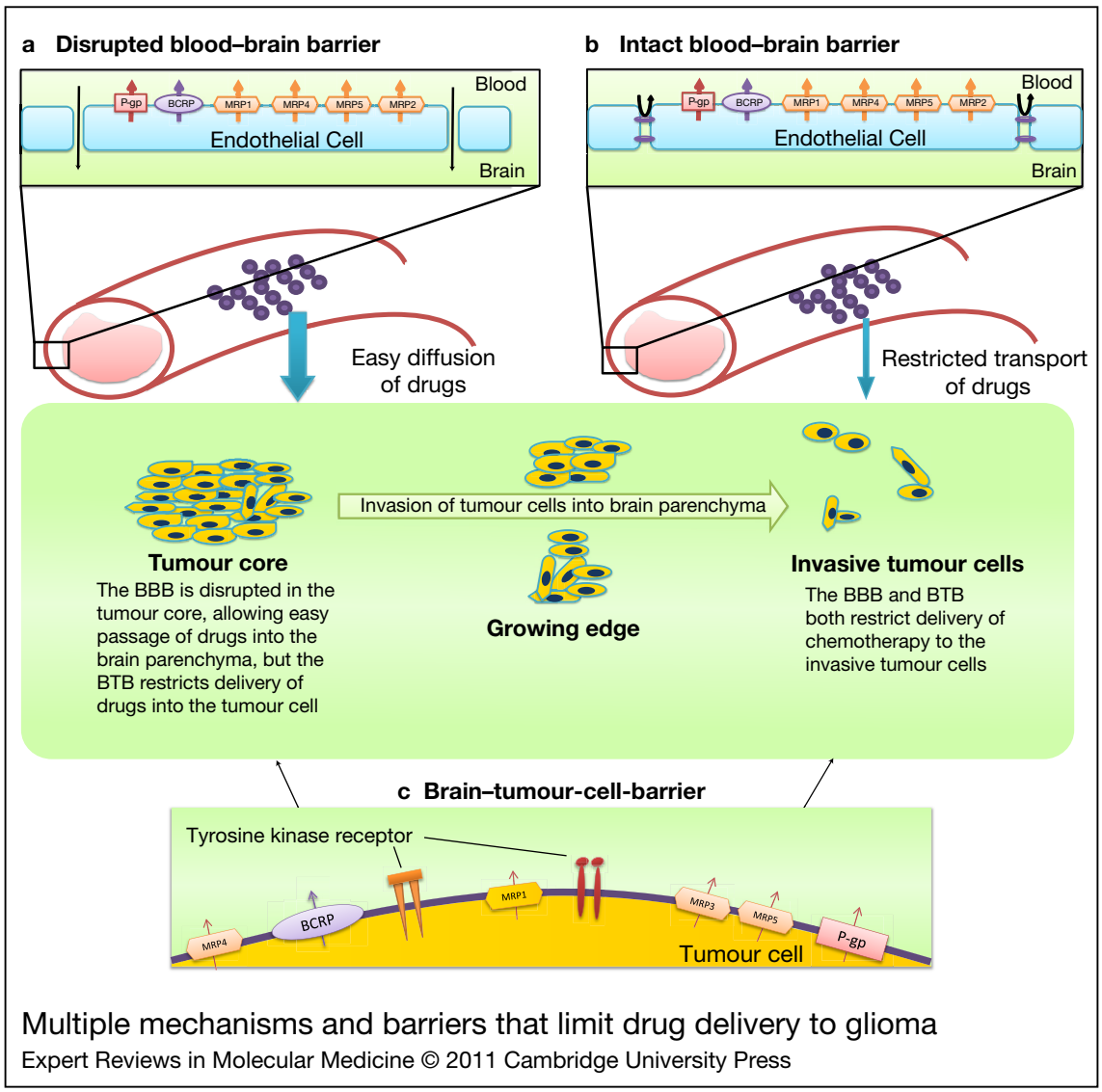


Multiple mechanisms and barriers that limit drug delivery to glioma



**Figure 3. Multiple mechanisms and barriers that limit drug delivery to glioma.** The blood–brain barrier (BBB) and the blood–tumour-cell barrier (BTB) form sequential barriers that a systemically administered drug must cross to reach the tumour. The centre of the figure shows the invasion of glioma cells from the tumour core into the normal brain parenchyma. Above this, parts a and b show how the integrity of the BBB affects drug delivery in these different locations; below, part c depicts the BTB, which restricts drug delivery in both locations. (a) The BBB is often disrupted at the site of the tumour, with the lack of tight junctions allowing for easy diffusion of drugs and small molecules into the tumour. However, this is also the part of the tumour that gets removed after surgery. (b) The BBB is intact in areas centimetres away from the tumour core. Drug delivery across this barrier is restricted by the presence of tight junctions between endothelial cells and more importantly by drug efflux transporters that pump drugs back into the blood. The amount of drug that is able to cross this barrier and reach the brain is usually a fraction of what reaches the tumour core. Nests of tumour cells in such locations protected by the intact BBB eventually give rise to the recurrent tumour after surgery. (c) The BTB represents the barrier between the brain parenchyma and the tumour cell. Drug efflux transporters present in the tumour cell are a major component of this barrier and restrict intracellular drug uptake. This second barrier is especially important for molecularly targeted agents that target intracellular domains of receptor tyrosine kinases. Abbreviations: BCRP, breast-cancer-resistance protein; MRP, multidrug-resistance-associated protein; P-gp, P-glycoprotein.



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for both P-gp and BCRP (Ref. 149), and then suggested that these two transporters work together at the BBB to limit brain penetration of dual substrates (Ref. 129). Thereafter, we showed that P-gp and BCRP work together to limit the brain penetration of several other TKIs, such as dasatinib, gefitinib and sorafenib (Refs 127, 130, 132). Subsequent studies have shown that this is true for tandutinib and erlotinib as well (Refs 150, 151). An extremely important finding in most of these studies is that pharmacological inhibition of the two transporters together significantly enhanced brain levels of the TKIs, over and above individual inhibition of one of the transporters. These preclinical studies used elacridar (GF120918), a dual inhibitor of P-gp and BCRP, and demonstrated that it increases the transport of the concurrently administered TKI to the brain. This suggests that coadministration of an inhibitor of P-gp and BCRP can be used as a strategy to enhance the delivery of these drugs to the brain.

Many promising TKIs are effective in treating non-CNS malignancies such as lung, breast and hepatic cancer. However, none of them shows any clinical efficacy against metastatic disease in the brain or against primary brain tumours such as glioma (Table 1). The complication of delivery across an intact BBB has made it difficult to apply peripherally acting chemotherapeutic agents to invasive cancers of the brain. The problem is confounded by the fact that the BTB has the ability to further restrict intracellular delivery of drug into invasive glioma cells. A more detailed understanding of these multiple barriers can help researchers devise strategies to overcome some of these barriers and thereby increase the effectiveness of these drugs against glioblastoma multiforme.

## Clinical implications

### TKIs in glioblastoma multiforme: hopes and disappointments

In May 2001, the first TKI, imatinib, was approved for treatment of a human malignancy (chronic myeloid leukaemia), raising hopes within the oncology community of the promise of similar target-directed chemotherapeutics for treating other devastating cancers such as glioblastoma multiforme. However, to date, none of the TKIs has been able to show any clinical benefit against this disease. Imatinib's success in chronic myeloid leukaemia started a wave of clinical trials that evaluated different TKIs either alone or in combination for therapy in glioma. The trials were backed by significant data showing efficacy of the compounds in preclinical models of glioblastoma multiforme, but most of them culminated in disappointing failures. The first clinical trial of a TKI in glioma tested gefitinib (Ref. 34), with the hope that inhibition of the highly deregulated EGFR signalling pathway would translate into improved patient survival. Its failure was soon followed by the clinical inefficacy of the other major EGFR inhibitor, erlotinib. Identification of newer targets led to the introduction of newer targeted therapies; the clinical outcomes, however, did not change.

Studies explaining the failure of these trials have suggested that some reasons for this could be the heterogeneous molecular characteristics of individual gliomas and the complexity of signalling cascades that feed the tumour. However, several questions remain. (1) Does the drug cross the BBB? (2) What are the drug concentrations in the brain? (3) What are the concentrations in the tumour? (4) Is the concentration sufficient to inhibit the target? Answers to these questions can help us gain an insight into the possible reasons behind the failure of these drugs. If therapeutic agents do not reach their intended molecular target, regardless of their potency they cannot possibly be effective. It is well accepted that the BBB evolved to protect the brain and will be a barrier in the CNS delivery of most drugs. As discussed earlier, many of the TKIs are substrates for important transporters at the BBB, and this significantly limits their concentrations in the brain. Whether these preclinical findings translate in humans and whether these transporters restrict penetration of drugs across a human BBB is still unknown. But there is no evidence to suggest otherwise, and the inefficacy of these agents against brain tumours in humans adds further credence to the hypothesis.

### Drug concentrations in the brain and the tumour

Many of the questions raised above can be explored if drug levels in the brain could be measured in patients receiving chemotherapy. Unfortunately, very few studies have evaluated drug concentrations in brain tissues. This is due in part to the difficulty in sampling drug levels in the brain tissue and to uncertainty in the prediction of drug levels in the brain from concentrations in surrogate tissues such as



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cerebrospinal fluid (Ref. 152). However, Hofer and colleagues recently presented a few case reports where they investigated the concentrations of chemotherapeutic agents in the brain and the tumour. The group measured gefitinib concentrations in tissue specimens from seven glioblastoma multiforme patients and reported tenfold higher concentrations in excised tumour tissue compared with plasma (Refs 136, 153). These findings were supported by preclinical reports describing gefitinib accumulation in the tumour (Ref. 154). These investigators concluded that delivery of drugs (gefitinib) to the tumour is not restricted in patients because the BBB is overcome by residual damage from radiotherapy and by the pathological infiltrative characteristics of glioblastoma multiforme, which compromise the functional integrity of the BBB. In the 1980s, a few studies by Stewart and co-workers measured the concentrations of cisplatin (Ref. 155), vinblastine (Ref. 156) and etoposide (Ref. 157) in brain tumours. All these studies reported high drug levels in the tumour, similar to those in the above report. But the group also presented a very interesting finding. Drug concentrations in regions immediately adjacent to the tumour were surprisingly lower than those in the tumour, with the concentrations decreasing with increasing distance from the tumour. In a similar study, Blakeley et al. used microdialysis to show that penetration of methotrexate was significantly lower in the brain areas adjacent to the tumour (Ref. 158). All these studies show significantly high drug levels in the tumour. So how does one explain the apparent contradiction that tumour distribution of drugs does not seem to be restricted by the BBB, yet at the same time their efficacy against the tumour is minimal and the recurrence of tumour after surgery, centimetres away from the original tumour, is inevitable even with intensive radio- or chemotherapy?

## Glioblastoma: a whole-brain disease

Given its invasive and infiltrating nature, we consider glioma as essentially a disease of the entire brain, and this idea can help understand the answers to some of the questions raised above. Apart from being one of the most malignant cancers, glioma is also one of the most infiltrative tumours. Even complete surgical resection of the tumour-bearing hemisphere inevitably leads to recurrence and has been abandoned (Ref. 159). Historical reports show that more than 50% of untreated brain tumours spread into the contralateral hemisphere (Ref. 160). Thus, one of the most important hallmarks of malignant glioma is local invasion, which has been described in studies as early as 1938: in a landmark study, Hans-Joachim Scherer described the diffuse invasion of glioblastomas by defining secondary patterns that reflected the growth of tumour in neighbouring brain tissue (Ref. 161). Thus glioblastoma multiforme is a disease of the whole brain. Tumour cells that migrate into the surrounding brain parenchyma escape surgical resection and are the putative source of the recurrent tumour (Figs 2, 3).

This pathological property of glioma can account for many of the pharmacokinetic findings mentioned above. First, the central core of the tumour is a highly necrotic mass and the BBB is most likely disrupted in this area. This allows systemically delivered chemotherapy to easily traverse the impaired barrier and reach the tumour, thus explaining the high concentrations seen in the tumour by Hofer and Frei (Ref. 136). This is almost always true because the very ability of contemporary imaging techniques to detect a brain tumour relies on the ability of the contrast agent (gadolinium) to leak through a disrupted BBB and enhance the tumour core (Ref. 162). Nevertheless, this is also the part of the tumour that is removed by surgical debulking, rendering less relevant any correlations between drug concentrations in this area to eventual efficacy or lack thereof.

Second, disruption of the BBB becomes increasingly insignificant in areas away from the tumour. This is a valuable finding in studies conducted by Stewart et al. and Blakeley et al. (Refs 155, 156, 157, 158). The fact that drug exposure in areas immediately adjacent to the tumour was an order of magnitude lower than exposure in the tumour confirms the presence of a functional BBB in these areas, capable of restricting the passage of drugs into the brain. This has been elegantly demonstrated by Lockman et al., where the authors show that the BBB remains sufficiently intact in satellite lesions of the metastatic tumour to significantly restrict drug delivery to the tumour cells (Ref. 163). This theory has also been supported by other recent



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studies that have shown that concentrations of paclitaxel (Ref. 90) and temozolomide (Ref. 164) in the tumour periphery were lower than those in the tumour core. A recent study by Pitz et al. summarises findings from clinical studies and shows that the concentrations of many anticancer drugs in contrast-enhancing areas of the tumour were severalfold higher than those in plasma (Ref. 91). More importantly, the study also reports that tissue-to-blood ratios were generally higher in contrast-enhancing regions than in non-contrast-enhancing regions, and in areas of brain distant from tumour (Ref. 91). Thus, in areas distant from the tumour core, where gadolinium does not cross the intact BBB, mechanisms that limit drug distribution (tight junctions and efflux transport) will still be operative and limit drug delivery. Consequently, less drug reaches the sites that harbour the infiltrated tumourigenic glioma cells, which continue to grow and ultimately reach a clinically significant size. Thus recurrence, an inevitable occurrence in glioma, might be due not only to tumour cells invading the adjoining brain areas but also to a lack of drug delivery in such areas.

Finally, there is a growing body of literature that suggests that a subset of these invasive cells have stem-like properties that allow them to repopulate the tumour (Ref. 165). The cancer stem cell hypothesis asserts that tumour development and maintenance in glioblastoma multiforme is controlled exclusively by these rare fractions of cells with unlimited proliferative and self-renewing capacities (Ref. 166). A basic tenet of this hypothesis is that these stem-like cells have an innate resistance to chemotherapy (Refs 167, 168), mainly due to the presence of drug transporters that efflux drugs out of the cells (Refs 169, 170, 171, 172). This indicates that even if a drug crosses the BBB to reach the brain parenchyma, its entry into an infiltrative tumour cell can be further restricted by drug efflux proteins present within such cells. These infiltrative cells, shielded by the BBB and the BTB, thus grow and eventually give rise to the recurrent tumour.

Thus, the two complex sequential barriers – the BBB and the BTB – are two important factors that govern the passage of drug from systemic circulation to the target site. The clinical failure of molecularly targeted therapy suggests two fundamental realities. One is that the BBB and the BTB can significantly limit drug delivery to the target site. The other is that regardless of how potent our targeted agents are, they will continue to be ineffective until strategies are devised to improve their delivery across the BBB and the BTB into invasive glioma cells. An excellent depiction of this predicament is given by Berens and Giese, where the authors explain that the clinical course of glioma patients after surgery is determined by residual, invasive tumour cells – that is, 'those left behind' (Ref. 7).

## Outstanding research questions

The realisation of the impact that the BBB and the BTB can have on chemotherapy in glioma has resulted in a renewed interest among researchers to pursue strategies that can overcome these barriers and increase the delivery of drug to tumour targets. Several innovative methods have been developed and used to circumvent the BBB and improve drug delivery to the brain. These techniques can be divided into three broad categories: administration of chemotherapy directly into the brain parenchyma, osmotic disruption of the BBB and inhibition of drug efflux.

Direct administration into the CNS is achieved by the use of biodegradable polymers, convection-enhanced delivery, or intrathecal and intraventricular administration. In 2002, the US Food and Drug Administration (FDA) approved Gliadel® wafers for use as an adjunct to surgery in the treatment of malignant glioma. These are biodegradable polymeric wafers that slowly release the DNA-alkylating agent BCNU in the space remaining after surgical resection; they have been shown to be well tolerated and offer a survival benefit in glioblastoma multiforme patients with concurrent chemotherapy (Refs 173, 174). However, new data indicate no survival benefit and significant adverse effects on treatment with these wafers (Ref. 175). Clinical evaluation of convection-enhanced delivery for enhancing tumoural delivery of chemotherapy has yielded similar results (Ref. 176). In a recent trial, convection-enhanced delivery afforded no survival benefit compared with Gliadel® (Ref. 177), whereas a separate clinical trial reported that treatment was associated with severe neurological complications (Ref. 178). Transient disruption of the BBB by intra-arterial infusion of a hyperosmotic solution of mannitol (Ref. 179) or



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the bradykinin analogue RMP-7 (Ref. 180) is a method used to enhance concentrations of chemotherapy in the brain. Recent studies have shown that treatment with carboplatin, etoposide (Ref. 181) and bevacizumab (Ref. 182) after disruption of the BBB resulted in prolonged time to progression and reduction in tumour volume.

A primary drawback common to the above approaches is that these are complex techniques and are associated with a significant incidence of treatment-related complications. Modulation of drug transporters at the BBB might be an alternative possible method to improve delivery of chemotherapy to the brain. Compounds such as valspodar (PSC833), zosuquidar (LY335979) and elacridar (GF120918), which are potent inhibitors of the drug transporters P-gp and BCRP, can significantly enhance systemic and brain concentrations of the concurrently administered chemotherapeutic agent (Refs 127, 128, 130, 131, 132). Consequently, several clinical trials have tested these chemical modulators with the aim of reversing multidrug resistance in haematological and solid tumours. The results from these clinical investigations have been disappointing, with many studies reporting no enhancement in drug efficacy and significant toxicities related to administration of the reversal agent. Treatment with valspodar has been associated with severe toxicities and no improvement in efficacy of concurrent chemotherapy (Refs 183, 184). The P-gp inhibitor zosuquidar has been reported to be relatively nontoxic but has again failed to show any improvement in treatment (Refs 185, 186). By contrast, coadministration of the dual P-gp and BCRP inhibitor elacridar resulted in significant enhancement in the oral bioavailability of topotecan and doxorubicin (Refs 187, 188). However, these effects were seen after high doses of elacridar, which were often toxic. Although most of these failures were in trials for peripheral solid tumours, the scenario might be different in brain tumours where a moderate enhancement in drug delivery across the BBB can dramatically increase relative drug concentrations in the brain. Furthermore, in many of the studies, it was not clear whether the observed toxicities were a result of the transport modulator or the simultaneously administered chemotherapeutic agent. Again, this may be different in brain tumours where the most common toxicity observed with the current TKIs is systemic and administration of an efflux inhibitor could even serve to reduce the chemotherapeutic dose if the desired brain concentrations were achieved at lower systemic doses. Clinical trials of modulation of multidrug resistance have been limited by two major factors: inability to achieve adequate nontoxic levels of the modulators to reverse drug resistance in patients, and the presence of multiple mechanisms of resistance (Ref. 189). The development of new, more potent inhibitors can help overcome some of these limitations. Further clinical studies are needed to better understand the benefit of increasing the delivery of chemotherapeutic drugs to tumours in the brain. Additionally, preclinical studies that will be used to justify these clinical trials must use intracranial models that exhibit appreciable tumour-infiltrated normal brain protected by the BBB in order to be most informative.

## Conclusion

The BBB and the BTB are two important obstacles that restrict the passage of molecularly targeted agents to the tumour. An increase in our understanding of the molecular biology of glioma has resulted in new potent compounds that intervene in various signalling pathways that drive tumour growth. However, regardless of potency, if the therapeutic agents do not reach their intended molecular target, they cannot possibly be effective. Numerous strategies have been devised to circumvent some of these barriers and improve the delivery of drug to tumour cells in the brain. Although some of these strategies have shown promising results in the preclinical setting, the results in patients have thus far been poor. The molecular heterogeneity in glioma calls for the use of multitargeted agents – 'dirty drugs' that can inhibit multiple signalling pathways simultaneously. However, we also need 'sharp needles' that can effectively deliver such drugs to the site of the invasive tumour. The next generation of clinical trials is exploring the use of multitargeted TKIs or combinations of single-targeting TKIs. Further research investigating the delivery of chemotherapeutics to the tumour will ensure that these clinical trials do not follow the same pattern as that of the previous trials. Approaching the treatment of glioma by assuming that the tumour is



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localised in the contrast-enhancing area (hence resection) will lead to continued failure. The dismal prognosis in glioma may remain unchanged until measures are taken to ensure that promising anticancer treatments are delivered effectively to invasive glioma cells – those hiding behind an intact BBB. We must effectively treat 'those left behind'.

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## Further reading, resources and contacts

Van Meir, E.G. et al. (2010) Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. CA: A Cancer Journal for Clinicians 60, 166-193
Reviews the latest developments in therapeutic options for glioma and highlights the current and future direction of clinical trials.

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A review on the role of ABC transporters at the BBB and the availability of newer research tools in the form of transgenic mice.

## Features associated with this article

**Figures**

Figure 1. Molecularly targeted therapy for malignant glioma.
Figure 2. Hypothetical schematics of regional drug delivery in glioma.
Figure 3. Multiple mechanisms and barriers that limit drug delivery to glioma.

**Tables**

Table 1. Molecularly targeted agents for tumours of the central nervous system.
Table 2. Selected ABC transporters at the blood–brain barrier (BBB) and the brain–tumour-cell barrier and their substrate chemotherapeutic agents.

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