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Chemotherapy Delivery Strategies to the Central Nervous System: neither Optional nor Superfluous

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Abstract: Malignant brain tumors including primary brain tumors (*e.g.*, glioblastoma multiforme) and metastases, are aggressive and lethal entities for the majority of affected patients. Current standard treatments involving combinations of surgery, radiotherapy and systemic chemotherapy offer only modest improvements in survival. Faced with dismal survival, great efforts are deployed to find interesting treatment alternatives. However, the blood-brain barrier (BBB) and the blood-tumor barrier (BTB) remain great obstacles to significant drug delivery to brain tumors. The need to optimize delivery strategies for better patient outcome in the treatment of malignant brain tumors is well acknowledged. Certain interesting strategies use surgical or physical techniques to enhance the distribution of therapeutic agents to the central nervous system. The following strategies will be discussed in this review: intra-arterial delivery, osmotic BBB disruption, intranasal delivery, convection-enhanced delivery and osmotic pumps, implanted polymers, magnetic microspheres and ultrasound BBB disruption. The purpose of this paper is to review the importance of the BBB and the BTB and to review the current status and future perspectives of these delivery procedures.

Keywords: Blood-brain barrier, blood-brain barrier disruption, brain tumor, chemotherapy, drug delivery, glioblastoma multiforme, targeted delivery.

INTRODUCTION

Primary malignant central nervous system (CNS) tumors have an annual incidence of up to 8.85 per 100 000 in the adult population of the United States [1]. Glioblastoma multiforme (GBM), grade IV astrocytoma according to the World Health Organization (WHO) classification, is the most common of these (incidence of 3.19/100 000 person/ year in the United States) [1-3]. This tumor expresses highly aggressive and invasive behavior, and gravely impacts the functional status and quality of life of affected patients.

The standard treatment of GBM involves cytoreductive surgery followed by external beam radiation and chemotherapy (mainly temozolomide). However, because of the infiltrative nature of this tumor, complete tumor removal remains a utopian concept. Tumor relapse is the norm. Surgery nonetheless remains of crucial importance. It allows a more precise molecular diagnosis as well as positively affects survival and quality of life of patients [4-10]. In 2005, Stupp *et al.* found an increased survival in patients with GBM after undergoing surgical resection followed by radiotherapy and concomitant temozolomide (median survival time [MST] 14.6 months *vs.* 12.1 months with radiotherapy alone) [11]. Despite these promising results, 5-year survival remains less than 10% and MST less than 2 years [2, 12]. Different reasons explain this rather poor prognosis and response to treatment. One of these stands and remains unsolved to this day: the presence of the blood-brain barrier (BBB) and blood-tumor barrier (BTB), both significant obstacles to drug delivery. Because of these restrictive entities, the role of potentially active chemotherapeutic agents remains marginal in the treatment of malignant astrocytomas.

The purpose of this paper is to review the importance of the BBB and the BTB as well as the current status and future perspectives of interesting physical and surgical strategies to circumvent these CNS barriers.

BRAIN TUMOR TREATMENT LIMITATIONS: CNS BARRIERS

Blood-brain Barrier

The BBB is responsible for regulating the cerebral microenvironment. It selectively transports substances into the brain, maintains the right concentration of essential compounds and protects the CNS from harmful substances. It is generally refered to as “the neurovascular unit”, and is comprised of a microvascular endothelium, basement membrane and neuroglial structures (astrocytes, pericytes and microglia) [13, 14]. The astrocytic projections and neuronal endings directly interact with the endothelial cells. This contributes to maintaining the phenotypic characteristics expressed by these cells and their function [15-17].

Certain particularities of the neurovascular unit are essential to its function. First, the endothelial cells of the brain capillaries are connected by tight junctions (TJ) [13, 18, 19]. Lacking fenestrations, these cells restrict paracellular transport of large hydrophilic compounds. They also exhibit

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very low pinocytotic activity and endosomal transport. This leads to tightly regulated and limited transcellular transport of substances needed for brain functioning [20]. The TJ are a major component of the protective layer and determine the permeability to hydrophilic molecules [21]. They do so because of the presence of adhesive molecules such as zona occludin-1 (ZO-1) [22], ZO-2, ZO-3, occludin [15], claudin [23] and junctional adhesion molecules [24, 25], which constitute the backbone of the TJ. The barrier possesses a high electrical resistance (1000-2000 Ohm/cm^2) due to its protein composition as well as other mechanisms [26]. The luminal surface of the endothelium presents a net negative charge, thereby creating an additional barrier to polar and ionic substances [27, 28].

The BBB exerts its protective functions by also acting as a metabolic barrier. Indeed, active peptides and peptidases inactivate proteins transiting across the barrier. Likewise, intracellular enzymes that inactivate neuroactive and neurotoxic substances are also present in brain endothelial cells [29]. Interestingly, in comparison to their systemic counterpart, the cerebral endothelial cells also possess a greater concentration of mitochondria to provide the energy needed for the maintenance of the barrier integrity as well as active transport [30].

Typically, a molecule weighing less than 180 Da and with sufficient liposolubility should be able to enter a normal BBB [31-33]. Lipophilicity is traditionally measured by the oil-water partition coefficient (optimal value of 1.6) [33-35]. In general, small and lipophilic molecules (O_2, CO_2, ethanol) diffuse freely. On the other hand, larger hydrophilic molecules need an active transcellular transport to gain access to the CNS. A molecule with a low solubility will require facilitated transport relying on ion channels, specific transporters, energy dependent pumps or receptor mediated endocytosis (*e.g.*, insulin, transferrin) for CNS transportation [35, 36]. Other factors impacting CNS delivery should also be considered such as the degree of ionization, plasma protein binding, local cerebral blood flow and affinity for dedicated carriers.

An extra layer of protection further limits CNS entry: efflux transporters (*e.g.*, P-glycoprotein, multidrug resistance protein and organic anion transporter). They are usually found on the luminal side of the brain capillary endothelial cell. Using an ATP-dependent process, these protein systems extrude a wide array of lipophilic compounds that have crossed the lipid bilayer of the endothelial cells [19, 37-39].

Blood-tumor Barrier

The BBB can be diseased in many pathological conditions such as trauma, multiple sclerosis, infections, Alzheimer's disease as well as brain tumors. In malignant primary brain tumors (mainly malignant gliomas), the vessels present in the tumor depict phenotypic ultrastructural alterations that distinguish them from the BBB. Indeed, these vessels show a thicker basal membrane, increased fenestrations, enlarged perivascular spaces, fewer perivascular glial end-feet, increased pinocytic vesicles, abnormal TJ morphology and distended capillary diameter [30]. As a consequence, these abnormal vessels thus present heterogenous leaky walls leading to an accumulation of interstitial fluid and an increased intratumoral interstitial pressure. This increased pressure can in turn further restrict the diffusion of a therapeutic agent into the tumor [40]. Interestingly, the BTB presents different expression profiles of efflux transporters compared to the BBB. The BTB's altered permeability returns to normal within a few millimeters of the tumor margin. It is unfortunately a dreadful hallmark of malignant gliomas: tumor cells freely permeate the brain parenchyma and are found further away from the edges of the tumor. These cells are thus typically protected behind a competent BBB [18, 41-46].

Further affecting delivery of therapeutics to tumor cells, a "sink effect" can be observed whereby administered agents preferentially accumulate in the leaky necrotic center of the tumor. Whereas the concentration at the periphery of the tumor is decreased due to a constant wash out by circulating cerebrospinal fluid (CSF) [13, 47].

Brain Compartments: not so simple, after all

Too often, the CNS is presented as a homogeneous single compartment. It is not unusual that the measured CSF concentration of a drug is considered similar to its parenchymal delivery. For example, temozolomide, an alkylating agent, was shown to have a CSF/plasma area under the curve (AUC) ratio of 20% [48]. This data is commonly cited to describe the propensity of TMZ to cross the BBB. Nevertheless, this oversimplification does not translate the complexity of the true pharmacokinetics of CNS therapeutic agents. Indeed, it must be kept in mind that it is the free concentration of drug in the brain interstitial fluid compartment that elicits its action rather than the total amount of drug. According to Reichel *et al.* [49], the concentration of brain interstitial fluid is regulated by a complex array of factors such as :

- plasma exposure (total clearance and distribution of the drug)
- plasma protein binding (unbound drug free to penetrate the brain)
- BBB transport rates (efflux transport, active uptake or passive diffusion)
- distribution to brain cellular and interstitial fluid compartments, and binding to receptors
- clearance from the CNS (brain metabolism and/or elimination *via* CSF)

The author also elegantly reviews and describes interesting concepts that could allow the design of compounds based on more coherent models of brain penetration and distribution.

Very few studies adequately account for all these factors. Too often, research results neglect the complexity of CNS delivery pharmacokinetics. This and many other challenges could explain why certain clinical studies fail despite promising pre-clinical results [50]. A more thorough approach to delivery in animal experimentation might indeed better predict the clinical outcome of experimental therapeutics.

BRAIN TUMOR DRUG DELIVERY

CNS delivery is increasingly acknowledged as a difficult and subtle topic in the literature. It needs to be addressed in an effort to promote better clinical outcome in patients with CNS disorders. This is essential in neuro-oncology. Many aim to develop new delivery strategies that allow better CNS distribution of therapeutic agents than the standard oral and intravenous (IV) routes with the hopes of achieving better survivals. We will review these surgical/physical strategies of delivery that are currently being studied.

Intra-arterial Delivery

Intra-arterial (IA) delivery implies a paradigm of arterial regional administration of chemotherapy in the vascular distribution of the tumor. *Via* a first pass effect, an increase in the local plasma peak concentration of the drug occurs. This will produce an improved AUC [13, 51-54]. Consequently, this translates in an increased local exposure of the target tissue to the therapeutic agent. It does so by a 3-5 fold factor [55-57]. The regional concentration of the delivered drug is obviously affected by the artery selected for infusion and the velocity of blood flow within that vessel. However, unfortunately, the IA distribution of the drug is nonuniform. Also, it can be increased in areas of normal brain supplied by the artery used for treatment. Complications related to this procedure are discussed later along with osmotic blood-brain barrier disruption.

Preclinical studies in animal models have explored delivery of agents by the IA route. In the early 1980's, Neuwelt *et al.* used a nude rat brain tumor model (human lung tumor xenograft) and showed an increased concentration of methotrexate (MTX) up to fourfold in the brain tumor with the use of this route. In this model, the increase in concentration in the peri-tumoral area reached 20-fold when compared to an IV infusion [58]. Similarly, Schuster *et al.* (4-hydroperoxycyclophosphamide, athymic rat with glioma xenograft) [59], and Charest *et al.* (platinum compounds, Fischer-F98 rat with cerebral glioma xenograft) [60] observed significant drug level increases with the use of IA delivery. These last authors demonstrated 18-fold and 91-fold increases of carboplatin and lipoxal^{TM} in tumor cell nucleus, respectively, when administered intra-arterially. This enhanced delivery was associated with increased MST of 31 days and 30.1 days with IA carboplatin and IA lipoxal^{TM}, respectively, compared to 23.2 days and 24.6 days with IV carboplatin and IV lipoxal^{TM}, respectively. Recently, using liquid chromatography with tandem mass spectrometry (LC-MS\MS), we measured a fourfold increase in temozolomide peak concentrations in brain tumor tissues of Fischer-F98 rats treated with IA temozolomide when compared to IV administration (Drapeau *et al.* unpublished data, manuscript in preparation).

Starting in the 1980s, multiple accounts of clinical studies using IA delivery of chemotherapy in the treatment of high grade gliomas and other brain tumor entities have been reported. A few interesting studies were executed in patients with newly diagnosed high grade gliomas. The majority of these studies took place prior to the acceptance of the Stupp regimen as a first-line standard of care. For example, Larner *et al.* reported results of a phase I/II study in 17 GBM and 8 anaplastic astrocytomas (AA) treated with a supraophthalmic infusion of fluorouracil (5-Fu, 200-600 mg every week for 4 cycles) combined with radiotherapy. They observed a MST of 60 weeks in GBM patients. Only four complications occured, with three of them during the first four treatments [61]. Others such as Kochii *et al.* and Imbesi *et al.* failed to show significant difference in survival in two randomized phase III trials of IA nimustine (80-100 mg/m^2) compared to IV nimustine [62, 63]. Madajewicz *et al.* observed a 48% response rate (4 complete responses [CR], 30 partial responses [PR]) in 71 high grade glioma patients treated with IA cisplatin (40 mg/m^2) and IA etoposide (20 mg/m^2) with minimal side effects (blurred vision 4.8% and focal seizures 6%) [64].

Other studies performed in the setting of progressive or recurrent malignant gliomas have also shown potential benefits. Newton *et al.* showed modest efficacy and minimal toxicity when using IA carboplatin (200 mg/m^2) combined to IV etoposide (100 mg/m^2) in 25 progressive or recurrent non-GBM gliomas. These authors reported a 20% objective response (1 CR, 3 PR and 1 minor response [MR]) and a median overall time to progression of 24.2 weeks, with 32 weeks for those in the response group (20% of all patients). Overall MST was 34.2 weeks [52]. In a recent prospective phase II trial, Fortin *et al.* treated patients at first (n = 39) or second relapse (n = 12) of GBM with an IA administration of carboplatin (400 mg/m^2) and melphalan (10 mg/m^2). They observed a median OS from initial diagnosis of 23 months and the MST from study entry was of 11 months. The progression free survival (PFS) after the IA treatments initiation was 4.2 months. Additionally, low toxicity was observed (8% grade II neutropenia, 12% grade II thrombocytopenia and 7% grade III thrombocytopenia). Thus, the authors considered this therapeutic strategy a great second line regimen in the treatment of recurrent malignant gliomas [65].

Despite divergent results, the use of IA delivery in the treatment of malignant brain tumors appears to be promising when used with the appropriate chemotherapeutic agents. Globally, recently published results of studies using this approach are summarized in Table **1**.

Osmotic Blood-brain Barrier Disruption

In order to improve on the delivery obtained with IA infusion, the idea of osmotic manipulations of the BBB has been explored. In 1972, Rapoport *et al.* first described the effect of an IA infusion of concentrated solutions on the BBB. They identified its potential application for the distribution of therapeutic agents through a transiently opened BBB [69]. It is believed that the IA infusion of a hyperosmolar agent causes a rapid diffusion of fluid out of the cells, shrinkage of the endothelial cells and widening of the TJ [18, 69-71]. Other mechanisms are probably also implicated such as the involvement of second messenger systems (intracellular calcium and nitric oxide) and cytoskeletal changes [72-74]. Of the many hypertonic solutions with potential to disrupt the BBB (*e.g.* arabinose, lactamide, saline, urea and radiographic contrast agents), mannitol is the typical choice in both preclinical and clinical studies because it is approved for administration to patients.

Table 1. Clinical studies using intra-arterial chemotherapy in high-grade gliomas.

Study	N	Tumor Types	Chemotherapy Regimen	Response	Toxicity
Fortin *et al.* 2014[a]	51	rGBM	IA carboplatin (400 mg/m^2) & IA melphalan (10 mg/m^2)	MOS from initial diagnosis 23 mo MS from study entry 11 mo PFS from start of treatment 4.2 mo	8% grade II neutropenia 12% grade II thrombocytopenia 7% grade III thrombocytopenia
Figueiredo *et al.* 2010[b]	16	rGBM	IA BCNU (250 mg/m^2)	MS from IA start 66.6 wks MOS 87.9 wks PR 6, SD 7	1 delayed visual loss 2 focal deficits (leuko-encephalopathy or tumor necrosis)
Imbesi *et al.* 2006[c]	33	nGBM	IA ACNU (80-90 mg/m^2, n = 17) *vs.* IV ACNU (n = 16)	Median TTP 6 mo (IA) *vs.* 4 mo (IV) MST 17 mo (IA) and 20 mo (IV)	1 delayed stroke
Newton *et al.* 2002[d]	25	9 AA, 7 grade II oligo, 4 mixed gliomas, 2 BSG, 2 PA, 1 ME (all recurrent)	IA carboplatin (200 mg/m2) + IV etoposide (100 mg/m2)	RR 20% (1 CR, 3 PR, 1 MR) SD 60% Overall median TTP 24.2 wks Median TTP in responders 32 wks MOS 34.2 wks	Grade III-IV leucopenia (12%) and thrombocytopenia (16%) <1% vascular complications (2 TIA, 1 tibial artery thrombosis)
Silvani *et al*. 2002[e]	30	nGBM	IA carboplatin + IA ACNU (n = 15) *vs.* IV cisplatin + IV BCNU (n = 15)	PR 3 (IA) *vs.* 5 (IV) SD 11 (IA) *vs.* 10 (IV) TTP 5.2 mo (IA) *vs.* 5.8 mo (IV) MS 18.3 mo (IA) *vs.* 18,6 mo (IV)	4 grade III-IV leucopenia 1 grade III thrombocytopenia 1 seizure 1 intracerebral hemorrhage
Ashby & Shapiro 2001[f]	25	11 GBM, 6 AA, 6 AOA, 2 AO (all recurrent)	IA cisplastin (60 mg/m^2) + oral etoposide (50 mg/m^2/day)	Overall RR 40% (PR 2, SD 6) Median TTP 18 wks (responders) MS 56.5 wks (responders), 11 wks (non-responders)	45% CNS toxicity 1 death (sepsis, renal failure) 4 DVT 7 grade III neutropenia 2 grade III thrombocytopenia 1 grade III anemia
Kochii *et al.* 2000[g]	84	nGBM	IA nimustine (80 mg/m^2) *vs.* IV nimustine	Median TTP 24 wks (IA) *vs.* 45 wks (IV) MS 59 wks (IA) *vs.* 56 wks (IV)	3 grade III-IV leucopenia 4 grade III-IV thrombocytopenia 1 granulocytopenic pneumonia (died) 1 hemolytic anemia and renal failure 1 grade IV liver dysfunction 1 temporary visual blurring
Madajewicz *et al.* 2000[h]	83	63 nGBM, 20 AA	IA cisplatin (60 mg/m^2) + IA etoposide (40 mg/m^2)	objective RR (4 CR, 30 PR) GBM MS 20 mo (IA pre-RT) *vs.* 7 mo (IA/RT) AA MS 45 mo (IA pre-RT) *vs.* 12 mo (IA/RT)	5 focal seizures 5 headache 4 blurred vision 6 groin hematomas 8 urinary retention 4 flushing of face

Abbreviations: N, number of patients included; rGBM, recurrent glioblastoma multiforme; nGBM, newly diagnosed glioblastoma; IA, intra-arterial infusion; IV, intravenous infusion; RT, radiotherapy; IA/RT, intra-arterial chemotherapy with concomitant radiotherapy; MOS, median overall survival; mo, months; wks, weeks; MS, median survival; PFS, progression free survival; TTP, time to progression; RR, response rate; CR – complete response; PR, partial response; MR, minor response; SD, stable disease; PD, progressive disease; AA, anaplastic astrocytoma; Oligo, oligodendroglioma; BSG, brainstem glioma; PA, pilocytic astrocytoma; ME, malignant ependymoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; CNS, central nervous system; DVT, deep vein thrombosis.
a - [65]; b – [66]; c – [63]; d – [52]; e – [67]; f – [68]; g – [62]; h – [64]

The combination of IA infusion of a drug with osmotic blood-brain barrier disruption (OBBBD) has been shown to increase the effect of first pass through the brain, increase maximal peak concentration as well as AUC of the administered drug [13, 52, 60]. Interestingly, Sato *et al.* showed *in vivo* that OBBBD produces a marked increase in permeability at the tumor edges. This area is typically associated with active tumor cells proliferation [43]. Theoretically, this concept is quite compelling, as it could help evade the "sink effect" [13, 47]. Indeed, by providing higher and more uniform delivery to the whole CNS, OBBBD allows a prolonged tumor exposure to higher concentrations of the administered drug. This includes the neoplastic cells at the tumor edges that are often the most proliferative and protected by an intact BBB and/or BTB [13, 43, 58, 75-77].

This increase in BBB permeabilization is transient in the ipsilateral hemisphere and lasts from 30 minutes to 2 hours [13]. Blanchette *et al.* elegantly studied OBBBD permeabilization with magnetic resonance imaging (MRI) in

real time, in the Fischer-F98 glioma model. These series of experiments demonstrated the important association of the molecular weight of a compound with its delivery [78-80]. More so, these authors found a relation between the length in the BBB opening window and the molecular weight of a compound. An acknowledged weakness in this approach is the high variability in the degree of OBBBD obtained from one treatment session to the other. Other factors are also known to influence the degree of OBBBD such as the chosen anesthetic agent, partial CO_2 pressure, osmolality of the infused agent as well as cardio-circulatory parameters [13, 81]. Interestingly, it was also found that antecedent cranial irradiation decreases the delivery of agents to the brain after an OBBBD [82].

Also, preclinical studies consistently show higher intratumoral concentrations of the infused therapeutic agent when OBBBD delivery is compared to IA infusions alone or to more traditional methods of administration (IV, intraperitoneal [IP]). Burkhardt *et al.* showed that IA bevacizumab combined to OBBBD led to significantly higher intratumoral concentrations at 24 hours after treatment in a tumor stem cell xenograft when compared to IA alone and to IP bevacizumab ($p < o.o5$) [83]. Studying the accumulation of different platinum compounds in the brain tumor and contralateral brain parenchyma of the Fischer-F98 glioma model, Charest *et al.* showed that IA infusions with OBBBD consistently increased delivery by a 2-5 and a 3.4 fold factor in each compartment, respectively [60]. More recently, we obtained similar results with temozolomide in the same animal model. We noted a 5-fold and 3-fold increase in brain tumor/plasma and brain tumor/contralateral brain AUC_{0-t} ratios, respectively, when comparing IA infusions with OBBBD to IV delivery (Drapeau *et al.* data not published, manuscript in preparation). Furthermore, our study is in agreement with previous literature demonstrating that the severity of neurotoxicity seen using this method varies greatly according to the chemotherapeutic agent delivered [13].

Questions raised in the clinical setting continue to guide the direction of preclinical research. Also, results of preclinical studies in animal models continue to find direct application in the treatment of patients. OBBBD is currently being used clinically to increase the delivery of chemotherapeutic agents for the treatment of brain tumors in humans.

Primary CNS lymphoma (PCNSL) is the first pathological entity that deserves to be addressed. As it is very chemosensitive, the results in treating this disease have been the most convincing. Angelov *et al.* reported the overall experience in the treatment of newly diagnosed PCNSL treated with OBBBD over 23 years in a consortium of four institutions. As a standard regimen, 149 patients were treated with OBBBD and IA MTX (5000 mg) with IV etoposide, IV cyclophosphamide, leucovorin rescue and granulocyte colony-stimulating factor. The overall response rate was 81.9% with 57.8% complete responses. The median OS was 3.1 years and the median PFS was 1.8 years with 31% 5-year PFS and 25% 7-year PFS. The regimen was well tolerated with 9.2% periprocedural focal seizures as the main side effect, and no long term sequelae [84]. In this series, patients of less than 60 years of age (n = 78) had a median OS of 5.2 years and a 5-year survival rate of 52%. Patients older than 60 years of age had less favorable outcomes with a median OS of 2.2 years and a 5-year survival rate of 30% ($P = 0.0019$). As can be appreciated from this data, age remains a powerful prognostic determinant. These authors also suggest that 13 patients may, indeed, have reached a "cure" as a plateau in OS was observed at 8.5 years in the group of patients less than 60 years old [84]. Moreover, when compared to trials using high dose IV MTX combined with radiation (RTOG 93-10 and EORTC 20962), the results reported by Angelov *et al.* were similar in terms of clinical outcome without the neurocognitive sequelae typically associated with radiation [85-87]. Thus, we suggest that avoiding or postponing cranial irradiation by using this alternative treatment strategy may enhance the quality of survival time.

Enhanced chemotherapy delivery with OBBBD has also been used in the treatment of malignant gliomas. In the pre-Stupp era, our group showed promising results using a carboplatin regimen (IA carboplatin 400 mg/m^2, IV etoposide 400 mg/m^2 and IV cyclophosphamide 330-660 mg/m^2) as a first line treatment in newly diagnosed GBM. We obtained a MST from diagnosis of 32.2 months for this subset of tumors [88].

In an attempt to understand the relation between survival outcome and the extent of increased barrier permeabilization brought by the OBBBD procedure, Kraemer *et al.* measured a delivery score in PCNSL patients. This score was based on post-procedural scans assessing the degree of barrier permeabilization and the number of treatment sessions. They found a correlation between the delivery intensity score and the long term survival in patients with PCNSL treated with this procedure and IA MTX [89]. These same authors treated 41 patients with high-grade gliomas (20 AA and 21 GBM) with IA carboplatin and etoposide, and a predisruption dose of IV cyclophosphamide. The regimen was combined to OBBBD in 28 patients and given alone to the other 13 patients. They showed that patients with an OBBBD procedure had significantly longer survival with a MST of 90 weeks *vs.* 50 weeks for patients with IA treatment alone ($p = 0.0113$) [90].

OBBBD is a complex invasive procedure requiring general anesthesia. Complications can be related to technical aspects of the procedure or to the BBBD itself. As it has been studied for many decades now, we can firmly conclude that it is a relatively safe procedure if performed in a standardized fashion in dedicated centers [13, 91]. The main complications related to the procedure include asymptomatic subintimal tear in 0.74 - 5%, groin hematoma in 0.5%, and parent vessel thrombosis in 0.5% of procedures. Those related to the OBBBD are seizures (when combined with the administration of MTX) in 2.7 - 13%, temporary obtundation and/or increase in neurological symptoms in 2.5% in the first 48 hours to 0.5% after 48 hours, and reversible motor deficits in 3.8%. Other possible complications reported are pulmonary embolism in 2.7%, renal toxicity in 1.8%, electrocardiogram changes in 4.3%, and headaches in 6% [91-93]. Overall, the technique appears safe with extremely low mortality rates and no short or long-term effects on

neurocognition [91, 94-96]. Potential for local neurotoxicity related to delivery of higher focal concentrations of drugs in the cerebral tissue remains. The administered drugs must thereby be carefully selected [97]. We must emphasize, however, that the OBBBD process *per se* is not toxic. The subsequently infused drugs are the culprits when faced with neurotoxicity. For example, such has been the case in studies held in the last decades with IA nitrosureas and cisplatin [98-100].

In short, many authors, most under the auspice of the international BBB consortium, led by E. Neuwelt, continue to push forward the use of this chemotherapy delivery technique. To date, they offer some of the best results in most clinical studies for the treatment of brain tumors. Table **2** summarizes some of the most recent clinical studies that used OBBBD combined with IA delivery of chemotherapeutic agents and measured impacts on survival or tumor response.

Intranasal Delivery

Intranasal (IN) delivery of therapeutic agents has been widely used in other disease processes such as postmenopausal osteoporosis (*e.g.* IN calcitonin) [105, 106]. There is a growing interest for its potential in brain tumor treatment. The IN delivery of agents to the CNS is thought to travel mainly *via* extracellular pathways (olfactory and trigeminal channels), thus bypassing the BBB [105, 107-111].

It shares many of the advantages of other direct locoregional methods of delivery. Indeed, it provides an easy, non invasive rapid and direct delivery to the CNS. It also avoids hepatic first pass drug metabolism [105, 112, 113]. The disadvantages of this method of delivery are mainly related to nasal anatomy and physiology. As such, this delivery approach can induce mucosal irritation. The delivery efficacy can be impacted by different factors such as the enzymatically active and low pH of the nasal epithelium as well as the mucociliary clearance of the administered agent [114].

Preclinical studies have demonstrated the utility of this method to administer drugs to the brain. For example, Jiang *et al.* showed that a herpes simplex virus-1 vector (QR9TO-LacZ) administered intranasally was found distributed to the olfactory bulb, hippocampus, striatum, cortex, medulla, cerebellum, ventricles and nasal septum of rats [115]. Hashizume *et al.* assessed the use of a telomerase inhibitor (GRN163) delivered intranasally. They demonstrated its rapid distribution in the brain and tumor of rats consistent with extraneuronal pathways of diffusion [112]. Shingaki *et al.* evaluated the effect of IN *vs.* IP delivered MTX in a rat tumor model. Overall, IN delivery showed higher concentrations of MTX in CSF and significant antitumor effect when compared to IP delivery [113].

The use of this delivery strategy has made its way into clinical trials with a phase I/II study by da Fonseca *et al.* These authors evaluated the effect of perillyl alcohol (POH), a Ras inhibitor, administered intranasally in 37 patients with recurrent malignant gliomas. The median 6-month PFS rate for GBM patients was 48.2%, 60% for AA patients and 66.6% for AO patients. The treatment was well tolerated for all patients with no signs of toxicity [116]. In 2011, these authors also showed interesting results on the efficacy of IN POH in 89 recurrent GBM *vs.* 52 matched untreated recurrent GBM (historical control group). Primary recurrent GBM patients treated with IN POH (93% of the INH POH treatment group) had a significant benefit in survival (mean 5.9 months) compared to the control group (mean OS 2.3 months [$p < 0.0001$]). The 6 secondary recurrent GBM patients also demonstrated encouraging survival advantage with a mean OS of 11.2 months after IN POH treatment [117].

Convection-enhanced Delivery and Osmotic Pumps

A very interesting mode of delivery developed in the early 1990s by Edward Oldfield *et al.*, convection enhanced delivery (CED), has already made its way into clinical studies [118]. It involves the local delivery of a solute containing a therapeutic agent through a continuous low rate perfusion under positive pressure. It uses a pump connected to one or multiple catheters targeted into the tumor or into the interstitial space of the brain around the resection cavity of such a lesion.

Many researchers have undertaken the task of elucidating multiple factors influencing the volume of distribution of drugs with this technique. The key factors identified are the catheter shape, size and placement, rate and volume of infusion, physical properties of the drug (*e.g.* molecular weight, size, lipophilicity, charge, viscosity) and structural properties of the target tissue (interstitial fluid pressure within and around the tumor, tissue anisotropy) [119-125].

The increasing enthusiasm surrounding the use of CED ensues from its ability to bypass the BBB and to allow a more sustained delivery of drugs of variable molecular weights. Nonetheless, this relatively young procedure remains limited by different technical factors such as the difficulty to achieve proper catheter placement and the inherent faults of the catheters (*e.g.* leakage, backflow). It is also restrained by complications related to the invasiveness of the procedure. More importantly, CED in brain tumor treatment battles with unpredictable drug distribution (*i.e.* heterogeneity of tumor or brain tissue, leakage into non-targeted brain regions, leakage of drug in wound tract or under scalp). This could lead to unwanted local neurotoxicity [120].

In fact, Shahar *et al.* showed that it was particularly difficult to adhere to a standard set of catheter placement guidelines when certain conditions were present. These were superficial or mesial temporal located lesions, proximity to CSF spaces and proximity to eloquent cortical areas. Also, these authors noted that the tissue density might interfere with the trajectory of the catheter. Other technical limitations observed were related to the use of stereotactic instruments (*e.g.* steep insertion angles) and to the placement of catheters through artificial dural implants. When reviewing complications related to the placement of 64 CED catheters in 25 patients, Shahar *et al.* noted increased edema in 31%, infection in 6.9%, bleeding in 6.9%, seizures in 13.8%, and significant neurologic deterioration in 13.8% [126].

In light of these difficulties, many have tried to establish key factors permitting an optimal CED delivery. Criteria for

Table 2. Clinical studies using intra-arterial chemotherapy with osmotic blood-brain barrier disruption in malignant brain tumors.

Study	N	Tumor Types	Chemotherapy Regimen	Response	Toxicity
Burkhardt *et al.* 2012[a]	14	rGBM	IA bevacizumab (2 – 15 mg/kg) followed by IV bevacizumab (in 12/14 patients)	Median PFS 10 mo MOS 8.8 mo	1 wound dehiscence 1 rash
Guillaume *et al.* 2010[b]	13	11 AO, 2 AOA	IA carboplatin (200 mg/m^2/dose) + IA melphalan (dose escalation) + IV etoposide (200 mg/m^2)	2 CR, 3 PR, 5 SD Median PFS 11 mo	1 asymptomatic subintimal tear 3 grade IV thrombocytopenia
Angelov *et al.* 2009[c]	149	nPCNSL	IA methotrexate (5000 mg) + IV etoposide (150 mg/m^2) + IV cyclophosphamide (15 mg/kg or 500 mg/m^2)	Overall RR 81.9% (57.8% CR, 24.2% PR) MOS 3.1 years Median PFS 1.8 years 5-year PFS 31% 7-year PFS 25%	9.2% focal seizures 7.4% stroke (4 permanent neurologic deficits; 0.2% per IA/OBBBD procedure) 3.6% red blood cell transfusion 2.8% granulocytopenic fever 2.6% DVT/PE
Fortin *et al.* 2007[d]	38	Metastasis (5 ovary, 18 lung, 4 breast, 8 lymphoma, 3 others)	Lymphoma : IA methotrexate (5000 mg) + IV etoposide (150 mg/m^2) + IV cyclophosphamide (500 mg/m^2) Other tumors: IA carboplatin (400 mg/m^2) + IV etoposide (400 mg/m^2) + IV cyclophosphamide (330-660 mg/m^2)	MOS from diagnosis 29.6 mo MOS from study entry 13.5 mo Mean TTP 127 days MS ovarian 42.3 mo MS lung 13.5 mo MS breast 8.1 mo MS lymphoma 16.3 mo	1 grade III anemia 1 Grade IV thrombocytopenia 3 grade III-IV neutropenia 1 severe neck pain 2 post infusion orbital pseudotumor syndrome
Hall *et al.* 2006[e]	8	DPG	IA methotrexate (5000 mg) + IV etoposide (400 mg/m^2) + IV cyclophosphamide (1000 mg/m^2) Or IA carboplatin (400 mg/m^2) + IV etoposide (400 mg/m^2) + IV cyclophosphamide (660 mg/m^2)	TTP 15 mo MS from diagnosis 27 mo MS from first IA treatment 16.5 mo	1 febrile neutropenia and pneumonia 1 multiple UTI 1 severe thrombocytopenia 1 neck pain, confusion and ataxia 2 hearing loss
Fortin *et al.* 2005[f]	72	Malignant glioma PNET Primary CNS lymphoma Metastatic disease	PCNSL : IA methotrexate (5000 mg) + IV etoposide (150 mg/m^2) + IV cyclophosphamide (500 mg/m^2) Other tumors: IA carboplatin (400 mg/m^2) + IV etoposide (400 mg/m^2) + IV cyclophosphamide (330-660 mg/m^2)	MOS from treatment start: GBM 9.1 mo, AO 13.9 mo, meta 9.9 mo, PCNSL not reached at time of publication TTP: GBM 4.1 mo, AO 9.2 mo, meta 3.3 mo, PCNSL 12.3 mo MST from diagnosis for GBM 32.2 mo	4 grade III-IV thrombocytopenia 2 grade III-IV neutropenia 1 neutropenic fever 5% seizures 2 postinfusion orbital myositis 2 carotid thrombosis (1 post-vasospasm, 1 ipsilateral monocular visual loss, 1 transient hemiparesis)
Kraemer *et al.* 2001[g]	74	PCNSL	Protocol I: IA methotrexate (2.5 g) + IV cyclophosphamide (15 mg/kg) x 2 days + oral procarbazine Protocol II: IA methotrexate (2.5 g) + IV or IA etoposide (150 mg/m^2/dose) + IV cyclophosphamide (500 mg/m^2/dose) x 2 days	Survival associated with the total intensity of OBBBD (number IA infusions or cumulative degree of OBBBD score)	N/A
Doolittle *et al.* 2000[h]	221	56 PCNSL, 18 PNET, 13 meta, 4 GCT, 3 BSG, 31 astrocytoma (WHO II, III), 73 GBM, 12 AO, 4 oligo, 7 others	PCNSL and BSG: IA methotrexate (5000 mg) + IV cyclophosphamide (1000 mg/m^2) + IV etoposide (300 mg/m2) Other tumors: IA carboplatin (400 mg/m^2) + IV cyclophosphamide (660 mg/m^2) + IV etopoisde (400 mg/m^2)	PCNSL: 75% CR All PNET, meta or GCT: SD or better GBM: 79% SD or better	5% asymptomatic subintimal tear 2.7% pulmonary embolism 18.2% DVT 1.7% renal toxicity 2.5% obtundation over 48 hrs 1.7% stroke

Abbreviations: N, number of patients included; rGBM, recurrent glioblastoma multiforme; nGBM, newly diagnosed glioblastoma; PNET, primitive neuroectodermal tumor; GCT, germ cell tumor; nPCNSL, newly diagnosed primary central nervous system lymphoma; IA, intra-arterial infusion; IV, intravenous infusion; OBBBD, osmotic blood-brain barrier disruption; RT, radiotherapy; IA/RT, intra-arterial chemotherapy with concomitant radiotherapy; MOS, median overall survival; mo, months; wks, weeks; MOS, median overall survival; MS, median survival; mo, months; wks, weeks; PFS, progression free survival; TTP, time to progression; RR, response rate; CR – complete response; PR, partial response; MR, minor response; SD, stable disease; PD, progressive disease; AA, anaplastic astrocytoma; Oligo, oligodendroglioma; BSG, brainstem glioma; PA, pilocytic astrocytoma; ME, malignant ependymoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; meta, metastasis; CNS, central nervous system; DVT, deep vein thrombosis; PE, pulmonary embolism; N/A, not available.

a – [101]; b – [102]; c – [84]; d – [103]; e – [104]; f – [88]; g – [89]; h – [91]

catheter placement have been established by Sampson *et al.* They are currently the main source of comparison available for clinical studies [127]. Moreover, the catheter design is continuously reviewed and most tend to agree that it should be made of fused silica tubing with a step design tip, or even a recessed-step tip. It should be primed to prevent air bubbles and should be fixed to the skull entry point (*e.g.* with bone wax) to avoid backflow [119, 128-131]. To further prevent backflow or leakage, most concur that the catheter should have an outer diameter smaller than 1 mm [119]. Finally, the optimal infusion rate in rat models has been within a range of 0.1 – 0.5 µL/min to avoid potential backflow and brain tissue damage [119]. However, recent data with the use of multiple catheters suggests that further increasing the flow (0.02-0.03 mL/hour/catheter) is possible and could yield interesting results in early clinical studies [130, 132-134]. Finally, according to most investigators, CED should be done in the peritumoral cerebral parenchyma after surgical tumor removal [120, 135].

An increasing number of preclinical studies have been designed to further explore the reach of this delivery strategy using all kinds of therapeutics. Oh *et al.* used CED of a bispecific ligand-directed toxin (DTEGF3) to treat U87 glioma rats and showed a 50% tumor eradication in this model [136]. Goldberg *et al.* used CED of Salirasib (farsnesyl thiosalicylic acid) in a 9L gliosarcoma rat model. This highly specific Ras inhibitor with suppressive effects on growth and migration of proliferating tumor cells seemed to be an efficient and non toxic treatment [137]. Allard *et al.* produced a significant increase in median survival in a 9L tumor rat model using CED of lipid nanocapsules encapsulating complexes of ^{188}Re [138]. Saito *et al.* used CED of nanoparticle liposomes containing Topotecan in orthotopic U87MG or U251MG xenografts models. By doing so, they inhibited growth or completely eradicated the tumor, thus prolonging median survival [139]. Inoue *et al.* showed a therapeutic advantage and prolonged survival in a 9L brain tumor rat model using CED of doxorubicin polymeric micell [140]. Noble *et al.* used CED of camptothecin derivative and topoisomerase I inhibitor (CPT-11) encapsulated in nanoliposome to an intracranial xenograft U87 glioma rat model. They showed a benefit with increased distribution and a longer tissue residence time [141]. Nguyen *et al.* showed a more homogeneous distribution of transduction when delivering adeno-associated vectors (AAV) into normal rat brains with CED [142]. White *et al.* demonstrated wide distribution and retention of carboplatin in the rat brain at 24 hours with CED. They also used a gadolinium-DTPA co-infusion to visualize the distribution of the drug by MRI [143]. Recently, Yang *et al.* combined radiotherapy with CED of carboplatin in F98 glioma bearing rats and showed increased MST of 83.6 days with CED (20 µg, 0.30 µl/min over 30 minutes) *vs.* 35.3 days for radiotherapy only (24.6 days for controls without treatment) [144]. On the other hand, Huo *et al.* showed that the delivery of Lipoplatin™ was very toxic when administered by CED in a F98 glioma rat model [145]. More recent work by Sonabend *et al.* showed survival advantage in mouse proneural glioma models treated with CED of etoposide [146]. In addition, many efforts are focused on developing real time imaging techniques to study the distribution of drugs with CED. Examples of such developments include co-infusion of contrast agents with MRI tracking, visualization of T2-WI MRI changes and the use of radiolabeled agents with SPECT/CT imaging [147-152].

Many preclinical studies have utilized osmotic minipumps (Alzet® osmotic pumps [Cupertino, CA, USA]) to achieve local delivery of therapeutic agents. The apparatus relies on an osmotic pressure difference between local tissue environment and the pump. This causes a shift of water into the pump, driving out the solution contained in the pump's reservoir. The predetermined permeability of the implantable pump allows continuous infusion of the solution at a controlled rate for multiple days. These subcutaneously implanted pumps can be used to infuse into the cerebral ventricles or directly into brain tissue. A study by Giussani *et al.* using osmotic minipumps for local administration of endogenous inhibitors (human hemopexin fragment of matrix metallaproteinase-2 or COOH-terminal fragment of platelet factor-4) in cerebral glioma bearing mice showed that this system was more effective than systemic delivery. It was also able to sustain a long-term control of tumor growth in the absence of toxicity or side effects [153]. Yang *et al.* achieved greater survival in F98 glioma bearing rats treated with Alzet® pump delivery of carboplatin (84 µg in 168 µl at 1 µl/h over 7 days) combined with radiotherapy (MST 107.7 days) than with CED (MST 83.6 days) or to radiotherapy alone (MST 35.3 days) [144]. An interesting study by Mairs *et al.* compared the extent of cellular uptake of 5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]IUdR) following a single injection, slow release PLGA polymer or osmotic pump delivery (C6 glioma rat model). They clearly demonstrated a significant increase in distrubtion of [^{125}I]IUdR with this method (mean labeling index 34.3% *vs.* 22.5% polymer implant *vs.* 6.2% single injection) [154].

The first phase III randomized clinical trial (PRECISE trial) compared the use of Gliadel® wafers *vs.* CED of cintredekin besudotox (IL13PE38QQR) in 296 patients with a first recurrence of GBM [135]. This trial did not show significant difference in the outcome between both groups, with MST of 35.5 weeks in the Gliadel® group *vs.* 36.4 weeks in the CED group ($p = 0.476$). The adverse event profile was similar between the groups with the exception of a higher occurrence of pulmonary embolism in the CED group (8% *vs.* 1%, $p = 0.014$). This was most likely due to a prolonged hospital stay for the CED infusion. However, the trial's methodology has been greatly criticized because of lack of complete adherence to the inclusion criteria, a steep learning curve in regards to catheter placement and the use of iPlan software in only a portion of patients. Nonetheless, Mueller *et al.* showed that the poor response in this trial could not be solely attributed to catheter positioning. They did not find a relation between catheter positioning and local tumor control, imaging change scores, OS and PFS data of the PRECISE trial [155].

Bruce *et al.* published a prospective phase Ib open-label non-randomized dose-escalation study of CED of Topotecan in the treatment of 16 patients with recurrent malignant gliomas (10 GBM and 6 WHO grade III gliomas) [156]. They observed an early response in 4 patients, progressive

disease in 5 patients and pseudoprogression in 7 patients with survival ranging from 13 weeks to more than 310 weeks. The median OS was 60 weeks and the OS at 6 months was 75%. The PFS ranged from 4 to 132 weeks with a median of 23 weeks. The PFS at 6 months for the GBM subgroup was 55%. Interestingly, they also showed that there was a relatively uniform delivery of the drug within the targeted tissue, a steep drop in the drug concentration outside of it and undetectable serum drug levels.

Furthermore, a growing number of case reports of CED used for the treatment of brainstem glioma in the pediatric population have been published [133, 157]. However, these studies are of short follow up and have yet to determine the safe volume and concentration of drugs to be used in these patients.

Implanted Polymers

Another known method of direct delivery to the CNS, implantable systems, provides a continuous drug delivery with a controlled sustained release of the drug. Many types of implants are being studied: wafers, gels, micro- and nanocarriers, and microchips [111, 158].

The interstitial pressure gradients and the drug concentration gradients favor the distribution of the agent enclosed in the implanted polymer from the center of the tumor to its periphery [158]. The controlled release of a therapeutic agent permits a slower elimination and an increased time of exposure to the agent. However, the major drawback of this approach is that local penetration of the agent is limited by diffusion into the local tumor environment [41, 159, 160].

These implantable devices are divided into two categories: degradable and nondegradable. Occasionally, they are combined into a copolymer that will modulate the degradation and release characteristics of the implant. Nondegradable polymers release the drug by diffusion of the agent through the polymer matrix. Their use is limited by the fact that the system needs to eventually be removed, thereby requiring an additional procedure [161]. The most common type is ethylene vinyl acetate (EVAc) and has been used to release DNA, antibodies and chemotherapeutic agents. Two studies looked at delivery of amsacrine and mitoxantrone in a rat glioma model and showed strong antitumor effect [162, 163]. Degradable polymer drug release depends on diffusion through the polymer and on erosion of the polymer. The most commonly used polymer is composed of polybis(p-carboxyphenoxy)propane-sebacic acid (p[CPP-SA]) [159]. Some formulations have included fatty acid dimer copolymers (FAD-SA) that can be made into a disk shape. These have been used for delivery of 4-hydroperoxycyclophosphamide (4-HC unstable with p[CPP-SA]) [164]. Others are composed of polylactic acid, polyglycolic acid or polylactic-co-glycolic acid (PLA, PGA or PLGA, respectively). These last formulations are biocompatible and hydrophobic. They have been used with carmustine (BCNU) and seem to have similar profiles as Gliadel® [165]. They can be made into nanoparticles or microparticles by single emulsion methods (encapsulate a hydrophobic compound) or by double emulsion methods (encapsulate a hydrophilic compound). They can also take other forms such as electrospun scaffolds (electrospinning uses a voltage differential to produce non-woven mesh that can conform to the shape of the resection cavity), thin films and wafers [154, 165-169].

Albeit being compelling devices, they are not without possible consequences such as infections (abscess, meningitis), impaired wound healing, CSF leak and tumor cyst accumulation [170].

These implants have been used to deliver a very large array of agents including chemotherapeutic agents such as mitoxantone, BCNU, 4-HC, paclitaxel, carboplatin and adriamycin. Many preclinical studies show the great variability in delivery profile between the different existing implants and the different drugs used. For example, PLGA-TMZ microparticles showed a biphasic release profile with an initial burst release followed by a linear release of the drug lasting up to 35 days [171]. PLGA-paclitaxel microparticles showed no such burst and a slower gradual release lasting 60 days due to the hydrophobic nature of the drug [172].

Gliadel® wafers are probably the most recognized and clinically used implantable device in clinical practice. These are p(CPP-SA) wafers combined with carmustine (7.7 mg), 14 mm diameter and 1 mm thick. Their use has shown modest survival benefits without significant increased toxicity. Carmustine is released over a period of 5 days and the implant is completely degraded over 6-8 weeks. Brem *et al.* showed that Gliadel® wafers were safe and well tolerated [173]. They also demonstrated that its combination to radiotherapy was safe and effective [174]. This was later confirmed in phase III trials leading to FDA approval in 1996 for the treatment of recurrent gliomas and in 2003 for the treatment of newly diagnosed gliomas [175, 176]. Further developments in a phase I trial and retrospective reviews demonstrate a safe and well-tolerated combination of Gliadel® with temozolomide in recurrent high grade gliomas [177-179].

Magnetic Microspheres

Magnetic microspheres (MM) are being studied as carriers with the potential to accumulate in a targeted tissue when delivered by selective methods of infusion. The magnetic agent is encapsulated within a polymeric matrix. It then targets the tumor site by being retained in the tumor capillaries with the application of an external magnetic field (MF). The advantage is not only related to its ability to selectively localize at the tumor site, but also to limit the systemic distribution of the agent [41, 180, 181]. Hassan and Gallo, and Devineni *et al.* were the first to show that magnetic microspheres could be used to selectively target brain and brain tumors. The main disadvantage is related to the need of an external MF [182-184].

In 1995, Devineni *et al.* created a MM containing MTX. They compared the IA infusion of MM-MTX (3 mg/kg) *vs*. MTX alone with the application of 6000 Gauss MF for 15 minutes. They showed that the concentration of MM-MTX was 3.5-5 fold greater than MTX-solution. They observed a tumoral distribution of MM-MTX suggestive of an extravascular uptake. This was in contrast to a capillary distribution in normal brain tissue. The hydrolysis of the

drug from the MM once in the targeted vessel creates a drug concentration gradient favoring distribution into the brain. This is favored by the formation of MM aggregates under the effect of the MF. These aggregates become trapped in the brain capillaries and are unable to advance in the venous circulation once the MF is removed. Other factors influenced the higher MTX concentrations such as endocytosis across the BBB or BTB (*in vitro* findings) as well as passive movement across the BTB or across endothelial gaps created by the MF [184].

The effect of the ionic charge of the delivered MM has proved to be important. Pulfer *et al.* studied the difference in delivery between magnetic aminodextran microspheres (MADM) and neutral magnetic dextran microspheres (MDM) infused intra-arterially (25 mg/kg) in RG2 tumor rats. MADM showed a higher concentration inside the tumor and were retained longer than MDM. The MADM concentration decreased by 4% after 6 hours *vs.* 32% for neutral MDM. This suggested a cationic:anionic interaction promoting tissue retention in the former. In general, the MF increased the concentration of MM in the targeted brain tumor tissue. They also observed a differential accumulation between both particles: MADM appeared mainly in the interstitial space while MDM seemed trapped in the vasculature, thus implying that endocytosis by the BBB had a minimal role [179].

In another series of experiments, this research team explored the use of uncharged small magnetic particles (SMP) infused intra-arterially (4mg/kg) with a MF of 0 or 6000 Gauss for 30 minutes. The results showed that SMP localized in tumor tissue. The MF appeared to increase the SMP tumor selectivity over non-target tissue as well as to increase particle retention over a greater length of time. Indeed, it increased the percentage of the dose per gram of tissue from 31 to 41% at 30 minutes, and from 23 to 48% at 6 hours. The influence of the particle size was also demonstrated by higher levels of SMP in the brain than MDM and MADM [180, 185].

As fascinating as this concept appears, the use of magnetic particles as a means to deliver therapeutic agents in the treatment of cerebral tumors remains mainly at a preclinical stage. There is much work to be done in creating a reproducible, stable, efficient and non-toxic drug-loaded MM. Furthermore, better characterization of the optimal external MF parameters is needed to ensure safe clinical use.

Ultrasound Blood-brain Barrier Disruption

There is interest in using the ultrasound (US) as a mean to improve molecular transport across the BBB. The US seems to produce a temporary increase in cell membrane permeability. Some of the advantages of focused ultrasound (FUS) delivery consist of providing a non-invasive, focal and targeted delivery of therapeutic agents through a transient and reversible BBB disruption.

To help disrupt the BBB, the US has been associated with the use of microbubbles (MB). MB are made of gas (air or perfluorocarbon) covered by a shell of albumin or lipids. Their usual diameter of about 1-10 μm allows them to pass through capillary networks of the tissue exposed to the US. US microbubble contrast agents, such as Optison™ (FDA approved, GE Healthcare), have been used to help induce a reproducible BBBD [186-188].

Aryal *et al.* recently reviewed the multiple potential mechanisms involved in the US-induced BBBD [188]. Many of the effects of FUS result from the interactions with the MB. The US wave causes MB to expand and contract in capillaries. This stretches the vessel wall and causes a mechanical opening of the TJ [189, 190]. More so, these MB oscillations can induce fluid microstreaming leading to indirect shear forces on the vascular endothelium [191]. However, when the MB oscillations increase above a certain threshold, the bubbles collapse due to the inertia of the surrounding fluid and shock waves result from this collapse. If these fluid jets are created too close to the wall, they have the potential to cause vascular wall damage [192, 193]. Interestingly, multiphoton microscopic studies showed that vasoconstriction accompanied the BBBD during sonication. Thus, this points to the possibility that changes in cerebral blood flow could influence FUS-BBBD [194, 195]. These interactions could ultimately lead to biochemical reactions implicated in the opening of the BBB. For example, Sheikov *et al.* studied the effect of FUS on the expression of TJ specific transmembrane proteins (occludin, claudin-1, claudin-5 and submembranous ZO-1) using immuno-electron microscopy. They confirmed the BBBD by observing leakage of molecules with different molecular weight (horseradish peroxidase at 40 000 Da and lanthanum chloride at 139 Da). These authors also observed that FUS caused a change in the immuno-electron microscopic expression of all 4 TJ examined. More specifically, it caused a transient disintegration of TJ complexes. These changes paralleled the transient BBB breakdown and paracellular leakage of the tracers into the brain parenchyma. All of these changes appeared reversible and lasted up to 4 hours after sonication [190]. Other phenomena observed include increased transcellular transport (*i.e.* increased cytoplasmic vesicles and fenestrae) [189, 196], involvement of intracellular signaling cascades (*e.g.* Akt pathway [197]) and of intracellular calcium changes [198, 199].

The following US parameters are commonly used and have been shown to trigger a BBBD without inducing vascular damage that might in turn result in ischemic or apoptotic death to neurons [187, 200-203]:

- Frequency of 1.63 MHz
- Burst length at 10-100 msec
- Pressure amplitudes < 1 MPa
- Durations of 20-30 seconds
- Pulse repetition at a frequency of 1 Hz

However, the optimal parameters still remain to be determined. Pressure amplitudes higher than 2.3 MPa have been shown to cause brain necrosis in about 70-80% of sonicated regions [204]. In a study by Liu *et al.*, there was no apparent intracerebral hemorrhage or brain surface hemorrhage when the pressure was 0.62 MPa or less [205]. They observed that most cases of intracerebral hemorrhage occurred at pressures exceeding 0.98 MPa. Post-mortem

Table 3. Focused-ultrasound blood-brain barrier disruption in preclinical studies.

Study	Model	Therapeutic Agent	Findings
Wei *et al.* 2013[a]	9L glioma (rat)	Temozolomide (TMZ)	3.8/2.1-fold increased accumulation of dye in normal/tumor tissues MS 23 days (FUS) *vs.* 20 days (TMZ only)
Aryal *et al.* 2013[b]	9L glioma (rat)	Liposomal doxorubicin (DOX)	MS 35 days (FUS+DOX) *vs.* 20.3 days (DOX only) *vs.* 18 days (control and FUS only)
Ting *et al.* 2012[c]	C6 glioma (rat)	BCNU-loaded microbubbles	Prolonged BCNU circulatory half-life *in vivo* (5-fold) MS 32.5 days (FUS+BCNU-MB) vs. 29 days (control)
Treat *et al.* 2012[d]	9L glioma (rat)	Liposomal DOX	MS 31 days (FUS+DOX) *vs.* 29 days (DOX only) *vs.* 25 days (control and FUS only)
Liu *et al.* 2010[e]	C6 glioma (rat)	BCNU	FUS-BBBD increased BCNU in brain tumor tissue (by 202%) MS 53 days (FUS+BCNU) *vs.* 32 days (BCNU only)

Abbreviations: TMZ, temozolomide; DOX, doxorubicin; BCNU, carmustine; FUS, focused ultra-sound; MS, median survival; MB, microbubbles.
a – [212]; b – [213]; c – [214]; d – [215]; e – [205]

animal light-microscopy studies showed that the number of extravasated red blood cells diminished with decreasing US frequency and increased with rising pressure amplitudes. The tissue absorbed the red blood cells over a period averaging four weeks without any detectable adverse reaction (no apoptosis or ischemia) [203].

Studies of the duration and extent of BBB opening after FUS have shown variable data. Using light and electron microscopy, Mesiwala *et al.* showed an opening of the BBB lasting up to 72 hours after sonication with high-intensity FUS [206]. In contrast, Hynynen *et al.* showed that the BBBD decreased after 6 hours using a MRI contrast model and US at 0.2-11.5 W, burst length of 10-100 ms and frequency at 1 Hz [207]. McDannold *et al.* also used MRI contrast to evaluate the BBBD after FUS. They showed that the process seems to be self-healing with decreasing contrast enhancement already at 6 hours after sonication. It remained without obvious delayed effects up to 4 weeks, as observed by MRI and histology [203].

One of the main limitations to the current use of this technique is the skull, which produces many distortions in US signals [208]. Recent efforts are geared toward studying devices that could correct for these drawbacks. For example, McDannold *et al.* showed that FUS-BBBD with MB was possible at deep and superficial targets through Rhesus Macaque skulls using the ExAblate 4000 low-frequency TcMRgFUS system (InSightec) coupled to a 3T MRI [209]. Beccaria *et al.* used an ultrasound device that is applied in the epidural space after trepanation to bypass this hurdle. Using this very same device, this group is now moving ahead in a phase I clinical study, combining this delivery approach to the administration of IV carboplatin [210, 211].

A growing number of preclinical studies can be found on the use of FUS-BBBD with the delivery of therapeutic agents in primary or metastatic brain tumor animal models showing potential benefits (Table **3**). However, despite being on the brink of clinical use, this strategy remains by and large at the preclinical investigational stage as many refinements are still required in regards to treatment planning, accurate targeted delivery and technique adjustment.

CONCLUSION

The blood-brain barrier is an incredibly complex entity and is of the utmost importance in neuro-oncology. It is imperative to consider its physiological and neuro-pharmacokinetic implications when discussing brain tumor treatments. Malignant astrocytomas, the most aggressive primary brain tumor in adults, remain a disease with no cure in sight. Different factors are at cause in this dreadful observation such as intrinsic tumor cell aggressiveness, invasiveness, heterogeneity as well as the presence of the BTB and BBB. Nonetheless, efforts toward developing alternative treatment strategies to bypass the BBB should continue unabated. This aspect remains at the forefront of considerations in the fight against brain tumors. As this review shows, the neuro-oncology community is prospering in this field, searching for better and more efficient CNS drug delivery systems. However, too often, devices or concepts developed toward that aim remain trapped at the preclinical level, never reaching the realms of human clinical experimentation. Furthermore, it is beyond doubt that these efforts call for further advancement in the development of therapeutic agents. The successful use of alternative strategies greatly depends on the efficacy and tolerability of the drug delivered. Thus, the overall goal must remain to find ways to better impact patient outcomes and survival.

Of course, the difficulties of showing clinical benefit of these delivery systems remains in the burdensome creation of randomized studies and recruitment of brain tumor patients. These are possible with the joint effort of many by building multi-center trials such as the international BBB consortium, led by the Oregon Health Sciences University [91]. This international multi-center group continues to show the advantages of using invasive alternative means to deliver chemotherapeutic drugs to treat patients with brain tumors (osmotic BBBD) and serve as a great example of collaboration in this field. Other consortia should see the day of light, born out of central ideas and common interests focusing on CNS delivery strategies. By encouraging such collaboration in the future, greater progress in preclinical and

clinical research will lead to tremendous findings and clinical impact.

CONFLICT OF INTEREST

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