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Delivery of Chemotherapeutics Across the Blood–Brain Barrier: Challenges and Advances

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Abstract

The blood–brain barrier (BBB) limits drug delivery to brain tumors. We utilize intraarterial infusion of hyperosmotic mannitol to reversibly open the BBB by shrinking endothelial cells and opening tight junctions between the cells. This approach transiently increases the delivery of chemotherapy, antibodies, and nanoparticles to brain. Our preclinical studies have optimized the BBB disruption (BBBD) technique and clinical studies have shown its safety and efficacy. The delivery of methotrexate-based chemotherapy in conjunction with BBBD provides excellent outcomes in primary central nervous system lymphoma (PCNSL) including stable or improved cognitive function in survivors a median of 12 years (range 2–26 years) after diagnosis. The addition of rituximab to chemotherapy with BBBD for PCNSL can be safely accomplished with excellent overall survival. Our translational studies of thiol agents to protect against platinum-induced toxicities led to the development of a two-compartment model in brain tumor patients. We showed that delayed high-dose sodium thiosulfate protects against carboplatin-induced hearing loss, providing the framework for large cooperative group trials of hearing chemoprotection. Neuroimaging studies have identified that ferumoxytol, an iron oxide nanoparticle blood pool agent, appears to be a superior contrast agent to accurately assess therapy-induced changes in brain tumor vasculature, in brain tumor response to therapy, and in differentiating central nervous system lesions with inflammatory components. This chapter reviews the breakthroughs, challenges, and future directions for BBBD.

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1. Introduction

The goal of blood–brain barrier disruption (BBBD) is maximizing the delivery of chemotherapy and antibodies to the brain while preserving neurocognitive function and quality of life and minimizing systemic toxicity. Taken together, preclinical and clinical blood–brain barrier (BBB) studies at Oregon Health & Science University (OHSU) evaluate (1) the toxicity of chemotherapeutics and chemoprotectants, (2) the potential for chemotherapy dose intensification in combination with chemoprotectants, and (3) the antitumor efficacy of chemotherapeutics in combination with chemoprotectants and/or monoclonal antibodies, in primary and metastatic brain tumors.

The BBB, consisting of tight junctions between endothelial cells of the cerebral vasculature, limits the access of blood-borne agents to the brain and brain tumor. The surrounding microenvironment plays a critical role in promoting the unique features of the BBB. The microenvironment (i.e., the neurovascular unit) consists of horizontal elements such as the endothelium, basal lamina, and astrocyte end-feet as well as vertical elements such as intervening astrocytes, neurons and their axons, and pericytes (Muldoon et al., 2013; Neuwelt et al., 2011). The barrier in malignant central nervous system (CNS) tumors can have extremely variable permeability, ranging from nearly the low permeability of normal BBB to nearly the high permeability of systemic vasculature. Low and inconsistent blood–tumor barrier (BTB) permeability is an effective impediment to drug entry. Blood–Brain Barrier Program preclinical and clinical research studies have focused on characterizing the role of the BBB in imaging, diagnosis, and therapy of brain tumors.

2. Blood-Brain Barrier Disruption

2.1. Delivering agents across the BBB: Preclinical studies

Delivery of agents across the BBB and the BTB in rats can be achieved by intracarotid infusion of hyperosmotic mannitol, resulting in transient shrinkage of cerebrovascular endothelial cells and opening of the BBB tight junctions (Fig. 1). Brightman et al. showed in the early 1970s that osmotic disruption of the BBB opened a clear channel between endothelial cells for the passage of agents from the blood to the brain (Brightman et al., 1973; Brightman & Reese, 1969; Rapoport & Robinson, 1986).

The maximum delivery and the time course for barrier opening vary depending on the size, charge, and protein and lipid binding characteristics of the agent delivered. Vascular permeability to water-soluble small molecular weight chemotherapeutics such as methotrexate (MTX) is increased maximally by 15 min after the infusion of 25% mannitol, after which it decreases to preinfusion levels within 2 h. We can measure 10- to 100-fold increases in the delivery of radiolabeled markers to intracerebral tumors and tumor-infiltrated brain, comparing intravenous (IV) administration to intraarterial (IA) administration with BBBD (Neuwelt, Barnett, et al., 1998; Remsen, Trail, Hellstrom, Hellstrom, & Neuwelt, 2000). Larger agents such as immunoconjugates (approximately 150 kDa), nanoparticles (30 nm diameter), or virus particles (200 nm diameter) have only a short window (15 min) of enhanced brain delivery.

We evaluated BBBD delivery of iron oxide nanoparticles magnetic resonance imaging (MRI) contrast agents as a model for visualizing the delivery of virus-sized particles to rat brain (Muldoon et al., 1999; Muldoon, Sandor, Pinkston, & Neuwelt, 2005; Fig. 2). Delivery of ferumoxytol nanoparticles across the BBB showed signal enhancement throughout the disrupted hemisphere that was maximal at 24 h and faded to baseline over 3 to 7 days. These particles, with a complete coating of modified carbohydrate that limits protein binding, disperse throughout the brain parenchyma after BBBD and are metabolized over time by brain cells (Muldoon et al., 2005). Ferumoxides iron oxide nanoparticles with an incomplete dextran coating cross through the endothelial tight junctions after osmotic BBBD but then bind to the basal lamina and do not actually enter the brain (Muldoon et al., 1999).

Drug delivery studies showed that BBBD was consistent in normal rat brain, with every animal attaining a good to excellent disruption, but was less consistent in rats with brain tumor xenografts, indicating that the BBBD procedure was not optimized when an intracerebral mass was present. We investigated pharmacologic and physiologic factors that may impact the quality and reproducibility of BBBD in implanted brain tumor xenograft models. Infusion flow rate and time, pCO₂, and osmotic agent (mannitol vs. arabinose or other sugars) were found to be important for BBBD. Anesthesia has a marked effect on cerebral blood flow and cerebral metabolic rate, and also impacts cardiac output and vascular tone. In rats with intracerebral tumors, propofol anesthesia was significantly superior to isoflurane for optimizing BBBD, yielding uniform Evans blue staining and enhanced drug delivery to tumor, brain around tumor (BAT), and brain distant to tumor (BDT) (Remsen et al., 1999).

2.2. Factors that impact chemotherapy delivery to brain tumors

Despite the rapid development of targeted therapeutics, chemotherapy remains the mainstay for brain tumor therapy, and our prediction is that it will remain widely used in adult and pediatric brain tumors. In fact, most of the new targeted therapies exclude primary brain tumors as an indication for their use. Therefore, it remains essential to maximize chemotherapy delivery and efficacy.

Numerous factors impact chemotherapy delivery to tumor including variable BBB permeability and drug concentrations achieved in the CNS. New vasculature within the tumor is often disordered and highly permeable, but infiltrating tumor makes use of the existing brain vasculature with a largely intact BBB. The magnitude of tumor vascular permeability varies within tumors both spatially and temporally, with the greatest permeability elevation in the tumor core and a relatively intact BBB at the proliferating edge of the tumor. The key to successful chemotherapy in brain tumors is drug delivery to the tumor-infiltrated BAT and the individual tumor cells and micrometastases distant from the main tumor mass in the BDT.

Chemotherapeutic drug concentrations within the CNS depend on multiple factors, including the permeability of the BBB to the chemotherapeutic agent, the extent to which the drug is actively transported out of the brain, and the drug volume of distribution in the brain parenchyma (Muldoon et al., 2007). Tissue concentrations of lipophilic agents are predominantly controlled by plasma protein binding, active efflux transport, and drug

metabolism. Delivery of water-soluble drugs to brain tumors is more complex, and pharmacokinetic data in this issue are scarce. MTX is an example of a widely used hydrophilic chemotherapeutic agent in primary central nervous system lymphoma (PCNSL); however, very high doses must be administered to achieve therapeutic drug concentrations in the tumor and surrounding brain. In contrast, MTX delivery to the CNS is enhanced four-to sevenfold when administered IA after BBBB when compared with IA administration without BBBB. For a review of the pharmacokinetics of common chemotherapeutic drugs in the brain and in brain tumors, see Muldoon et al. (2007).

Drug concentration in brain tumors can vary by the route of drug delivery. For example, therapeutic concentrations of etoposide were found in glioblastomas and astrocytomas after IV delivery, but concentration decreased with increasing distance from the tumor (Zucchetti et al., 1991). The etoposide concentration was found to be four times higher after IA administration than IV administration (Savaraj, Lu, Feun, Burgess, & Loo, 1987). Route of delivery has been shown to impact brain delivery of cisplatin, with IA administration increasing delivery to glioma twofold compared with IV administration (Nakagawa et al., 1993). One study reported the results of brain pharmacokinetics of cytarabine, comparing different routes of administration (Groothuis et al., 2000). After IV administration of cytarabine, a diffuse pattern of low drug concentrations was detected throughout the brain (Groothuis et al., 2000).

Animal studies have also shown that antecedent cranial irradiation decreases agent delivery to the brain (Remsen, Marquez, Garcia, Thrun, & Neuwelt, 2001; Remsen et al., 1995). The studies evaluated long-term effects of various sequences of radiation therapy and BBBB chemotherapy in rodents. Drug delivery, acute toxicity, and long-term (1 year) neuropathological effects of MTX or carboplatin plus etoposide were evaluated. External beam whole brain radiotherapy (WBRT) of 2000 cGy as a single fraction using parallel opposed portals, either 30 days before or concurrent with BBBB, resulted in a statistically significant decrease in drug delivery compared to animals not receiving cranial irradiation. Seizures were observed in 26% of the animals that received irradiation before or concurrent with BBBB and MTX, but not carboplatin. The mortality rate for animals receiving radiotherapy 30 days prior to chemotherapy was significantly higher than the mortality rate for animals receiving only BBBB chemotherapy without irradiation (Remsen et al., 1997, 1995).

Increasing dose intensity with BBBB enhances antitumor efficacy in animal models. BBBB delivery of BR96–DOX, a tumor-specific monoclonal antibody (mAb)–doxorubicin immunoconjugate, significantly increased antitumor efficacy compared with IV or IA administration without BBBB (Remsen et al., 2000). We evaluated whether prior irradiation would decrease the dose intensity and efficacy of antibody-targeted chemotherapy given with BBBB. Results showed that BR96–DOX administered prior to WBRT significantly increased survival compared to rodents receiving irradiation prior to chemotherapy or compared to those receiving chemotherapy concurrently (Remsen et al., 2001). These findings were later supported in the clinic when subjects with PCNSL who received cranial irradiation before beginning BBBB chemotherapy had significantly decreased median

survival time compared to those who received initial BBBD chemotherapy as first-line treatment (Dahlborg et al., 1996).

2.3. Preclinical BBBD chemotherapy neurotoxicity studies

The choice of chemotherapy agents is extremely important in the setting of BBBD for malignant brain tumors. The BBB preclinical team carefully conducts toxicity studies in rodents. Chemotherapy agents that can be safely administered with BBBD with acceptable toxicity are determined. Neuro-toxicity can be caused by the chemotherapy itself or by agents such as detergents or strong salts in the diluent. Important knowledge was gained when laboratory studies showed severe neurotoxicity when doxorubicin (IA) (Neuwelt, Pagel, Barnett, Glassberg, & Frenkel, 1981), cisplatin (IA), or 5-FU (IA) (Neuwelt, Barnett, Glasberg, & Frenkel, 1983) was administered as single agents after BBBD. Fortin, McCormick, Remsen, Nixon, and Neuwelt (2000) reported unexpected neurotoxicity in the preclinical setting, when etoposide phosphate (IA) was administered in combination with melphalan (IA), MTX (IA), or carboplatin (IA) after BBBD, when propofol anesthesia was used. Neurotoxicity was minimized by appropriate timing of drug administration, with etoposide phosphate prior to BBBD, and carboplatin and melphalan immediately after BBBD (Fortin et al., 2000).

2.4. The clinical technique of osmotic BBB opening

The technique of clinical BBBD used by Neuwelt et al. is based on extensive preclinical toxicity and efficacy studies (Neuwelt, 2004). BBBD involves infusing hyperosmolar mannitol (25%, warmed) IA in the carotid or in the vertebral arteries. The infusion of mannitol is theorized to cause osmotic shrinkage of the endothelial cells which line CNS capillaries, with resultant separation of the tight junctions between the endothelial cells (Rapoport & Robinson, 1986). To date, BBBD has shown very promising clinical results especially as front-line treatment in chemosensitive brain tumors such as PCNSL (Angelov et al., 2009; Doolittle, Korfel, et al., 2013; Neuwelt et al., 1991).

The BBBD treatment is conducted on two consecutive days (24 h apart) every 4 weeks for 12 months. BBBD is performed under general anesthesia to ensure patient comfort and safety during the rapid (30 s) IA infusion of a large volume of mannitol. A femoral artery is catheterized, and a selected intracranial artery (either an internal carotid or a vertebral artery) is accessed. Mannitol is delivered IA via an infusion device at a predetermined flow rate of 3–12 cc/s into the cannulated artery for 30 s. The precise mannitol flow rate is determined by fluoroscopy, to just exceed cerebral blood flow. Following the administration of mannitol, chemotherapy is infused IA over 10 min. Immediately following the mannitol, nonionic contrast dye is administered IV.

Following completion of the chemotherapy infusion, patients undergo a computed tomography (CT) brain scan. Contrast enhancement in the disrupted territory of the brain is compared to the nondisrupted territory and graded using the results reported by Roman-Goldstein et al. (1994). During each monthly treatment, one of the intracranial arteries (right or left internal carotid or a vertebral) is infused on the first day of BBBD treatment and a different artery is infused on the second day, depending on the tumor type, extent, and

location. Since many tumors such as PCNSL have widespread microscopic infiltration of the brain, infusion of the arteries is rotated such that during a year of BBBD treatment, each of the three intracranial arteries is infused eight times, thus providing global delivery to all cerebral hemispheres.

Chemotherapy agents used most frequently in conjunction with BBBD in the clinical setting are MTX (IA, 2500 mg/day $\times$ two consecutive days), carboplatin (IA, 200 mg/m²/day $\times$ two consecutive days), melphalan (IA, a dose of 8 mg/m²/day $\times$ two consecutive days is currently under study), cyclophosphamide (IV, 500 mg/m²/day $\times$ two consecutive days when given with MTX; 330 mg/m²/day $\times$ two consecutive days when given with carboplatin), etoposide and etoposide phosphate (IV, 150 mg/m²/day $\times$ two consecutive days when given with MTX; 200 mg/m²/day $\times$ two consecutive days when given with carboplatin). Depending on the brain tumor histology and according to the specific IRB-approved protocol, a combination of the above drugs is given with BBBD. These agents, infused by the respective routes and doses, have been routinely used in the clinical setting with minimal toxicity (Angelov et al., 2009; Dahlborg et al., 1996, 1998; Doolittle, Korfel, et al., 2013; Doolittle et al., 2000; McAllister et al., 2000; Tyson et al., 2003).

2.5. Safety of BBBD in a multicenter setting

Utilizing BBBD treatment and managing the care of patients treated with the BBBD procedure require a multidisciplinary team approach including a neurooncologist, neurosurgeon, pharmacist, neuroradiologist, anesthesiologist, nurse coordinator, audiologist, physical therapist, and social worker. Uniform guidelines for anesthesia, transfemoral arterial catheterization, IA infusion of mannitol and chemotherapy, radiographic assessment of disruption and tumor response, and patient care guidelines must be used by participating centers when performing BBBD. In the setting of rapidly progressing brain tumor with associated rapid neurological deterioration, there is a risk of increasing mass effect following BBBD. Thus, BBBD is safest before tumor burden becomes excessive (Doolittle et al., 2000).

As part of the treatment regimen, neuroimaging studies are performed before each monthly treatment course, after the final course, and at specified follow-up intervals depending on the type of tumor and tumor response. Routine follow-up includes complete blood counts and audiologic and neurologic examinations. Ophthalmologic evaluations and cerebrospinal fluid (CSF) cytopathology are conducted when clinically indicated. The frequency and severity of toxicities associated with BBBD treatment are well described (Angelov et al., 2009; Guillaume et al., 2010). Indeed the exceptional preservation of cognitive function in the long-term PCNSL survivors treated with BBBD as well as the preservation of hearing in brain tumor patients treated with carboplatin (IA) with BBBD and delayed high-dose sodium thio-sulfate (STS) have formed the basis of numerous publications, ongoing studies, and future research (Doolittle, Korfel, et al., 2013; Doolittle, Muldoon, et al., 2001; Neuwelt, Brummett, et al., 1998; Neuwelt et al., 1991).

3. Primary CNS Lymphoma

PCNSL is a generally diffuse, aggressive large B-cell lymphoma confined to the brain, leptomeninges, spinal cord, and eyes at the time of presentation, with a characteristic pattern of scattered and perivascular infiltration. High-dose MTX (IV) is the most widely used drug for PCNSL. In combination with WBRT, high-dose MTX improved survival rates over WBRT alone. However, delayed treatment-related neurotoxicity emerged as a significant disabling complication of the combined treatment especially in patients older than 60 years (Correa et al., 2012; Harder et al., 2004; Morris & Abrey, 2009; Thiel et al., 2010).

There is a trend toward more intensive first-line treatments for PCNSL such as dose-intensive chemotherapy and myeloablative chemotherapy followed by stem cell transplantation, as a strategy to achieve durable remissions while avoiding the neurotoxicity associated with WBRT. As treatment regimens intensify, durable remission rates and survival are expected to increase. However, reducing toxicity from intensive induction and high-dose chemotherapy regimens will be critical to achieving acceptable long-term outcomes.

Many clinical investigators have added the CD20⁺ antibody rituximab to the first-line PCNSL regimens. Inclusion of this mAb is primarily based on the increased survival when rituximab was added to chemotherapy for patients with systemic CD20⁺ diffuse large B-cell non-Hodgkin lymphoma (Coiffier et al., 2002), and on evidence of radiographic response to rituximab monotherapy in patients with recurrent PCNSL (Batchelor et al., 2011). Although initially thought to have poor CNS penetration because of its large molecular size, rituximab has a long plasma half-life with notable binding and accumulation at target B cells.

3.1. Development and characterization of a CNS lymphoma rat model

In order to evaluate PCNSL biology and new treatment approaches, we developed an animal model that closely mimics the clinical situation (Jahnke et al., 2009; Muldoon, Lewin, et al., 2011; Soussain et al., 2007). We implanted MC116 human B-cell lymphoma cells either intracerebrally or intracerebroventricularly in athymic rats. The intracerebral CNSL tumor model is inconsistent with ~25% of rats showing no tumors, and the tumors that grow are unpredictable, ranging in size from tiny (2 mm³) to huge (175 mm³) at 19–26 days after tumor implantation. In the intraventricular model, all rats ($n = 4$) showed weight loss and behavioral changes such as agitation in response to noise.

In very large intracerebral tumors (>100mm³), T2/FLAIR signal changes at the inoculation site (caudate nucleus) and in the cortex and ventricles indicated tumor infiltration and edema throughout the inoculated hemisphere and also in the contralateral side along white matter tracts. Gadolinium enhancement was found primarily in the central region of large tumors, indicating relative low permeability in most of the tumor-infiltrated brain. In smaller tumors (2–50 mm³), FLAIR sequences appeared to be the most sensitive in the delineation of the tumor-infiltrated brain and showed a good visual correlation with hematoxylin staining. After intraventricular cell implantation, FLAIR MRI showed large ventricles with periventricular enhancement suggesting tumor infiltration.

The MC116 CNSL model showed positive staining for a variety of human B-cell markers, including CD19, CD20, CD22, and CD45 (Fig. 3). Immunohistochemistry showed diffuse infiltration of the MC116 cells from the inoculation site spreading into the cortex, meninges, sub-arachnoid space, and tracking along the corpus callosum into the contralateral hemisphere. Perivascular infiltration was found in brain tissue up to 5 mm from the inoculation site. In the intraventricular model, immunocytochemistry for CD20 showed diffuse tumor cell infiltration into the brain parenchyma around both the right and left ventricles and in the subarachnoid space.

To replicate and confirm findings in the MC116 CNSL model, we are developing a second animal model utilizing intracerebral implantation of NALM-6 human pre-B cell line. This cell line forms a model of acute lymphoblastic leukemia when injected IV in immunocompromised mice.

3.2. Therapy studies in the rat CNS lymphoma model

MC116 cells were sensitive to radiation *in vitro*, with toxicity seen 3–4 days after a single treatment in the dose range of 2–10 Gy. *In vivo*, WBRT (20 Gy) was effective in five animals with MRI-confirmed tumor. One week after WBRT, histochemistry showed minimal tumor with scattered enlarged and necrotic CD45-positive cells near the inoculation site. Although radiation appeared effective, because of the short time frame in the pilot study, it remains unknown whether tumor regrowth could take place.

MC116 cells were also highly sensitive to MTX *in vitro*, with most cells killed by a clinically relevant dose of 0.1 μ M within 5 days of treatment (Jahnke et al., 2009; Soussain et al., 2007). In contrast, three independent studies demonstrated a lack of responsiveness of the CNSL model to MTX *in vivo*. In the initial study, five rats with MRI-confirmed tumor received one dose of IV MTX (3 g/m²). Immunocytochemistry demonstrated histologically infiltrative tumor in all five animals 1 week after treatment, including clusters of tumor cells throughout the inoculated hemisphere. The second study assessed MRI tumor volumetrics 1 week after treatment with a single dose of MTX (1 g/m² IV). MTX treatment appeared to reduce tumor growth compared to the control, but several animals showed an increase in the area of enhancement or no change in enhancement. Histology showed the presence of viable tumor infiltrated into the brain parenchyma. A third study assessing the impact of BBBD-enhanced delivery of MTX with or without rituximab on survival in the rat model is described below.

3.3. mAb delivery and efficacy in the preclinical setting

A study of IV rituximab with or without MTX chemotherapy was evaluated in the rat CNSL model. Both agents were given IV as a single dose and outcome was determined by MRI 1 week after treatment (Jahnke et al., 2009). Control tumors showed approximately a doubling of volume over the 1-week assessment period. Overall, rituximab-treated tumors did not grow compared to baseline, and included 60% of rats with an objective response (tumor shrinkage on MRI). We hypothesized that improving delivery would enhance the efficacy of rituximab in the brain tumor model.

To evaluate the effect of BBBB on the delivery of mAb to CNSL tumor mass and tumor-infiltrated rat brain, we used ^{90}Y -labeled mAb ibritumomab tiuxetan (Zevalin), which targets CD20 on B cells similar to rituximab (Muldoon, Lewin, et al., 2011; Fig. 4). BBBB improved ^{90}Y -ibritumomab delivery throughout the disrupted hemisphere compared with IV mAb administration. At 10 min after BBBB, there was a significant increase in mAb levels in tumor, tumor-infiltrated BAT, and BDT. Levels of mAb in brain were elevated at 10 min and 24 h after BBBB, but by 3 days mAb concentrations in brain were no different than IV infusion. IV mAb gave 10–20% increased concentration in tumor-infiltrated brain compared to the normal contralateral hemisphere, but the difference was not significant. We conclude that BBBB was effective for the delivery of high-molecular-weight agents such as mAbs, giving a rapid two- to fourfold increase in the delivery of mAb to brain tumor. However, elevated localization of the mAb was not maintained compared to IV delivery over time. IV delivery alone gave slightly elevated mAb localization in tumor, BAT, and BDT, but this was not significantly different from the contralateral normal brain levels.

We tested the hypothesis that BBBB-enhanced delivery would improve the effectiveness of rituximab, MTX, or combination therapy. Five groups were evaluated: (1) BBBB control (IA saline); (2) MTX 1 g/kg IA with BBBB; (3) rituximab 375 mg/m² IV with BBBB; (4) rituximab plus MTX with BBBB; and (5) rituximab IV. Animals were followed for survival, with an interim outcome measure of MRI at 1 week after treatment, with follow-up MRI if possible. We found three patterns of response: (a) increased tumor size on MRI with short survival. Many control rats fit this phenotype; (b) increased tumor size on the 1-week MRI coupled with long survival. Some animals with this phenotype showed later tumor shrinkage on subsequent MRI; and (c) decreased tumor size and long survival. No rat showed decreased tumor size on MRI coupled with short survival. Control tumors grew by $201 \pm 102\%$ at the 1-week assessment period, and both the MTX and rituximab groups had significantly smaller tumors than control. The tumors treated with MTX had a reduced growth rate on MRI, with $70 \pm 42\%$ increase in volume, but this did not translate to improved survival (Fig. 5). Overall survival (OS) in the control group was 14 days (range: 6–33 days). Survival time after MTX was actually decreased (median: 7 days; range: 5–19 days), but this was not significantly different from controls ($P=0.18$). The reasons for the lack of MTX efficacy in the rat CNSL model are not clear, given that the cells are chemosensitive. Possible reasons include the lower dose compared to humans, lack of delivery across the BBB, and that we are limited to a single dose in the rat model.

The impact of rituximab on early tumor volumetrics was inconsistent. Tumors in the combined rituximab groups showed both increased and decreased size on MRI, with an overall $32 \pm 122\%$ increase in volume at 1 week. In contrast, rituximab improved survival in all groups. Survival times ranged from 37.5 days (20–60 days, predetermined end of study) for rituximab BBBB, 42 days (27–60 days) for rituximab plus MTX BBBB, and undefined (26–60 days) for IV rituximab. The study lacks adequate power to determine whether there were differences between the three rituximab groups. Thus, this study demonstrated that a single dose of rituximab was effective at prolonging survival in the CNSL model, whether or not it was delivered with BBBB, and whether or not MTX was included. We conclude that the long slow leak of mAb across the minimally disrupted BBB of the brain tumor was sufficient to prove an effective dose. Of interest, many long-term survival rats showed tumor

regression, even complete response (CR), at the inoculation site, with tumor growth in cortex or contralateral hemisphere. This tumor histology suggests that tumor was responsive where vasculature was leaky, but infiltrating tumor far from the initial mass was protected by a more normal BBB.

3.4. PCNSL: Clinical studies

The first PCNSL patients successfully treated with BBBD-enhanced delivery of MTX-based chemotherapy were reported by Neuwelt et al. in the early 1980s (Neuwelt, Balaban, Diehl, Hill, & Frenkel, 1983). Since then, excellent clinical outcomes have been obtained in PCNSL which is a highly chemosensitive brain tumor, using IA MTX in conjunction with BBBD without the use of WBRT (Angelov et al., 2009; Doolittle, Fu, et al., 2013; Doolittle, Korfel, et al., 2013; Neuwelt, Balaban, et al., 1983; Neuwelt et al., 1991). We reported our multi-institutional experience using BBBD in conjunction with IA MTX-based chemotherapy in 149 patients newly diagnosed with PCNSL (Angelov et al., 2009). The patients achieved an overall response rate of 82% (58% CR; 24% partial response). The median OS was 3.1 years (25% estimated survival at 8.5 years). The median progression-free survival (PFS) was 1.8 years, with 5-year and 7-year PFS of 31% and 25%, respectively. In low-risk patients (age <60 years and Karnofsky Performance Score [KPS] ≥ 70), median OS was approximately 14 years, with a survival plateau after approximately 8 years (Fig. 6). The BBBD procedures were generally well tolerated; peri-procedural focal seizures in 9.2% of the patients almost exclusively after MTX infusion were the most frequent side effect and lacked long-term sequelae. Durable remissions have been achieved with stable or improved cognitive function, a median of 12 years (range: 2–26 years) after diagnosis (Doolittle, Dosa, et al., 2013; Doolittle, Korfel, et al., 2013).

Isolated brain parenchyma relapse is a rare complication of systemic non-Hodgkin lymphoma. A large multinational retrospective analysis of isolated brain relapse cases was conducted by our group in collaboration with the International Primary CNS Lymphoma Collaborative Group (Doolittle et al., 2008). MTX was found to be the optimal treatment for isolated brain relapse. Although the number of patients treated with MTX (IA) in conjunction with BBBD included in the report was small, the majority of the patients treated with this approach were listed as long-term survivors.

3.5. mAb delivery to brain in the clinical setting

Data from anti-CD20 radioimmunotherapy support the idea that the BBB limits efficacy. Clinical studies of ⁹⁰Y-labeled anti-CD20 antibody ibritumomab tiuxetan provide evidence of target accumulation of the antibody within the brain as assessed by single photon emission computed tomography (SPECT) imaging with ¹¹¹In-labeled ibritumomab tiuxetan in recurrent PCNSL, in the absence of BBBD (Doolittle et al., 2007; Maza et al., 2009; Muldoon et al., 2007; Fig. 7). We studied ⁹⁰Y-ibritumomab tiuxetan in two patients with refractory PCNSL (Doolittle et al., 2007; Muldoon et al., 2007). SPECT imaging in one patient at 45 h and one patient at 48 h showed uptake of ¹¹¹In-ibritumomab localized at enhancing lesions, which were detected on brain MRI, providing evidence of mAb leakage across the BBB. The original lesions achieved CR to ⁹⁰Y-ibritumomab; however, recurrence was detected on brain MRI in multiple locations distant to the original lesions. An

independent report showed ^{90}Y -ibritumomab efficacy in four of six PCNSL patients (Maza et al., 2009). The ^{90}Y -ibritumomab study provides insight into the leakage and efficacy of rituximab in brain. At the original lesion, the BBB was leaky secondary to lymphomatous infiltration and ^{90}Y -ibritumomab provided local efficacy. However, the BBB was mostly intact at distant brain areas at the time of ^{90}Y -ibritumomab infusion and may have received less ^{90}Y -ibritumomab for efficacy. Impaired BBB integrity at the original lesion improved mAb delivery.

Based on the safety and efficacy using rituximab in combination with increased delivery to the CNS with BBBD in recurrent PCNSL as well as our translational laboratory studies including the development of a rodent model of human B-cell CNS lymphoma, we treated PCNSL patients with the first-line rituximab in combination with MTX-based chemotherapy and BBBD (Doolittle et al., 2007; Jahnke et al., 2009; Muldoon, Lewin, et al., 2011; Soussain et al., 2007). The rituximab was infused IV approximately 12 h prior to day 1 of monthly BBBD. The addition of rituximab safely improved the CR rate (74%) and median OS (61 months) in newly diagnosed PCNSL patients when compared with our previous series, with an acceptable toxicity profile (Doolittle, Fu, et al., 2013). Immunotherapy with rituximab improved clinical outcomes even in high-risk patients without escalating chemotherapy doses.

3.6. Neurocognitive outcomes in long-term PCNSL survivors

Since the goal of BBBD is enhanced CNS delivery while preserving neurocognitive function, BBBD clinical protocols include a standardized neuropsychological test battery, which is conducted at study entry and at follow-up. Long-term PCNSL survivors were prospectively evaluated to assess changes in neuropsychological scores and the association with pretreatment and long-term neuroimaging outcomes. Survivors who were a minimum of 2 years postdiagnosis and in complete remission after BBBD treatment were evaluated with neuropsychological tests and brain MRI or CT (Doolittle, Dosa, et al., 2013; Doolittle, Korfel, et al., 2013). Complete disease remission was required to assess neurotoxicity without the confounding presence of infiltrative, often multifocal CNS disease.

Neuropsychological scores obtained pretreatment and long-term were available on 23 of 26 long-term PCNSL survivors. The median interval from diagnosis to long-term evaluation was 12 years (min 2 years, max 26 years); eight survivors (35%) were evaluated 15 years or more after diagnosis. There was significant improvement in tests of attention/executive function from pretreatment to long-term. The majority of survivors showed stable or improved cognitive status at long term (Fig. 8). Of the eight survivors evaluated 15 years or more after diagnosis, five were working in high-level occupations as surgeon, attorney, registered nurse, law-enforcement agent, and optician. Three were retired (Doolittle, Dosa, et al., 2013).

On neuroimaging, the total T2 MRI hyperintensities or CT hypo-densities decreased or resolved by the end of treatment in 75% of survivors. Total T2 MRI hyperintensities or CT hypodensities did not change from the end of treatment to long term. There was no association between neuropsychological scores and neuroimaging pretreatment and long term. We are not aware of studies that have evaluated cognition and neuroimaging with such

lengthy follow-up, showing preserved or improved cognitive functioning in this rare cancer (Doolittle, Dosa, et al., 2013).

In addition, 80 PCNSL survivors from four MTX-based treatment groups (one group with WBRT and three groups without WBRT) who were a minimum of 2 years after diagnosis and in complete remission underwent prospective cognitive testing and brain MRI evaluation (Doolittle, Korfel, et al., 2013). The patients who had been treated with BBBB ($n = 25$) showed long-term cognitive, quality of life, and imaging outcomes that were as good as if not better than the outcomes in the other three treatment groups (Fig. 9). The patients treated with BBBB were a median of 12 years (min 2 years, max 26 years) after diagnosis. This group had the longest median interval from diagnosis to long-term evaluation of the four treatment groups. Overall, the PCNSL patients treated with WBRT had significantly poorer cognitive performance when compared with the non-WBRT treatment groups ($P = 0.05$).

3.7. Anaplastic oligodendroglioma and CNS embryonal tumors: BBBB outcomes

Anaplastic oligodendroglioma and oligoastrocytoma, especially in patients demonstrating 1p and/or 19q deletion, are chemosensitive brain tumors, which respond well to alkylating agents. We have reported acceptable toxicity and encouraging efficacy in patients with anaplastic oligodendroglioma and oligoastrocytoma who were treated with melphalan (IA), carboplatin (IA), and etoposide phosphate (IV) in conjunction with BBBB (Guillaume et al., 2010). The patients had undergone prior treatment with temozolomide. During the completed phase I component of the study, 13 patients who had undergone 147 BBBB treatments were assessed. The most common grade 3 and grade 4 adverse events (graded according to Common Terminology Criteria for Adverse Events [CTCAE]) was thrombocytopenia in 20% and 12% of BBBB procedures, respectively. The maximum tolerated dose (MTD) of melphalan (IA) when administered in combination with carboplatin (IA, 200 mg/m²/day) and etoposide phosphate (IV, 200 mg/m² day) was determined to be 4 mg/m². In terms of tumor response, 5 of 13 patients demonstrated complete or partial response, five patients remained stable, and three developed disease progression. The 2 efficacy component of the study, using the MTD of melphalan (4 mg/m) determined during the phase I component, is ongoing.

We have reported outcomes in 54 patients with primitive neuroectodermal tumor (PNET), medulloblastoma, and germ cell tumor who were treated with MTX (IA)-based chemotherapy and carboplatin (IA)-based chemotherapy in conjunction with BBBB (Jahnke et al., 2008). Many of the patients had adverse prognostic factors and received IA/BBBB as salvage treatment. Nonetheless, the response, survival, and toxicity data are encouraging. A plateau in the survival curves in conjunction with the long median follow-up suggests possible cure for some patients with PNET and germinomas. Long-term survival may be achieved with focal or reduced dose radiotherapy in some IA/BBBB patients (Jahnke et al., 2008).

3.8. CNS metastases

The occurrence of CNS metastases of systemic cancers far exceeds the number of primary malignant brain tumors. Current therapies such as radiosurgery are effective for short-term

palliation of CNS metastases, however often do not provide long-term disease control. The use of WBRT to treat CNS metastases has been associated with neurotoxicity. BBBD may offer a new treatment strategy for CNS metastases, since BBBD enables global delivery to all cerebral circulations. Based on the results of preclinical studies, preirradiation BBBD chemotherapy may provide improved drug penetration to CNS metastases in the clinic.

Metastasis of solid tumors from the periphery to the CNS requires a complex series of events, including extravasation of tumor cells from the primary site, travel through the blood stream, binding to and infiltrating through the cerebral vasculature, and growth in the foreign site. In the laboratory, we have investigated the hypothesis that cell adhesion proteins, particularly α_v integrin, are involved in multiple steps in the metastatic process. The pan- α_v integrin mAb intetumumab blocks integrin-mediated cell migration and signaling *in vitro* and *in vivo*. We used a hematogenous breast cancer brain metastasis model to test the impact of intetumumab as a preventive agent (Wu et al., 2012). Rats received human breast cancer cells into the internal carotid artery either alone or in combination with intetumumab. MRI at 5–7 weeks after tumor cell infusion showed multiple brain metastases in control animals (Fig. 10), while animals receiving IV intetumumab prior to tumor cell infusion showed few metastases even at 11 weeks after infusion. Intetumumab decreased the number of metastases on histology, with 32% of intetumumab-treated rats showing no metastases at 11 weeks. Rats given intetumumab had significantly longer survival compared to rats with untreated hematogenous metastases. Our results suggest that cancer patients at risk of metastases would benefit from early intetumumab treatment.

4. Chemoprotection Studies

Platinum-based chemotherapy is associated with progressive and irreversible ototoxicity, and can also cause bone marrow toxicity, renal toxicity, and hepatotoxicity. Platinum-induced toxicities are mediated at least in part by free radical damage. Sulfur-containing thiol chemoprotective agents that mimic activities of the endogenous antioxidant glutathione can protect against free radical damage and chemotherapy toxicity. Our preclinical and clinical studies have evaluated chemoprotection using STS and N-acetylcysteine (NAC).

4.1. Preclinical chemoprotection studies with thiols

Early rat and guinea pig studies showed that STS (8 g/m² IV) blocked platinum-induced damage to the cochlea when administered as late as 8 h after carboplatin but not at 24 h after carboplatin (Dickey, Wu, Muldoon, & Neuwelt, 2005; Muldoon et al., 2000; Neuwelt et al., 1996; Neuwelt, Pagel, Kraemer, Peterson, & Muldoon, 2004). In a rat model using IA infusion of cisplatin (6 mg/kg) to induce ototoxicity, NAC (400 mg/kg IV) protects against hearing loss when administered 15 or 30 min prior to chemotherapy or 4 h after chemotherapy (Dickey, Muldoon, Kraemer, & Neuwelt, 2004). NAC protects against cisplatin-induced weight loss (Dickey et al., 2004) suggesting that it may decrease mucositis. Pretreatment with high-dose NAC (1200 mg/kg) protected against bone marrow toxicity induced by a cocktail of chemotherapeutics (carboplatin, etoposide phosphate, and melphalan) (Neuwelt, Pagel, Hasler, Deloughery, & Muldoon, 2001). Rescue of white blood cells and platelets was found with NAC even if animals were pretreated with buthionine

sulfoximine to lower glutathione synthesis (Neuwelt et al., 2001). The combination of NAC plus STS improved bone marrow chemoprotection (Neuwelt, 2004). STS alone did not protect against nephrotoxicity (Dickey et al., 2005). In contrast, pre-treatment or 4 h posttreatment with NAC significantly decreased cisplatin-induced kidney damage as determined by measurement of the kidney blood urea nitrogen and creatinine and by pathological assessment (Dickey et al., 2005). We showed that NAC delivered IA in the descending aorta is more efficacious than IV NAC for kidney chemoprotection, while oral administration is ineffective (Dickey et al., 2008; Fig. 11). Nephroprotection correlated with blood concentrations of NAC, showing the importance of high dose and IV route of delivery.

Clinical use of chemoprotection has been limited by the possibility of protecting the cancer against chemotherapy toxicity. Our in vitro studies demonstrated that while both STS and NAC are protective to tumor cells if administered at the same time as chemotherapy, tumor cell protection was lost if the thiols were delayed by 2–4 h (Muldoon et al., 2001; Wu, Muldoon, & Neuwelt, 2005). STS was not tumor protective in a mouse model of neuroblastoma if delayed until 6 h after cisplatin (Harned et al., 2008). In a rat model of lung cancer brain metastasis, 8 h delayed STS, 1 h pretreatment with NAC, or the combination of NAC pretreatment plus STS posttreatment did not impact the antitumor efficacy of carboplatin chemotherapy (Neuwelt et al., 2004). We further assessed the impact of the timing of NAC on the efficacy of cisplatin in rat models of pediatric tumors (Muldoon, Wu, Pagel, & Neuwelt, submitted for publication). We found that pretreatment with NAC significantly decreased cisplatin efficacy in both a systemic solid tumor model (neuroblastoma) and an intracerebral tumor model (medulloblastoma). In contrast, delay of NAC until 4 h after cisplatin did not decrease chemotherapy efficacy in either tumor model.

4.2. Clinical chemoprotection studies with thiols

Dose intensive chemotherapy strategies for the treatment of malignant brain tumors necessitate minimizing CNS and systemic toxicities. Carboplatin has shown efficacy in malignant brain tumors. However, carboplatin causes myelosuppression including severe thrombocytopenia, often requiring platelet transfusions and dose reductions of subsequent carboplatin treatments. When administered in conjunction with BBBD, carboplatin (IA) causes irreversible hearing loss in a large proportion of subjects (Doolittle, Muldoon, et al., 2001; Neuwelt, Brummett, et al., 1998).

Clinical studies have shown hearing protection when high-dose STS (16–20 g/m²) was administered as part of a two-compartment model in adult patients with malignant brain tumors (Doolittle, Muldoon, et al., 2001; Neuwelt, Brummett, et al., 1998; Fig. 12). Carboplatin was administered IA in conjunction with BBBD. High-dose STS was administered IV in a delayed fashion, 4 h (or 4 and 8 h) after carboplatin, thus providing spatial and temporal separations between chemotherapy and chemoprotectant. The study showed a protective effect against carboplatin-induced hearing loss. We later reviewed hematologic data from patients with malignant brain tumors treated with carboplatin IA with BBBD, with or without delayed high-dose STS for hearing protection. The rate of grade 3 or 4 platelet toxicity (CTCAE) without STS was 47.8% and with STS was 17.2% there was a significant association of grade 3 or 4 platelet toxicity in patients without STS treatment

($P=0.0018$). The rates of dose reduction of carboplatin, controlling for prior chemotherapy, were statistically significant between the two groups ($P=0.0046$). These results suggest that STS may protect against severe thrombocytopenia, decreasing the number of platelet transfusions and dose reductions of carboplatin (Doolittle, Tyson, et al., 2001).

Our studies led to the implementation of two phase III trials of STS for protection against cisplatin-induced hearing loss. The Children's Oncology Group Study ACCL0431 evaluated the efficacy of STS treatment compared with observation in children diagnosed with childhood cancers typically treated with cisplatin therapy (Freyer 2014). The study is completed and concluded that STS protects against cisplatin-induced hearing loss in children. STS was not associated with a change in OS in patients with localized disease. However STS treatment was associated with lower OS in patients with disseminated disease. The COG study raised the bar for supportive hearing protection in children, while pointing out the role of disease extent (non-disseminated versus disseminated) in possible tumor protective effect when STS is administered on this dose and schedule. A second trial conducted by Societe Internationale d'Oncologie Pediatrique is continuing (Maibach 2014). In this trial, children with standard-risk hepatoblastoma (ie localized tumor) are treated with cisplatin monotherapy or cisplatin and STS. An Independent Data Monitoring Committee has periodically reviewed the efficacy results to assess any potential adverse impact of STS on cisplatin efficacy. To date, the periodic safety checks indicate no evidence of tumor protection. The expected end of accrual for this trial is December 2014.

A phase I clinical study of NAC in adult patients undergoing endovascular procedures is almost complete. Sixteen patients were randomized to receive IV or IA NAC in a standard dose escalation study. As the study nears completion, it appears that the NAC MTD is in the range of 300–450 mg/kg. A phase I NAC dose escalation study is underway in children with a variety of cancer diagnoses undergoing treatment with cisplatin-based chemotherapy.

5. Advances In Neuroimaging

Neuroimaging techniques are critical in assessing biologic and physiologic aspects of brain tumors as well as other neurologic diseases. The ability to accurately image infiltrative disease and to assess the true extent of disease and actual tumor volume is essential. Conventional MRI allows visualization of brain areas with abnormal BBB leakage but does not actually show tumor vasculature. New MRI techniques can characterize physiological changes in the vasculature in brain tumors and CNS lesions.

Dynamic MRI techniques use very rapid signal acquisition to track a bolus of contrast agent as it passes through the brain vasculature. Dynamic contrast-enhanced (DCE) MRI of CNS lesions with gadolinium-based contrast agents (GBCAs) provides a noninvasive mechanism to measure vascular permeability and pharmacokinetic properties, such as the contrast agent transfer rate constant (K^{trans}), with high spatial and temporal resolutions. A second technique is perfusion-weighted dynamic susceptibility-weighted contrast-enhanced (DSC) MRI, which can be used to noninvasively measure relative cerebral blood volume (rCBV) in brain lesions relative to normal-appearing white matter, providing a measure of blood vessel size and density. The high permeability of GBCA limits its use in rCBV measurements.

Instead we use the ultrasmall superparamagnetic iron oxide nanoparticle ferumoxytol (Feraheme) as a blood pool contrast agent. Ferumoxytol is a virus-sized carbohydrate-coated particle with an iron oxide core, which serves not only as a contrast agent for MRI but can be identified histologically and ultrastructurally by electron microscopy (as shown in Fig. 2). It is FDA-approved for iron replacement therapy, and is safe for neuroimaging in both rats and patients.

5.1. Preclinical studies of dynamic MRI

Standard GBCAs have limitations in medical imaging involving inconsistent measurement of rCBV. In a landmark preclinical study, we investigated rCBV measurement with ferumoxytol in comparison to GBCA in order to evaluate the technique of contrast leakage correction that is used clinically with GBCA (Gahramanov, Muldoon, Li, & Neuwelt, 2011). We found that ferumoxytol improves the consistency of rCBV measurements in rats before and after treatment with the anti-VEGF mAb bevacizumab and does not require contrast preload or leakage correction. This rat study showed that the technical and mathematical manipulations necessary to use GBCA for vascular MRI are not necessary using ferumoxytol and demonstrated the utility of DSC-MRI in measuring vascular changes.

We have used both the DCE-MRI and DSC-MRI techniques to assess brain tumor vasculature and vascular-targeting agents in animal models. DCE-MRI permeability measurements showed that glyburide decreases edema in brain metastasis models, which may allow reduced doses of steroids and lessen the morbidity associated with steroids in brain tumor patients (Thompson, Pishko, Muldoon, & Neuwelt, 2013). In a glioma model, we showed that bevacizumab significantly decreased the blood volume and permeability of the brain tumor vasculature, analogous to high-dose dexamethasone (Varallyay et al., 2009). In a lung metastasis model, using intracerebral implantation of human LX-1 small cell lung carcinoma cells, bevacizumab decreased blood volume and vascular permeability on dynamic MRI, which correlated with a 10-fold decrease in microvessel density on histology (Muldoon, Gahramanov, et al., 2011; Fig. 13). The changes in vasculature correlated with a significantly increased volume of tumor necrosis, yet the brain metastases continued to grow over the week assessment period after bevacizumab treatment (Muldoon, Gahramanov, et al., 2011). In another study, we used DCE-MRI and DSC-MRI to evaluate the effects of the anti- α_v integrin mAb intetumumab on tumor vasculature in the lung cancer brain metastasis model (Muldoon, Lewin, et al., 2011; Muldoon et al., 2005). Intetumumab increased tumor vascular permeability and blood volume on dynamic MRI and increased blood vessel size but not the number of vessels on histology. We hypothesize that alterations of tumor vasculature with intetumumab will increase the delivery of chemotherapy in brain tumors and may thus enhance chemotherapy efficacy. These results demonstrated the opposite effects of targeting brain tumor vasculature with differing agents and clearly show the interrelationship of vascular permeability and relevant tumor phenotype.

5.2. Clinical imaging studies using ferumoxytol

In the clinic, MRI using ferumoxytol shows good correlation with GBCA-enhanced scans 24 h postinjection and improves visualization of vasculature associated with malformations, tumors, inflammation, as well as rCBV measurements (Dosa, Guillaume, et al., 2011; Dosa,

Tuladhar, et al., 2011; Hamilton et al., 2011). Our clinical findings of the use of ferumoxytol give both anatomic and physiologic information about the BBB and CNS vasculature parameters (Neuwelt et al., 2007, 2009; Weinstein et al., 2010). Ferumoxytol shows signal changes on T1W and T2W sequences in demyelinating disease as well as in PCNSL and lymphoproliferative disorders (Farrell et al., 2013). Different enhancement patterns compared to GBCA-enhanced scans have been observed in some patients. Such differences might distinguish patients with differing degrees of inflammation, a characteristic which has prognostic or therapeutic importance.

In patients with malignant brain tumors, radiographic worsening after radiation therapy can be caused by true tumor progression or by pseudoprogression. Unlike true tumor progression, MRI signal changes in pseudoprogression reflect treatment-induced inflammatory change with increased permeability of the BBB. These changes stabilize spontaneously and are associated with a favorable prognosis (Fig. 14). The inability to differentiate tumor progression from pseudoprogression can lead to the continuation of ineffective therapy or early discontinuation of chemotherapy; it can cause inclusion of patients with pseudoprogression in experimental protocols, with further false-positive response to experimental treatment. Two recent studies showed the benefit of perfusion MRI with ferumoxytol for CBV assessment in the differential diagnosis of true tumor progression and pseudoprogression (Gahramanov et al., 2013; Gahramanov, Raslan, et al., 2011). We introduced dual-contrast imaging during a single MRI session: GBCA for BBB integrity assessment and ferumoxytol for CBV assessment. Dual-contrast imaging may be the beginning of a multicontrast imaging era when different contrast agents are applied for specific purposes, to confirm or rule out certain tumor types, establish the presence and magnitude of inflammation, or evaluate angiogenesis (Gahramanov et al., 2013; Gahramanov, Raslan, et al., 2011).

Dynamic MRI with ferumoxytol is a major advance toward the goal of delineating tumor response, but the technique has significant limitations. Because the entire brain must be scanned in a very short time frame (2 s) the images are necessarily low resolution. We have utilized the strong susceptibility effect of ferumoxytol to develop a new steady-state technique for imaging the cerebrovasculature. Our aim is to improve CBV maps by substantially eliminating image distortion and increasing resolution. We found that high-resolution CBV maps can be achieved using clinically applicable doses of ferumoxytol and in comparison with DSC-CBV (Fig. 15). The high spatial resolution and distortion-free parametric maps will help differentiate active tumor from necrotic tissue and better localize most malignant tumor regions, therefore increasing accurate targeted biopsy (Christen et al., 2013; D'Arceuil et al., 2013; Varallyay et al., 2013).

6. Conclusion

Many important observations regarding BBBD have been made in animal studies. For example, (1) a marked increase in brain and CSF concentrations of MTX were documented after BBBD with IA chemotherapy administration (Kroll & Neuwelt, 1998; Neuwelt, 1989; Neuwelt, Frenkel, Rapoport, & Barnett, 1980), (2) disruption of the BBB provides global delivery throughout the disrupted hemisphere, but delivery is variable depending on the

brain region and the type and size of tumor, (3) vascular permeability to small molecules such as MTX, as well as large molecules such as mAbs, is increased maximally by 15 min after mannitol infusion, and (4) BBB permeability rapidly decreases, returning to preinfusion levels within 2 h after BBBD.

Delivery of chemotherapy in conjunction with BBBD has provided excellent clinical outcomes especially in PCNSL. Long-term follow-up has shown that patients with this disease treated with enhanced chemotherapy delivery can maintain stable if not improved cognitive function and quality of life. The addition of rituximab to chemotherapy with BBBD treatment for PCNSL can be safely accomplished with excellent OS. The use of delayed high-dose STS has shown hearing protection in patients undergoing treatment with carboplatin-based chemotherapy when administered with BBBD. This approach laid the groundwork for chemoprotection studies using thiols such as STS and NAC to protect against cisplatin-based hearing loss in children.

Neuroimaging using dynamic MRI techniques and the new steady-state MRI technique using ferumoxytol will have important implications for monitoring patient outcomes and response to therapy. These techniques improve the detection of pseudoprogression, a treatment-induced inflammatory response that is associated with markedly improved survival. The measurements of tumor blood volume clearly differentiate pseudoprogression from true tumor progression, and pseudoresponse (decreased tumor vascular permeability) from true response to therapy. One of the major implications is that patients with pseudoprogression will continue with their effective therapy and will not be placed in trials of experimental therapy. We anticipate that improved neuroimaging will be incorporated into standard of care for assessing therapy-induced changes in brain tumor vasculature, improving detection of brain tumor response to therapy, and differentiating CNS lesions with an inflammatory component.

BBBD treatment is not without challenges. The procedure should be undertaken only by trained multidisciplinary teams at centers where neuro-oncology, interventional neurosurgery/neuroradiology, neuroanesthesia, and experienced oncology nursing are available. Uniform chemotherapy protocols and comprehensive guidelines for anesthesia, transfemoral arterial cannulation, mannitol/chemotherapy infusion, pre- and post-BBBD procedure patient care, and follow-up cognitive and neuroimaging assessment must be followed by the trained teams. Nonetheless, BBBD has been safely conducted at several institutions, an active BBBD Consortium is in place, and scientific meetings are convened annually to discuss preclinical and clinical advances in delivering agents across the BBB. Regarding imaging, ferumoxytol has not yet received FDA approval for neuroimaging, and image analysis is not yet standardized. However, processes are currently well underway to turn these goals into reality.

Future directions include preclinical and clinical dose intensification of platinum-based chemotherapeutics with thiol chemoprotection and further development of steady-state MRI techniques with ferumoxytol that provide high-resolution images of CNS lesions to improve rCBV measurements and measure absolute CBV. In addition, further preclinical and clinical investigations of the role of mAbs (1) as first-line treatment for PCNSL, (2) as maintenance

immunotherapy as a mechanism to improve clinical progression-free and OS in PCNSL, and (3) for the prevention of brain metastases are planned.

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Abbreviations

BAT	brain around tumor BBB, blood–brain barrier
BBBD	blood–brain barrier disruption
BDT	brain distant to tumor
BTB	blood–tumor barrier
CNS	central nervous system
CR	complete response
CSF	cerebrospinal fluid
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCE	dynamic contrast-enhanced
DSC	dynamic susceptibility-weighted contrast-enhanced
GBCA	gadolinium-based contrast agent
IA	intraarterial
IV	intravenous
mAb	monoclonal antibody
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MTX	methotrexate

NAC	N-acetylcysteine
OS	overall survival
PCNSL	primary central nervous system lymphoma
PFS	progression-free survival
PNET	primitive neuroectodermal tumor
rCBV	relative cerebral blood volume
SPECT	single photon emission computed tomography
STS	sodium thiosulfate
WBRT	whole brain radiotherapy

Figure 1.

The anatomical basis of osmotic blood–brain barrier disruption. (A) Lanthanum passage from cerebral capillary lumen to neuropil prevented by a tight junction between two endothelial cells. (B) Opening of tight junction between endothelial cells to peroxidase tracer, by intracarotid perfusion of 3 M urea in the rabbit. Arrows indicate tight junctions; BM, basement membrane; L, lumen. *Panel (A) is reprinted with permission from Brightman and Reese (1969), © 1969 Rockefeller University Press. Panel (B) is reprinted with permission from Brightman, Hori, Rapoport, Reese, and Westergaard (1973).*

Figure 2.

Delivery of nanoparticles across the blood–brain barrier (BBB). Normal rats received hyperosmotic mannitol to open the BBB, followed by intraarterial administration of the iron oxide nanoparticles ferumoxytol (A) or ferumoxides (B, C). After ferumoxytol administration, MRI signal intensity peaks by 24 h then fades over 3 to 7 days as the nanoparticles are metabolized (A, T1W indicates T1-weighted MRI). In contrast, ferumoxides induce signal dropout in the rat brain for over a month (B, T2* indicates T2*-weighted MRI). Electron microscopy shows that ferumoxides particles (Fe) are trapped between the vascular endothelial cells and the basement membrane after BBB disruption. *Panels (A) and (B) are adapted with permission from Lippincott Williams and Wilkins/ Wolters Kluwer Health: Neurosurgery (Muldoon et al., 2005), ©2005. Panel (C) is modified with permission from Muldoon et al. (1999), © by American Society of Neuroradiology.*

Figure 3.

MRI and histology of the rat CNS lymphoma model. Intracerebral implantation of human MC116 B-lymphoma cells forms an infiltrative brain tumor that shows minimal leakage to gadolinium contrast on T1-weighted MRI (A). Prominent hyperintensity on T2-weighted MRI (B) correlates with tumor extent as determined by immunohistochemistry for CD20 (C). The tumor shows infiltration along fiber tracts and around blood vessels distant to the tumor mass (D and E). *Panels (A)–(C) are reprinted with permission from Jahnke et al. (2009) by the permission of Society for Neuro-Oncology. Panels (D) and (E) are reprinted from Soussain et al. (2007).*

Figure 4.

Blood–brain barrier disruption (BBBD) increases antibody delivery in the rat model of CNS lymphoma. Rats with intracerebral MC116 xenografts received ^{90}Y -ibritumomab intravenously with or without BBBD. (A) Radiolabel in tumor, brain around tumor (BAT), ipsilateral brain distant to tumor (BDT), and contralateral left hemisphere (LH), was determined at 10 min after antibody administration. There was a significant effect of BBBD in the repeated measures ANOVA model ($P=0.0361$). (B) Radiolabel localized in BDT is shown at 10 min, 24 h, and 3 days after antibody administration. *Reprinted from* Muldoon, Lewin, et al. (2011).

Figure 5.

Rituximab increases survival in the rat model of CNS lymphoma. Rats with MRI-confirmed MC116 CNS lymphoma were randomized to treatment groups and followed for survival. The Kaplan–Meier survival curves show that methotrexate (MTX) was ineffective in this model ($P=0.18$), whereas all rituximab (RTN) groups improved survival compared with control (RTN BBBD [blood–brain barrier disruption], $P=0.013$; RTN +MTX BBBD, $P=0.0042$; RTN IV [intravenous], $P=0.0049$). *Reprinted from Muldoon, Lewin, et al. (2011).*

Figure 6.

Overall survival (OS) in patients newly diagnosed with primary CNS lymphoma according to risk groups. OS is from the date of first intraarterial in conjunction with blood-brain barrier disruption treatment stratified by age and Karnofsky Performance Score (KPS). Low risk, age younger than 60 years with KPS ≥ 70 (47 patients); moderate risk, age older than 60 years with any KPS or age younger than 50 years with KPS <70 (89 patients); high risk, age 50 to less than 60 years with KPS <70 (13 patients) ($P>0.0001$). In low-risk patients, median OS was approximately 14 years, with a survival plateau after approximately 8 years. Symbols on lines indicate censored observations. *Reprinted with permission from Angelov et al. (2009), © 2009 American Society of Clinical Oncology.*

Figure 7.

^{111}In -ibritumomab to assess delivery and ^{90}Y -ibritumomab to assess efficacy in refractory primary CNS lymphoma. (A) Brain MRI, gadolinium-enhanced T1 axial view, 11 days prior to ^{111}In -ibritumomab showing large enhancing tumor in the genu of the corpus callosum. (B) Brain axial SPECT image, 48 h after 5.2 mCi of ^{111}In -ibritumomab showing uptake in the genu of the corpus callosum. (C) Brain MRI, gadolinium-enhanced T1 axial view, 8 weeks after 5.2 mCi of ^{111}In - and 23.1 mCi of ^{90}Y -ibritumomab showing progressive disease distant from the genu of the corpus callosum. *Reproduced with permission from Doolittle et al. (2007), © 2007 Informa Healthcare.*

Figure 8.

Long-term cognitive outcomes in patients treated with blood-brain barrier disruption (BBBD). Patients with newly diagnosed primary CNS lymphoma (PCNSL) were treated with intraarterial (IA) methotrexate-based chemotherapy with BBBD. The median time from diagnosis to long-term evaluation was 12 years (range: 2–26 years). (A) Raw cognitive test scores were converted to z-scores based on the normative values demographically adjusted to age. A z-score is the number of standard deviations above or below the mean for a population of similar age. A domain score was obtained by averaging all test z-scores in each domain, for each participant. The z-scores (mean, $\pm$ SD) across survivors at baseline (pretreatment), long-term follow-up, and the change score are shown. The asterisks indicate statistical significance. There was improvement in Trail-making A, $P=0.0085$; Trail-making B, $P=0.0411$; and attention/executive function domain, $P<0.001$. (B) The z-scores (mean, $\pm$ SD) across the PCNSL survivors at baseline, long-term follow-up, and the change score for verbal memory, learning; verbal memory, delayed; and verbal memory domain are shown. There was no significant change from baseline to long-term follow-up. (C) Number of PCNSL patients declined (z-score declined one SD or more), stable (z-score remained within one SD of baseline score), and improved (z-score improved one SD or more) from baseline to long-term for the following tests: digit span forward, digit span backward, trail making a, trail making b, verbal memory learning, and verbal memory delayed. *Reprinted with permission from Doolittle, Dosa, et al. (2013), © 2013 American Society of Clinical Oncology.*

Figure 9.

Long-term neuropsychological outcomes in primary CNS lymphoma survivors, according to the treatment type. Neuropsychological domain (attention/executive function, verbal memory, and motor skills) and neuropsychological composite z-score results (crude mean, SD) are shown, according to the following treatment groups: HDMTX (high-dose methotrexate) alone; HDMTX IA (intraarterial) with BBBB (blood-brain barrier disruption); HDMTX+HDT/ASCT (high-dose chemotherapy with autologous stem cell transplantation); and HDMTX+WBRT (whole brain radiotherapy) group. A z-score is the number of standard deviations above or below the mean for a population of similar age. Asterisks indicate a statistically significant difference ($P < 0.05$) between the WBRT group and the non-WBRT groups in attention/executive function, motor skills, and composite score. *Reprinted with permission from Doolittle, Korfel, et al. (2013).*

Figure 10.

Effect of intetumumab anti- α_v integrin mAb on breast cancer brain metastasis in the rat. (A) MRI shows that the control rat had multiple metastases at 7 weeks (top; arrows). The intetumumab-treated rat showed no metastases at 7 weeks and 2 lesions at 11 weeks (bottom; arrows). Histochemistry for human mitochondrial antigen showed 15 ± 9 metastases in control rats (B) and 4 ± 5 metastases in intetumumab-treated rats (IV INT, intravenous intetumumab) (C). *Material was originally published in Wu et al. (2012). Reprinted with kind permission from Springer Science and Business Media.*

Figure 11.

Effect of dose and route of administration on N-acetylcysteine (NAC) chemoprotection. (A) Nephroprotection. Rats received a nephrotoxic dose of cisplatin followed in 4 h by no NAC or NAC 400 mg/kg given by oral or IV route of administration. NAC significantly reduced cisplatin-induced kidney toxicity, when given by IV but not by oral route of administration. (B) NAC pharmacology. Serum NAC concentrations were measured by HPLC 15 min after IV or oral administration. Chemoprotective doses of NAC (400–1000 mg/kg) gave peak blood concentrations of 2 mM or greater only when administered IV and were not effective when administered intraperitoneal or oral. *Panels (A) and (B) were originally published in Dickey et al. (2008), © with kind permission of Springer Science and Business Media, adapted figure 4.*

Figure 12.

Sodium thiosulfate (STS) shows hearing protection in adults with malignant brain tumors. Comparison of hearing threshold shift against carboplatin treatment number, at 4000 Hz, in historical comparison brain tumor patients who were treated with carboplatin (intraarterial [IA]) with blood–brain barrier disruption (BBBD) without STS and brain tumor patients treated with delayed STS 2 h (STS2) or 4 h (STS4) after carboplatin (IA) with BBBD. There was a significant difference in hearing protection between the STS treatment groups and the historical comparison group ($P=0.0075$). *Reprinted from Doolittle, Muldoon, et al. (2001).*

Figure 13.

Effect of intetumumab and bevacizumab on brain tumor vasculature. Rats with intracerebral LX-1 SCLC xenografts were randomized to no treatment, intetumumab anti- α_v integrin mAb, or bevacizumab anti-VEGF mAb. Rats underwent serial dynamic contrast-enhanced MRI with gadolinium-based contrast agent to evaluate vascular permeability (A) and dynamic susceptibility-weighted contrast-enhanced MRI with ferumoxytol to evaluate relative cerebral blood volume (rCBV) (B). Bevacizumab decreased vascular permeability and rCBV within 24 h, while intetumumab increased both permeability and rCBV over a week following treatment. *Reprinted from Muldoon, Gahramanov, et al. (2011) by the permission of Society for Neuro-Oncology.*

Figure 14.

Survival of glioblastoma multiforme patients according to the relative cerebral blood volume (rCBV) cutoff value >1.75 . Brain tumor rCBV was measured using fer-umoxytol vs. gadoteridol. Kaplan–Meier survival curves show the best survival prediction by using rCBV values obtained with ferumoxytol ($P<0.001$). By using gadoteridol, survival prediction is similar but not statistically significant. *Reprinted with permission from Gahramanov et al. (2013).*

Figure 15.

Comparison of steady-state-cerebral blood volume (SS-CBV) and dynamic susceptibility contrast (DSC)-CBV maps in a glioblastoma patient. (A) T1-weighted post-gadoteridol scan describes the multifocal signal abnormalities. In corresponding slices, the SS-CBV (B) and DSC-CBV (C) maps show increased areas of CBV referring to highly vascular tumor areas. Note the mismatch between the most enhancing region (arrow) and the highest CBV values. *Reprinted from Varallyay et al. (2013).*