

# New Frontiers in Translational Research in Neuro-oncology and the Blood-Brain Barrier: Report of the Tenth Annual Blood-Brain Barrier Disruption Consortium Meeting

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## ABSTRACT

**The blood-brain barrier (BBB) presents a major obstacle to the treatment of malignant brain tumors and other central**

nervous system (CNS) diseases. For this reason, a meeting partially funded by an NIH R13 grant was convened to discuss recent advances and future directions in translational research in neuro-oncology and the BBB. Cell biology and transport across the BBB, delivery of agents to the CNS, neuroimaging, angiogenesis, immunotherapy, and gene therapy, as well as glioma, primary CNS lymphoma, and metastases to the CNS were discussed. Transport across the BBB relates to the neurovascular unit, which consists not only of endothelial cells but also of pericyte, glia, and neuronal elements.

## INTRODUCTION

Although recent basic science advances in understanding mechanisms of the blood-brain barrier (BBB) have been substantial, much research remains to be done on translating cellular mechanisms to improve the treatment of malignant brain tumors and other neurologic diseases. A meeting was convened on March 17 to 20, 2004 in Sunriver, Oregon, to discuss recent preclinical and clinical research in neuro-oncology and the BBB. More than 120 basic science and clinical researchers from 50 institutions attended the meeting. Seventy-five attendees presented their research findings. Senior scientists provided overviews and led discussions on advances and future directions in specific neuro-oncology and BBB topics, then summarized the discussions for this meeting report. The cell biology of central nervous system (CNS) endothelial tight junctions is shown in Fig.1; clinical implications of the BBB are the topics of recent reviews (1, 2).

## BLOOD-BRAIN BARRIER: CELL BIOLOGY AND TRANSPORT

Recent cellular studies of the BBB have focused on (a) molecular properties of transport systems in both luminal and abluminal plasma membranes, (b) new and refined *in vitro* models, (c) regulation of tight junctions, (d) cellular mechanisms of disease, and (e) methodologies for global analyses of BBB function.

It was discussed that a molecular approach to defining transport properties of the BBB has improved our understanding of organic nutrient delivery to the brain, fluid and electrolyte homeostasis, the transcytotic process, and the role of efflux transporters (1, 2). Net fluid influx and brain extracellular fluid homeostasis may be regulated by hormones produced in the CNS that influence blood-brain transport. Transcytosis of insulin and transferrin has been well defined, and these pathways have been utilized for targeted delivery to the brain and brain tumors. The presence of active efflux transporters in the BBB prevents many systemically administered drugs from entering the brain and is a major obstacle in designing drugs to treat neurologic disorders (3). In addition, efforts have been directed at developing

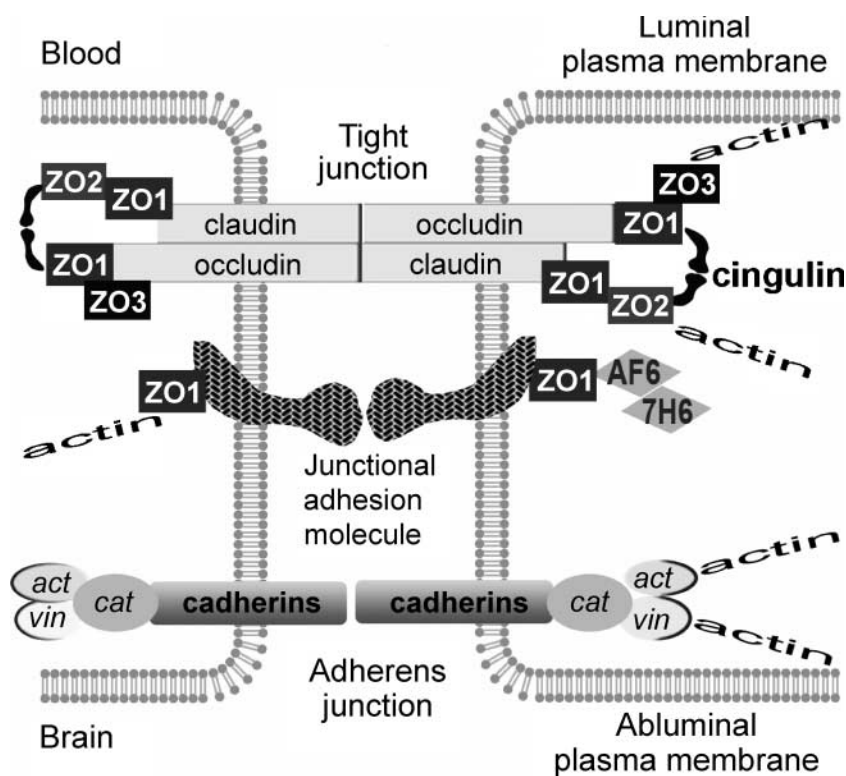
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*Fig. 1* Molecular model of BBB structures. The major proteins of the BBB tight junctions are the transmembrane claudins and occludins, which dimerize with opposite members on adjacent endothelial cells. Zonula occludens protein ZO1 binds both claudins and occludins as well as the less well characterized junctional adhesion molecule. Additional structural support for the tight junction is provided by zonula occludens proteins ZO2 and ZO3, the cingulin linking protein, and the cytoskeletal protein actin. Adherens junctions consist of transmembrane cadherin proteins linked to  $\alpha$ ,  $\beta$ , and  $\gamma$  catenins (*cat*). Structural support for adherens junctions is provided by  $\alpha$ -actinin (*act*) and vinculin (*vin*) linking to actin. AF6 and 7H6 are proteins of unknown function associated with ZO1. Reprinted with permission from Elsevier.<sup>1</sup>

better *in vitro* models to simulate BBB function in a highly controlled environment.

The relationship between the cytoskeleton and tight junctions apparently allows for regulated alterations in BBB function by modifying paracellular transport (1). Recent data indicate that abnormal permeability changes accompanying ischemia and reperfusion may utilize calcium-dependent processes that alter cytoskeletal and tight junctional functions. More importantly, a cellular and molecular approach to studying the brain endothelium has enabled characterization of specific mechanisms for a variety of pathologic states. Diseases may have different initiating mechanisms, but a single common pathway to final pathology (2). Apoptosis seems to play a key role in seemingly divergent conditions such as ischemia-reperfusion injury (stroke), multiple sclerosis, and CNS tumors. This implies common pathways including mitochondrial damage and caspase activation.

Meeting participants agreed that a major area to be addressed will be regulatory mechanisms of transport across the brain endothelial cell. The differences in endothelial cell properties as a function of development, anatomic location, and response to disease need to be ascertained. These advances will have major importance in neuropharmacokinetics and drug delivery (3). Progress is being made in applying genomics and proteomics to BBB function and disease (4). A highly structured experimental approach using proteomics may lead to identification of specific cellular mechanisms and therapeutic targets. Future studies will likely consider the "neurovascular unit," defined as the integration of multiple components including the endothelial cell, pericyte, basement membrane, and surrounding glial and neuronal elements. The neurovascular unit is dependent on specific expressed genes and gene products.

## BLOOD-BRAIN BARRIER IN DISEASE

Although a compromised BBB has been reported under numerous pathologic conditions, the role of a disrupted BBB in the pathogenesis of neurologic illnesses is mostly unknown. Alteration of the barrier tight junctions is a hallmark of many CNS pathologies, including tumor, stroke, HIV, encephalitis, Alzheimer's disease, multiple sclerosis, vasogenic edema, and bacterial meningitis (2).

Mechanisms for BBB breakdown (or tight junction protein rearrangement) seem to involve both direct and indirect effect of stress responses and inflammatory mediators. Huber et al. (1) and Witt et al. (5) have used *in vitro* and *in vivo* methodologies in concert to examine hypoxia/post-hypoxic reoxygenation to reveal alterations in the BBB tight junctional protein complex (Fig. 1), resulting in increased paracellular permeability. Transcription factor alterations have also been noted that indicate significant BBB activation/compensatory mechanisms resulting from hypoxia/post-hypoxic reoxygenation in both *in vitro* and *in vivo* models. Defining disruption of the BBB under these conditions may provide potential targets for new treatment paradigms.

Data described by Friedman et al. at the meeting suggest that long-lasting BBB opening may result in astrocytic dysfunction followed by epileptogenesis. Janigro et al. (6) described data showing that S100- $\beta$  is directly related to the extent and temporal sequence of hyperosmotic blood-brain barrier disruption (BBBD), suggesting that S100- $\beta$  is a marker of BBB function and that elevated S100- $\beta$  may indicate the presence of radiologically detectable BBB leakage. Skold et al. described that traumatic brain injury leads to an up-regulation of cell vascular endothelial growth factor (VEGF), VEGF receptor 1 (VEGFR1) and VEGFR2 mRNA and protein in and around the

lesion. This finding provides important knowledge about the mechanisms of posttraumatic angiogenesis and new potential targets for therapeutic interventions.

There was consensus that knowledge of preclinical BBB models should be applied to human diseases. Changes in neurovascular status and in regional BBB permeability and transport systems should be incorporated in clinical thinking to better understand neurologic disease pathophysiology and disease-specific differences in BBB cellular mechanisms.

## DELIVERY OF THERAPEUTICS TO THE CENTRAL NERVOUS SYSTEM

Several strategies were discussed at the meeting for improving drug delivery to brain tumors through circumvention of the BBB (7, 8). There was agreement that one approach that offers considerable promise is selective inhibition of multidrug-resistance transporters, such as P-glycoprotein, multidrug resistance-associated protein, and/or breast cancer resistance protein. P-glycoprotein and breast cancer resistance protein are highly expressed at the BBB and act to markedly restrict brain and brain tumor uptake of a broad range of anticancer drugs (9). Inhibition of active efflux transport has been shown to increase both CNS anticancer drug delivery and therapeutic efficacy. For example, Fellner et al. (10) showed a 90% reduction in tumor volume in nude mice implanted with intracerebral human glioblastoma when treated with paclitaxel together with the second-generation P-glycoprotein pump inhibitor, valsopodar (PSC 833). In the absence of valsopodar, paclitaxel was without effect on brain tumor volume. Similarly Kemper et al. (11) reported a 5-fold elevation in brain paclitaxel uptake in mice treated with the third-generation P-glycoprotein inhibitor elacridar (GF 120918). However, efflux transport inhibition can compromise chemotherapeutic drug clearance from plasma, resulting in elevated plasma anticancer drug concentration and increased anticancer drug toxicity. Furthermore, efflux transporters have critical roles in protecting the brain from a wide range of potentially toxic molecules (3). Thus, patients on efflux transporter blockade therapy will need to be closely monitored. Several third-generation multidrug resistance reversal agents have been developed (e.g., biricodar, elacridar, and zosuquidar) that are reported to have less effect on anticancer drug pharmacokinetics than the earlier agents (12).

Convection-enhanced delivery, by increasing convective fluid flow within the brain interstitial space, distributes the drug over a greater volume than would be achievable by simple diffusion. Furthermore, it provides a means of delivering protein immunotoxin and nucleic acid therapeutics to the brain. However, the approach is invasive, cannot deliver drug over the entire brain, and may result in variable drug delivery in brain regions in which the BBB is compromised, or in tumors (13).

## CHEMOPROTECTION AND PEDIATRIC TUMORS

Chemoprotectants may have a role in increasing chemotherapy dose intensity. Preclinical and clinical studies of hearing and bone marrow chemoprotection have been done with thiols,

which are small-molecular-weight agents with reactive sulfur groups. Several issues seem to be important for chemoprotection.

(a) *Dose of chemoprotectant:* N-Acetylcysteine (NAC) requires 400 to 1200 mg/kg, yielding 2 to 15 mmol/L peak blood concentration, for chemoprotection in various animal models (14, 15). Clinically, sodium thiosulfate is given at 20 g/m<sup>2</sup> for otoprotection (16).

(b) *Route of administration:* An aortic infusion method enhances bone marrow chemoprotection with thiols (15, 17), whereas i.v. or intra-arterial delivery may target chemoprotection to other organs or brain (14).

(c) *Timing:* Sodium thiosulfate is otoprotective when given 4 hours after carboplatin in patients (16) and when given up to 8 hours after cisplatin in rats. NAC is bone-marrow protective only if given before alkylating chemotherapy but shows otoprotection when given 4 hours after cisplatin (14).

(d) *Isomer:* For chiral thiols, such as D- versus L-methionine, or L- versus D-NAC, the isomer may be important for cellular uptake and/or metabolism that affects chemoprotection.

(e) *Genetic factors:* No high-frequency alleles with significant effect conferring susceptibility to chemotherapy-induced hearing loss have been detected; however, genes in glutathione and antioxidant pathways may have an effect.

A major issue is the potential to interfere with antitumor efficacy. A rat brain tumor study showed no change in chemotherapeutic efficacy when NAC was given before chemotherapy and/or when sodium thiosulfate was given 4 and 8 hours after chemotherapy (15). Amifostine (WR-2721) has the advantage of selectively targeting nontumor cells, thus avoiding chemoprotection of cancer, including CNS malignancies (18). However, results from a recently closed Children's Oncology Group protocol indicate amifostine lacks otoprotective activity. This may have been because very little amifostine is able to penetrate the intact BBB and the blood-inner ear barrier.

There was consensus that new clinical trials should be developed to address the potential for chemoprotection in pediatric patients with cancer. Chemoprotection of normal brain may reduce adverse neurotoxic effects of chemotherapy or ionizing radiation, especially in children in whom protection of the vulnerable developing CNS is a greater challenge. A phase I trial was proposed for protection of normal brain from cranial radiotherapy with the use of intrathecal amifostine. In a proposed phase III Children's Oncology Group trial, delayed sodium thiosulfate will be evaluated in pediatric histologies treated with cisplatin chemotherapy. This trial will determine whether otoprotection can be achieved in the pediatric population without reducing antitumor efficacy.

## ANGIOGENESIS AND ANGIOLYSIS

Angiogenesis is important to the survival of many tumors including gliomas (19). Antiangiogenesis therapy can theoretically overcome two problems in neuro-oncology: drug delivery and drug resistance (20). Normal nonmalignant endothelial cells are presumed to be genomically stable, whereas actively

proliferating angiogenic vessels are associated with the multidrug resistance (*MDRI*) phenotype (21).

Brain tumor angiogenesis has traditionally involved the tumor cell and the endothelial cell. Dore-Duffy showed the importance of the CNS microvascular pericyte in regulation of angiogenesis (22). Using a three-dimensional culture system, they showed that primary endothelial cells are unable to form new vessels in the absence of pericytes; however, the addition of pericytes to endothelial cell cultures resulted in three-dimensional verified vessels. Furthermore, unlike normal pericytes, pericytes in microvessels of glioblastoma express VEGF mRNA and constitutively express VEGF protein. Under normal conditions, translation of pericyte VEGF protein is under tight regulation, whereas in the tumor-altered pericyte, *VEGF* gene expression may perpetuate angiogenesis in malignant brain tumors.

Endothelial progenitor cells derived from bone marrow can migrate to brain tumor and control angiogenesis (23). At the meeting, Glod showed that a subset of CD14<sup>+</sup> cells could form a functional barrier characteristic of the cerebral endothelium. These endothelial-like monocytic cells incorporate themselves into the neovascular network in xenografts of brain tumors. Nag and colleagues reported on angiopoietin-1 and -2 regulation of vascular homeostasis (24). The angiopoietins function as ligands for endothelial cell-receptor-specific tyrosine kinase Tie-2. As a key mediator of breakdown of the BBB, angiopoietin-1 maintains vascular hemostasis and is antiapoptotic, whereas angiopoietin-2 promotes apoptosis after traumatic brain injury.

Brem presented two novel pharmacologic inhibitors of angiogenesis,  $\alpha$ -amyloid and GFA-116 (25). GFA-116, a low molecular weight inhibitor of angiogenesis, blocks experimental growth and metastatic spread of experimental tumors and will be evaluated for the treatment of brain tumors.

Angiolytic is a new approach to therapy that exploits the structural differences between tumor vasculature and normal brain vasculature. Antagonists against cell adhesion molecules, such as VE-cadherin, which mediates endothelial cell interactions, and N-cadherin, which mediates endothelial cell-pericyte interactions, may cause rupture of tumor blood vessels. Studies are in progress using serial magnetic resonance imaging (MRI) with high and low molecular weight contrast agents (ferumoxtran-10 versus gadolinium) to evaluate the effect of the angiolytic agent Exherin (Adherex Technologies Inc, Durham, NC) on the growth and permeability of rat brain tumor models.

Future directions include improved methods of imaging angiogenesis and surrogate markers to determine optimal dosage and efficacy of antiangiogenesis drugs in clinical trials. Angiogenesis inhibitors may be most effective in combination with chemotherapy (21) or with other angiogenesis inhibitors (26). These inhibitors normalize the tumor's vascular supply, making cells less hypoxic, decreasing expression of *MDRI*, and potentially making brain tumors more susceptible to drug and radiation therapy.

## GLIOMA

Translational work in gliomas has led to promising new targeted therapies such as the epidermal growth factor antagonists and their use as sole agents or as part of combination

therapy. The small molecular weight inhibitor OSI774 is currently being evaluated in a phase II trial with recurrent glioblastoma multiforme patients and has shown some promise; however, mechanisms of failure need further evaluation. This class of drugs is also being evaluated as a radiosensitizer in combination therapy with temozolomide and radiation. The protein-based agent herstatin, which blocks receptor dimerization and activation of epidermal growth factor receptors, has been evaluated in a rat glioblastoma model. Herstatin blocked growth of intracerebral glioma expressing the normal epidermal growth factor receptor, whereas *in vivo* and *in vitro* growth of cells expressing a mutant constitutively active epidermal growth factor receptor were resistant to herstatin (27).

Recent advances in molecular analysis of gliomas have begun to affect treatment strategies (28). For oligodendrogliomas that are 1p19q deleted, the emphasis has moved to treatment with temozolomide up front and using radiation as salvage therapy.

From a glioma modeling perspective, standardization of an animal model would be beneficial. Fortin et al. discussed a tumor implant model using F98 astrocytic cell lines syngeneic to Fischer rats, which showed predictable and reproducible tumor growth. There was consensus that more adult patients with glioma should be placed on clinical trials whenever possible to match the enrollment progress in pediatric tumor clinical trials.

## IMMUNOTHERAPY AND THE BLOOD-BRAIN BARRIER

Malignant gliomas have been difficult to treat with immunotherapy because of their immunosuppressive nature and the immune privilege provided by the BBB. Immunotherapy for malignant gliomas includes targeted toxin therapy and anticancer vaccines (29). Targeted toxins have been primarily administered against malignant gliomas because of their ability to be administered directly into target tissue by convection-enhanced delivery, thereby bypassing the BBB. These agents are well developed and are currently under investigation in phase III clinical trials that target either the interleukin-13 or transferrin receptor. Despite the mature nature of these trials, there are still concerns regarding basic issues such as the optimal number of catheters for infusion and location for catheter placement. It is also unclear whether slow flow (1  $\mu$ L/h) or fast flow (10  $\mu$ L/h) better influence the distance of distribution of drugs such as doxorubicin or a transferrin-doxorubicin conjugate. Anticancer vaccines have been developed for a variety of cancers and have shown both safety and efficacy in early-phase clinical trials including those against malignant gliomas. In malignant gliomas, initial vaccine trials have been dendritic cell-based or have used genetic engineering to modify cytotoxic T-lymphocytes (CTLs) that recognize the interleukin-13  $\alpha$ 2 receptor expressed on malignant glioma cells (30).

Transmigration of virally infected monocytes and T cells across the BBB plays a major role in HIV infection of the brain. Enhanced matrix metalloproteinase (MMP) expression is seen with HIV infection and the statins may reduce the migration of cell-associated virus into the brain by suppressing MMP expression. In animals infected with the simian immunodeficiency virus, the identification of macrophages or T cells that



produce specific cytokines such as interleukin-6, tumor necrosis factor  $\alpha$ , and IFN- $\gamma$  may help elucidate the nature of the immune system in the brain.

## GENE THERAPY

Initial gene therapy approaches for brain tumor therapy involved therapeutic, gene-expressing, replication-incompetent viral vectors; this approach has proven inefficient. Mechanisms of tumor selectivity have included the use of (a) viral gene deletion mutants targeting cancer-related pathways, (b) tumor/tissue-specific promoters to control viral replication, and (c) viral coat modifications to engineer tumor-selective uptake. The results of early clinical trials with G207 and the challenges for future study were discussed by Martuza (31). Unlike for other cancer therapeutics, the BBB or even the blood-tumor barrier significantly limits delivery and efficacy. Rabkin et al. studied intracarotid injection of oncolytic herpes simplex virus vectors after osmotic BBBD for the treatment of metastatic tumors in the brain in mouse models. They showed that BBBD is necessary for efficient delivery of herpes vectors into brain tumors. Muldoon et al. have shown that the distribution of dextran-coated iron oxide particles the size of the adenoassociated virus in rat brain mimics adenovirus and herpes virus distribution, allowing MRI of virus delivery (32).

All gene therapy strategies depend on having defined targets. Stanimirovic et al. showed that unique “targets” including vascular targets for tumor-selective drug and gene therapy could be identified by molecular fingerprinting of glioblastoma vessels using a combination of laser capture microdissection, genomics, and proteomics (33). Mukhtar et al. described a new gene therapy vector for the brain based on spleen necrosis virus, which can integrate into nondividing cells. Pseudotyping with rabies virus glycoproteins allowed targeting to neural cells.

Jensen described a novel genetic engineering approach for redirecting the antigen specificity of CD8<sup>+</sup> cytolytic T cells to the glioma-restricted interleukin-13  $\alpha$ 2 cytokine receptor and the application of these technologies to a clinical adoptive therapy trial using autologous IL13Ra2-specific CTL clones. Genetic engineering was used to express varied luciferases in both tumor cells and lymphocytes to track cell migration using optical imaging.

As discussed at the meeting, a broad range of gene therapy approaches is being pursued. Their success will depend on further identification of molecular targets, efficient delivery to the brain, and imaging of the vector, gene expression, and disease end points.

## PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

Non-AIDS primary CNS lymphoma (PCNSL) is a rare type of extranodal non-Hodgkin's lymphoma that occurs exclusively in the brain, eyes, or spinal fluid. Although more than 90% of PCNSL is of B- cell origin, the molecular characteristics are poorly understood, in stark contrast to systemic non-Hodgkin's lymphoma. Preliminary evidence suggests that PCNSL shares some characteristics of the germinal center phenotype and that the expression of *bcl-6* may have prognostic importance (34). New techniques such as comparative genomic hybridization

offer the possibility of improving our understanding of the molecular phenotype of PCNSL.

The optimal treatment of PCNSL, particularly tumor localized in the eyes or cerebrospinal fluid space, remains controversial. There was consensus that the use of high-dose methotrexate-based chemotherapy with or without the addition of whole-brain radiotherapy (WBRT) is the most effective approach. However, disease control and overall survival are inferior to that seen in similarly aggressive extranodal non-Hodgkin's lymphoma suggesting that either the ability to administer therapeutic doses is limited by the blood-brain and blood-cerebrospinal fluid barrier or that there is an inherent biological resistance. Furthermore, PCNSL treatment carries a significant risk of neurotoxicity that is generally attributed to the combination of high-dose methotrexate and WBRT; however, some investigators have reported similar rates of neurotoxicity in patients treated with chemotherapy alone (35).

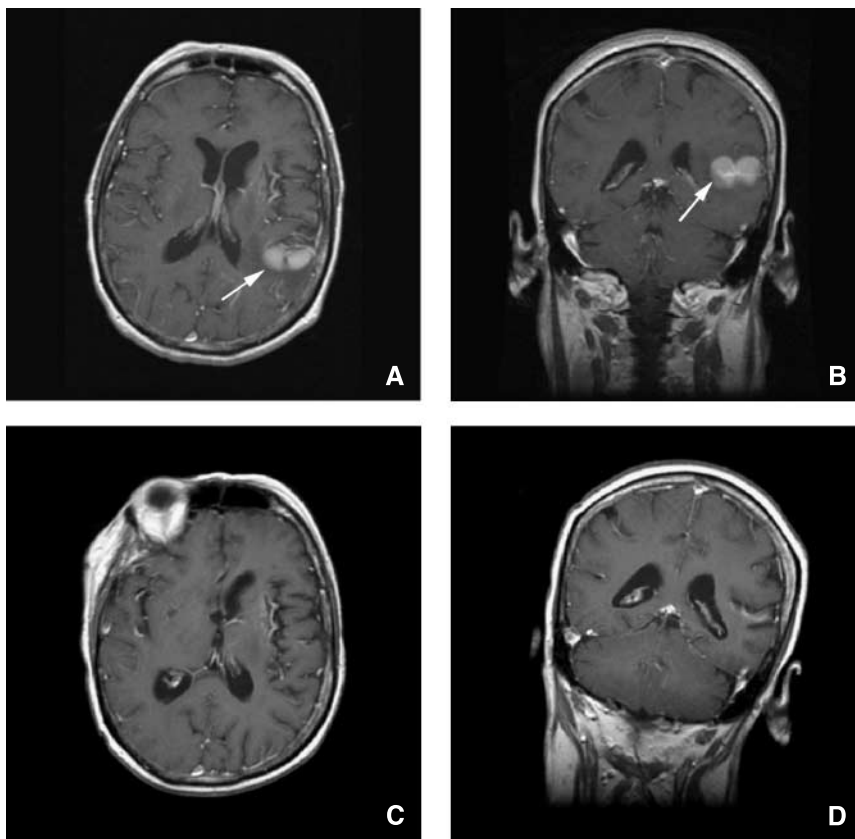
Many groups have sought to improve outcome by intensifying the delivery and dose of chemotherapy administered. BBBD, high-dose chemotherapy with autologous stem cell support, and use of monthly maintenance chemotherapy are all strategies that have been used with varying but comparable degrees of success (36–39) and are evaluated in ongoing trials. BBBD-enhanced methotrexate delivery has shown increased CNS dose intensity (40), with 86% of patients in complete response at 1 year demonstrating no cognitive loss (39). New Institutional Review Board-approved protocols are under way using BBBD to (a) deliver rituximab (an anti-CD20 monoclonal antibody) across the BBB in recurrent PCNSL (Fig.2) and (b) clear CNS relapse before systemic high-dose chemotherapy with peripheral blood stem cell rescue in patients with systemic non-Hodgkin's lymphoma. In an effort to eradicate disease involving the vitreal or cerebrospinal fluid compartment, investigators are exploring the delivery of chemotherapy directly into the eye (41) as well as the delivery of rituximab into the cerebrospinal fluid (42).

An international effort to improve the understanding and treatment of PCNSL has resulted in the establishment of the International PCNSL Collaborative Group (43). Efforts are under way to establish tissue databanks for molecular analysis, to standardize clinical trial reporting and to establish a prospective patient database.

## CENTRAL NERVOUS SYSTEM METASTASES

There was consensus that CNS metastases is an increasing clinical problem. The discussion on brain metastases emphasized (a) landmark and future therapeutic trials, (b) regional perfusion studies, and (c) the biology of brain metastases. For certain subgroups of patients, radiation sensitizers such as motexafingadolinium and efaproxiral with WBRT is superior to WBRT alone (44). Several randomized trials of chemotherapy alone or with WBRT have shown higher response rates but no survival advantage. Current studies will compare WBRT plus temozolomide or topotecan, respectively, with WBRT alone.

It was agreed that further trials of chemotherapy, particularly preirradiation, should be pursued. New phase I and II studies utilize intra-arterial chemotherapy, dose-intense temozolomide + vinorelbine (recurrent), temozolomide + thalidomide +



*Fig. 2* MRI of a subject with recurrent non-AIDs PCNSL (*arrows*), who previously failed methotrexate-based chemotherapy. *A* and *B*, axial T1 with gadolinium enhancement and coronal T1 views (January 27, 2004) before rituximab in conjunction with carboplatin-based BBBB treatment. *C* and *D*, the same sequences and views approximately 4 weeks after treatment with rituximab (February 25, 2004) demonstrating a complete response.

1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (melanoma) or intrathecal radioactive ligands (leptomeningeal carcinoma). Newton et al. (45) described results using intra-arterial carboplatin and i.v. etoposide in patients who had prior irradiation for a variety of brain metastases. Thirteen of 24 evaluable patients had objective responses (54.2%) with a median time to progression of 30 weeks in responders (range, 6-118 weeks), and the treatment was well tolerated (45). A study is under development for patients with metastatic breast cancer to the CNS, utilizing BBBB to deliver herceptin across the BBB in HER-2/*neu* positive patients. Participants agreed that quality of survival is paramount; therefore, clinical trials in CNS metastases must include quality of life as well as neuro-psychometric end points.

Studies of the regional behavior of brain metastases using dynamic perfusion computed tomography scans have correlated symptoms with altered regional perfusion during WBRT. Future studies will use dynamic perfusion MRI to better define the local behavior of brain metastases. Elucidation of the metastatic cascade as well as the genetic control of metastasis should lead to the development of rational and directed therapies. For example, discovery of the role of MMP in tumor cell invasion after tumor cells breach the BBB suggests a strategy using MMP inhibitors to inhibit tumor cell invasion. Such a strategy could be used as treatment, as an adjunct to initial definitive therapy, or as an adjunct to WBRT for established brain metastases. An important goal of future trials should be primary prevention or

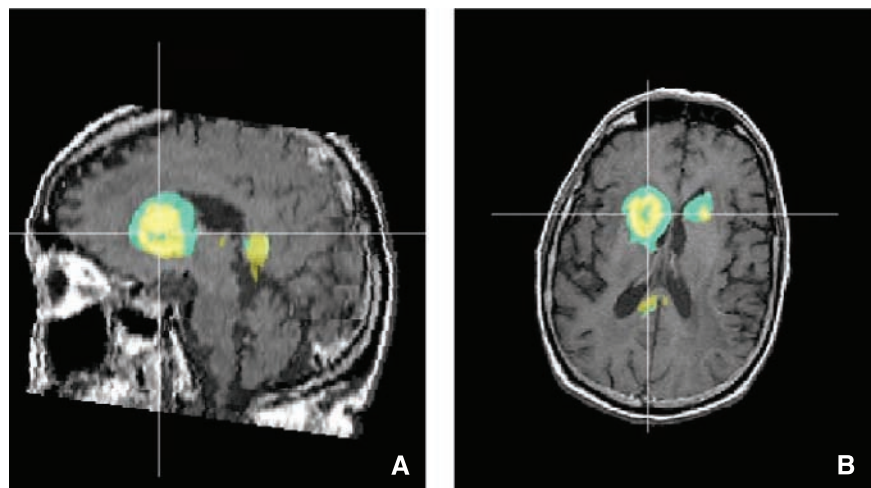
treatment of micrometastatic disease in the brain, a goal that will require delivery across an intact BBB.

## NEUROIMAGING

As neuroimaging techniques have developed and evolved they have become increasingly important in assessing the biological and physiologic properties of brain tumors. Imaging studies now allow for determination of important clinical parameters such as perfusion, metabolism, proliferative activity, and vascular permeability. Neuroimaging assessments, although important, have not provided information that has significantly improved brain tumor survival. The following advances are needed to affect survival: (*a*) the ability to image infiltrative disease, (*b*) better assessment of the true extent of disease and the actual tumor volume, and (*c*) molecular biological information relevant to treatment strategies.

An overview of the current state of brain tumor imaging included (*a*) recent data on fluorodeoxyglucose positron emission tomography (PET) and its prognostic capabilities, (*b*) new PET tracers and how they may be helpful in differentiating low-grade from high-grade tumors as well as differentiating radiation necrosis from recurrent tumor (46), (*c*) new MRI contrast agents that show superior image quality (47), and (*d*) a recent publication showing the utility of an optical imaging technique that could improve brain tumor delineation (48). Two presentations focused on high-field-strength MRI (8 T), which allowed for high-resolution imaging of tumor microvasculature and delineation of

**Fig. 3** Saggital (A) and transaxial (B) MRI views of two i.v. contrast agents (gadolinium and ferumoxtran-10), which do not cross the normal BBB, using coregistration by Brain Voyager QX (Brain Innovations, Maastricht, Netherlands) in a subject with PCNSL. Two different image sets are reconstructed. The enhancement of the two contrast agents is color coded, and the two image sets are aligned and added to each other. Yellow areas, enhancement with both gadolinium and ferumoxtran-10; green areas, enhancement only with ferumoxtran-10. The volume of enhancement of tumor is greater with ferumoxtran-10, a dextran-coated iron oxide crystal, which is superparamagnetic.



areas of increased cellularity and vascular proliferation. It was felt that this information could be used preoperatively in selecting sites to perform image-guided stereotactic biopsy.

In an animal imaging experiment at 8 T, it was shown that by using an ultrasmall iron oxide particle (USPIO) contrast agent there was additional improvement in imaging tumor microvasculature. In a separate presentation using another USPIO contrast agent in patients with brain tumor (49), investigators showed larger areas of enhancement in some cases (Fig. 3). With delayed imaging, the USPIO is taken up into tumor macrophages and reactive astrocytes, as visualized histochemically with iron staining (50). Due to the virus-like size of the USPIO it may be possible to monitor penetration of viral vectors when using gene therapy. It was agreed that newer molecular imaging techniques should be integrated into brain tumor management, thus providing critical information that may significantly improve the survival and care of patients with brain tumors.

## CONCLUSION

With regard to CNS malignancy, endothelial cells along with glia, pericytes, and neurons should be viewed as a neurovascular unit that impedes delivery of therapeutics. Delivery of most chemotherapy across the BBB is modest, and macromolecule and gene vector delivery to the CNS are an order of magnitude more difficult than drug delivery. Translational research involving the neurovascular unit is greatly needed because the CNS is, to a large extent, a sanctuary, “immune” from many neuro-oncologic therapies.

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## REFERENCES

- Huber JD, Egleton RD, Davis TP. Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci* 2001;24:719–25.
- Neuwelt EA. Mechanisms of disease: the blood-brain barrier. *Neurosurgery* 2004;54:131–42.
- Taylor EM. The impact of efflux transporters in the brain on the development of drugs for CNS disorders. *Clin Pharmacokinet* 2002;41:81–92.
- Shusta EV, Boado RJ, Mathern GW, Pardridge WM. Vascular genomics of the human brain. *J Cereb Blood Flow Metab* 2002;22: 245–52.
- Witt KA, Mark KS, Hom S, Davis TP. Effects of hypoxia-reoxygenation on rat blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol* 2003;285: H2820–31.
- Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, Janigro D. Peripheral markers of blood-brain barrier damage. *Clin Chim Acta* 2004;342:1–12.
- Lee HJ, Pardridge WM. Monoclonal antibody radiopharmaceuticals: cationization, pegylation, radiometal chelation, pharmacokinetics, and tumor imaging. *Bioconj Chem* 2003;14:546–53.
- Ningaraj NS, Rao MK, Black KL. Adenosine 5’-triphosphate-sensitive potassium channel-mediated blood-brain barrier permeability increase in a rat brain tumor model. *Cancer Res* 2003;63:8899–911.
- Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. *Oncogene* 2003;22:7468–85.
- Fellner S, Bauer B, Miller DS, et al. Transport of paclitaxel (Taxol) across the blood-brain barrier *in vitro* and *in vivo*. *J Clin Invest* 2002;110:1309–18.



11. Kemper EM, van Zandbergen AE, Cleypool C, et al. Increased penetration of paclitaxel into the brain by inhibition of P-glycoprotein. *Clin Cancer Res* 2003;9:2849–55.
12. Robert J, Jarry C. Multidrug resistance reversal agents. *J Med Chem* 2003;46:4805–17.
13. Vavra M, Ali MJ, Kang EW-Y, et al. Comparative pharmacokinetics of <sup>14</sup>C-sucrose in RG-2 rat gliomas after intravenous and convection-enhanced delivery. *Neuro-oncol* 2004;6:104–12 [serial on the Internet]. 2004 [cited 2004 Mar 3]; Available from: <http://neuro-oncology.mc.duke.edu>. [posted to Neuro-Oncol Doc. 03-044].
14. Dickey DT, Muldoon LL, Kraemer DF, Neuwelt EA. Protection against cisplatin-induced ototoxicity and nephrotoxicity by *N*-acetylcysteine in a rat model. *Hearing Res* 2004;193:25–30.
15. Neuwelt EA, Pagel MA, Kraemer DF, Peterson DR, Muldoon LL. Bone marrow chemoprotection without compromise of chemotherapy efficacy in a rat brain tumor model. *J Pharmacol Exp Ther* 2004;309:594–9.
16. Doolittle ND, Muldoon LL, Brummett RE, et al. Delayed sodium thiosulfate as an otoprotectant against carboplatin-induced hearing loss in patients with malignant brain tumors. *Clin Cancer Res* 2001;7:493–500.
17. Neuwelt EA, Pagel MA, Hasler BP, Deloughery TG, Muldoon LL. Therapeutic efficacy of aortic administration of *N*-acetylcysteine as a chemoprotectant against bone marrow toxicity after intracarotid administration of alkylators, with or without glutathione depletion in a rat model. *Cancer Res* 2001;61:7868–74.
18. Alberts DS, Bleyer WA. Future development of amifostine in cancer treatment. *Semin Oncol* 1996;23:90–9.
19. Bello L, Giussani C, Carrabba G, Pluderi M, Costa F, Bikfavi A. Angiogenesis and invasion in gliomas. *Cancer Treat Res* 2004;117:263–84.
20. Brem S. Angiogenesis and brain tumors. In: Winn HR, editor. *Youmans neurological surgery*, Vol. 1. 5th ed. Philadelphia (PA): Elsevier Inc; 2004. p. 771–89. Chap. 38.
21. Kabbinar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003;21:60–5.
22. Balabanov R, Dore-Duffy P. Role of the CNS microvascular pericyte in the blood-brain barrier. *J Neurosci Res* 1998;53:637–44.
23. Ferrari N, Glod J, Lee J, Kobiler D, Fine HA. Bone marrow-derived, endothelial progenitor-like cells as angiogenesis-selective gene-targeting vectors. *Gene Ther* 2003;10:647–56.
24. Nourhaghighi N, Teichert-Kuliszewska K, Davis J, Stewart DJ, Nag S. Altered expression of angiopoietins during blood-brain barrier breakdown and angiogenesis. *Lab Invest* 2003;83:1211–22.
25. Sun J, Blaskovich MA, Jain RK, et al. Blocking angiogenesis and tumorigenesis with GFA-116, a synthetic molecule that inhibits binding of vascular endothelial growth factor to its receptor. *Cancer Res* 2004;64:3586–92.
26. Abdollahi A, Lipson EE, Sckell A, et al. Combined therapy with direct and indirect angiogenesis inhibition results in enhanced anti-angiogenic and antitumor effects. *Cancer Res* 2003;63:8890–8.
27. Justman QA, Clinton GM. Herstatin, an autoinhibitor of the human epidermal growth factor receptor 2 tyrosine kinase, modulates epidermal growth factor signaling pathways resulting in growth arrest. *J Biol Chem* 2002;277:20618–24.
28. Cairncross JG, Ueki K, Zlatescu MC, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst* 1998;90:1473–9.
29. Hall WA. Extending survival in gliomas: surgical resection or immunotherapy? *Surg Neurol* 2004;61:145–8.
30. Jensen MC, Clarke P, Tan G, et al. Human T lymphocyte genetic modification with naked DNA. *Mol Ther* 2000;1:49–55.
31. Kim D, Martuza RL, Zwiebel J. Replication-selective virotherapy for cancer: biological principles, risk management and future directions. *Nat Med* 2001;7:781–7.
32. Muldoon LL, Nilaver G, Kroll RA, et al. Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus and iron oxide particles to normal rat brain. *Am J Pathol* 1995;147:1840–3.
33. Mojsilovic-Petrovic J, Nestic M, Pen A, Stanimirovic D, Zhang W. Development of rapid staining protocols for laser-capture microdissection of brain vessels from human and rat coupled to gene expression analyses. *J Neurosci Methods* 2004;133:39–48.
34. Braaten KM, Betensky RA, de Leval L, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. *Clin Cancer Res* 2003;9:1063–9.
35. Korfel A, Thiel E. Chemotherapy for primary CNS lymphoma (PCNSL) [abstract]. *J Cancer Res Clin Oncol* 2004; Suppl 130:ISO96.
36. Soussain C, Suzan F, Hoang-Xuan K, et al. Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma. *J Clin Oncol* 2001;19:742–9.
37. Abrey LE, Moskowitz CH, Mason WP, et al. Intensive methotrexate and cytarabine followed by high-dose chemotherapy with autologous stem-cell rescue in patients with newly diagnosed primary CNS lymphoma: an intent-to-treat analysis. *J Clin Oncol* 2003;21:4151–6.
38. Batchelor T, Carson K, O'Neill A, et al. Treatment of primary CNS lymphoma with methotrexate and deferred radiotherapy: a report of NABTT 96-07. *J Clin Oncol* 2003;21:1044–9.
39. McAllister LD, Doolittle ND, Guastadisegni PE, et al. Cognitive outcomes and long-term follow-up results after enhanced chemotherapy delivery for primary central nervous system lymphoma. *Neurosurgery* 2000;46:51–60; discussion 60–1.
40. Kraemer DF, Fortin D, Doolittle ND, Neuwelt EA. Association of total dose intensity of chemotherapy in primary central nervous system lymphoma (human non-acquired immunodeficiency syndrome) and survival. *Neurosurgery* 2001;48:1033–41.
41. Smith JR, Rosenbaum JT, Wilson DJ, et al. Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. *Ophthalmology* 2002;109:1709–16.
42. Rubenstein JL, Shen A, Abrey L, et al. Results from a phase I study of intraventricular administration of rituximab in patients with recurrent lymphomatous meningitis. *Proc Am Soc Clin Oncol* 2004;23:578; abstract 6593.
43. Ferreri AJ, Batchelor T, Zucca E, Cavalli F, Armitage J. International collaborative group against primary CNS lymphomas. *J Clin Oncol* 2003;21:1649–50.
44. Suh JH, Stea BD, Kresl JJ, et al. A phase 3, randomized, open-label, comparative study of standard whole brain radiation therapy (WBRT) with supplemental oxygen (O<sub>2</sub>), with or without RSR13, in patients with brain metastases. *Neuro-oncol* 2003;5:345. Abstract RT-13.
45. Newton HB, Slivka MA, Volpi C, et al. Intra-arterial carboplatin and intravenous etoposide for the treatment of metastatic brain tumors. *J Neurooncol* 2003;61:35–44.
46. Becherer A, Karanikas G, Szabo M, et al. Brain tumor imaging with PET: a comparison between [<sup>18</sup>F]fluorodopa and [<sup>11</sup>C]methionine. *Eur J Nucl Med Mol Imaging* 2003;30:1561–7.
47. Knopp MV, Runge VM, Essig M, et al. Primary and secondary brain tumors at MR imaging: bicentric intraindividual crossover comparison of gadobenate dimeglumine and gadopentetate dimeglumine. *Radiology* 2004;230:55–64.
48. Kircher MF, Mahmood U, King RS, Weissleder R, Josephson L. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. *Cancer Res* 2003;63:8122–5.
49. Varallyay P, Nesbit G, Muldoon LL, et al. Comparison of two superparamagnetic viral-sized iron oxide particles ferumoxides and ferumoxtran-10 with a gadolinium chelate in imaging intracranial tumors. *AJNR Am J Neuroradiol* 2002;23:510–9.
50. Neuwelt EA, Varallyay P, Bago A, Muldoon LL, Nesbit G, Nixon R. Imaging of iron oxide nanoparticles by MR and light microscopy in patients with malignant brain tumors. *Neuropathol Appl Neurobiol* 2004;30:70.