Implications of the blood–brain barrier in primary central nervous system lymphoma

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✓The optimal treatment of primary central nervous system lymphoma (PCNSL), a rare form of extranodal non-Hodgkin lymphoma, has yet to be defined. Whole-brain radiation therapy (WBRT) has limited efficacy as a single therapeutic modality and is associated with a high risk of delayed neurotoxicity. Methotrexate-based chemotherapy regimens yield poor drug penetration across the blood–brain barrier (BBB), thus necessitating administration of high doses with the concomitant risk of increased systemic and neurological toxicity. Combined-modality therapy (WBRT plus chemotherapy) can improve response and survival rates, yet it is associated with a high risk of neurotoxicity. The aim of chemotherapy in conjunction with BBB disruption is to maximize drug delivery to the brain and improve the agent's efficacy, while preserving neurocognitive function and minimizing systemic toxicity. Methotrexate-based chemotherapy regimens administered in conjunction with BBB disruption have shown promising results in PCNSL. Animal models of central nervous system lymphoma and drug neurotoxicity offer new possibilities to study the effects of various treatments on PCNSL and normal brain and can also help understand biological and pathophysiological aspects of this disease. Because the intact BBB is even less permeable to antibodies than it is to drugs, preclinical and clinical studies of monoclonal antibody delivery (for example, rituximab and ⁹⁰Y ibritumomab tiuxetan) to the brain in conjunction with BBB disruption offer a new possibility to make these novel treatments more efficient against PCNSL. Regarding the evaluation of more sensitive and specific diagnostic imaging tools, iron oxide–based contrast agents for magnetic resonance imaging have shown promise for better differentiation of PCNSL from other white matter diseases.

KEY WORDS • primary central nervous system lymphoma • blood–brain barrier • methotrexate • rituximab • radiation therapy

THE incidence of PCNSL, an extranodal non-Hodgkin lymphoma confined to the CNS at the time of diagnosis, increased almost threefold from 1973 to 1984,²³ but has stabilized recently.³² The optimal treatment for PCNSL has yet to be defined. Postoperative WBRT improves the median survival duration by up to 18 months, but responses are short-lived.⁴³ For methotrexate-based intravenous chemotherapy alone, a 5-year survival rate of up to 43% has been reported.⁶⁶ Combined-modality therapy with high-dose methotrexate-based chemotherapy and WBRT improves response and survival rates compared with WBRT alone, but is associated with delayed neurotoxicity in 8 to 50% of patients,^{1,16,29,61,79} and the 5-year survival rates are 9 to 32%.^{1,7,16,64} Long-term survivors older than 60 years of age are at particular risk for experiencing late neurotoxicity;^{1,7,67} however, detailed neurocognitive outcomes and quality of life evaluations are rarely reported in published clinical trials.

Abbreviations used in this paper: BBB = blood–brain barrier; CNS = central nervous system; CSF = cerebrospinal fluid; CT = computed tomography; ICA = internal carotid artery; KPS = Karnofsky Performance Scale; MR = magnetic resonance; MS = multiple sclerosis; OHSU = Oregon Health & Science University; PCNSL = primary CNS lymphoma; VA = vertebral artery; WBRT = whole-brain radiation therapy.

Osmotic BBB disruption–enhanced chemotherapy delivery for PCNSL has been ongoing at the OHSU since 1982, and is currently being performed at eight institutions worldwide that are participating in the International Blood-Brain Barrier Disruption Consortium.¹⁹ The BBB Program at OHSU is a translational clinical and research model with preclinical and clinical research teams reciprocally informing each other of new developments. The key paradigm for the BBB Program is that the efficacy of chemotherapy in brain tumors is disappointing overall, due in part to the fact that the BBB limits the passage of therapeutic agents to the tumor and surrounding brain. Exclusion of molecules from the brain by the BBB is based on molecular weight, charge, and lipid solubility.⁴⁴ The goals of BBB disruption are as follows: 1) enhanced delivery of therapeutic agents to the brain, resulting in improved treatment outcomes; 2) preservation of neurocognitive function and quality of life; and 3) minimizing systemic toxicity. The BBB disruption technique is especially important in the delivery of high-molecular-weight agents, like monoclonal antibodies and radioimmunoconjugates^{44,48} that already are established treatment options in systemic lymphoma.^{24,60} We present an overview and outlook regarding preclinical and clinical results in the treatment of PCNSL reported by investigators in the BBB

Program, covering methotrexate- and carboplatin-based treatment regimens, the addition of monoclonal antibodies and radioimmunoconjugates to treatment regimens, neurotoxicity issues, and novel imaging techniques.

Preclinical Studies

Delivery of Methotrexate-Based Chemotherapy to the Brain With and Without BBB Disruption

Several observations regarding BBB disruption that have clinical implications have been made in animal studies.^{37,44,45} It has been shown that disruption of the BBB results in delivery of the respective agent throughout either the VA or ICA distribution. Variables that influence delivery are type, size, and location of the tumor. The concentration of methotrexate in brain and CSF can be substantially increased using BBB disruption.^{47,51,54,55,62,68–70} In normal dogs (Fig. 1), 4 mg/m² methotrexate injected into the carotid artery without BBB disruption resulted in concentrations in the ipsilateral brain that were lower by a log factor than in the serum. However, 6 to 12 hours after disruption, cerebral concentrations exceeded those in serum by a log factor. The slower clearance of drug from the brain implies that therapeutic concentrations can be maintained for a longer period of time. With disruption of the VA, delivery to the brain areas it supplies increased 12- to 18-fold.⁵³

Similarly, in a rat glioma model there was a significant increase in delivery of methotrexate to the tumor, brain around the tumor, and brain distant to the tumor in the lesion-bearing hemisphere compared with uptake for tumor-bearing control animals in which the BBB was not disrupted. In the same study, administration of dexamethasone prior to BBB disruption resulted in a 40 to 60% decrease of methotrexate uptake in the tumor and, to a lesser extent, in the brain around and distant to the tumor.⁴⁶ This study provided evidence that the BBB is partially intact in tumors, and that steroid drugs can alter the permeability of the disrupted BBB. Similar results were found in a small cell lung cancer xenograft model of CNS metastasis.⁴⁹ These findings are important because a clear dose-response relationship has been demonstrated in several studies of animal and human tumors (including in PCNSL), and even a 20% reduction in drug dose can have a dramatic impact on outcomes.^{18,25,27,35,36} A relationship between the degree of BBB opening and drug delivery in animals and the association of increasing dose intensity with increasing survival were demonstrated by our group in a nude rat model and in a human brain tumor xenograft model.^{37,72} Furthermore, a relationship between enhanced delivery of borinated compounds after BBB disruption and the efficacy of boron/neutron capture therapy in an animal brain tumor model has been reported.⁴

The exact timing of BBB disruption and drug administration is important. After BBB disruption, vascular permeability reaches maximum levels at 15 minutes after osmotic shock and then rapidly decreases, returning to pre-infusion levels within 2 hours after disruption. These observations apply to small molecules such as methotrexate as well as to large molecules such as monoclonal antibodies.^{37,44,45}

FIG. 1. Photograph obtained at necropsy showing an example of BBB disruption in a dog brain. The disrupted left hemisphere has a blue appearance due to extravasation of Evans Blue dye across the BBB, whereas Evans Blue is excluded from brain tissue in the nondisrupted right hemisphere.

Impact of Radiation Therapy and Sequence of Treatment Modalities on Drug Delivery, Efficacy, and Neurotoxicity

A PCNSL is both radio- and chemosensitive. If radiotherapy is used, the sequence of radio- and chemotherapy influences the delivery of therapeutic agents to the brain and the degree of acute and delayed neurotoxicity. In a rodent model, two clinically relevant chemotherapy regimens (intraarterial methotrexate [1 g/m²] and a combination of intravenous etoposide and intraarterial carboplatin) were given after BBB disruption. A single fraction of external-beam radiation (20 Gy) was administered 30 days before (Group 1), concurrent with (24 hours prior; Group 2), or 30 days after (Group 3) chemotherapy. Radiation administered either before or concurrent with chemotherapy resulted in a significant ($p < 0.01$) decrease in drug delivery to the brain compared with the levels found in animals that did not undergo brain irradiation. Furthermore, in Groups 1 and 2, seizures were observed in 26% of the animals, and the mortality rate in animals receiving radiotherapy 30 days before chemotherapy was significantly ($p = 0.03$) higher than in the control animals that did not receive radiation.⁷³ Similarly, the long-term toxicity evaluation in the same rodent study confirmed that radiation before chemotherapy is the most toxic sequence, and additionally, that there were significant increases ($p = 0.001$, 0.006 , and 0.013 , respectively, for the aforementioned treatment groups) in long-term toxic effects when

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comparing the treatment groups that received radiation plus chemotherapy with the control groups that received only radiation or chemotherapy.⁷²

There is evidence that the sequence of therapy modalities also influences treatment outcomes. In nude rats bearing the human lung cancer cell line LX-1, cranial radiotherapy (20 Gy, single fraction) was administered either before, concurrent with, or after BBB disruption–enhanced treatment with the antibody-targeted chemotherapy agent BR96-doxorubicin. Results indicated that disruption–enhanced delivery of immunoconjugates is more effective in combination with WBRT. Moreover, administration of BR96-doxorubicin before WBRT significantly increased survival compared with the treatment group that received WBRT and immunochemotherapy concurrently ($p < 0.05$).⁷¹ Although these findings were observed in a lung cancer and not in a PCNSL model, the results were later confirmed in the clinical setting in patients with PCNSL. Participants who underwent WBRT before BBB disruption–enhanced chemotherapy had a significantly decreased median survival duration compared with patients who received BBB disruption chemotherapy initially (see *Clinical Studies* for details).^{14,58}

New Animal Models for PCNSL and Neurotoxicity Studies

Because the optimal clinical treatment for PCNSL remains controversial, animal models that closely mimic the clinical situation could prove useful for evaluating biological, pathophysiological, and therapeutic aspects of PCNSL. In the past, animal models of PCNSL have mainly been focused on T-cell models,^{3,81,90} however, T-cell PCNSL is rare and accounts for less than 5% of all tumors of this type.⁸³ The only established human xenograft B-cell model of PCNSL in which human B lymphoma cells have been used focused on pathological aspects of the disease, and interventional studies were not performed.⁷⁷ Thus, there is a need for a new B-cell lymphoma model to generate preclinical data for possible translation to clinical practice.

The investigators in the BBB Program at OHSU recently released preliminary data on a new B-cell PCNSL model in which the human MC116 lymphoma cell line was used.⁸⁴ In this model, female nude rats were pretreated with WBRT, compared with a group that received no pretreatment, 24 hours before intracerebral (right caudate nucleus) inoculation of MC116 human B-cell lymphoma cells. Tumor growth and permeability were evaluated using MR imaging at various time points ranging from 1 to 4 weeks after tumor inoculation. To evaluate tumor response, selected rats were treated with WBRT (20 Gy) or high-dose intravenous methotrexate (3 g/m²). The rat brains were assessed 3 to 5 weeks after tumor implantation for lesion volumes and immunoreactivity. The MC116 cells formed infiltrative CD20- and CD45-positive brain tumors that spread through the cortex in the inoculated hemisphere, along the meninges, and into the ventricles. The tumor growth rate and pattern of infiltration were independent of preinoculation irradiation status. The MR images demonstrated that tumor permeability was heterogeneous, but generally demonstrated gadolinium enhancement at the inoculation site in the cortex and in the ventricles. A single treatment with WBRT markedly reduced

MR imaging enhancement and tumor volume, but intravenous methotrexate was not effective. In summary, this human B-cell PCNSL model in rodents displays growth, imaging, and immunological characteristics that closely resemble human PCNSL. This model will be useful for evaluating chemotherapy and immune-targeted therapies for PCNSL. A study testing the efficacy of methotrexate- and rituximab-based immunochemotherapy with or without BBB disruption is currently in progress.

Clinical Studies

The Clinical BBB Disruption Technique and Treatment Protocols for PCNSL

The care of patients undergoing BBB disruption–enhanced chemotherapy has been described in detail elsewhere.^{21,58} Briefly, the BBB disruption treatment is done on 2 consecutive days every 4 weeks for up to 1 year. The care of patients treated with this technique requires a multidisciplinary team approach. The BBB disruption is performed after induction of general anesthesia to ensure patient comfort and safety. A femoral artery is accessed, and an intracranial artery (ICA or VA) is catheterized. On the 1st day one artery is infused, followed by a different artery on the 2nd day. Hypertonic (25%), warmed mannitol is delivered at a predetermined flow rate of 3 to 12 ml/second into the chosen artery for 30 seconds. After administration of mannitol, the intraarterial chemotherapy agent(s) are infused, each for 10 minutes. Intravenous chemotherapy is begun directly after induction of general anesthesia to allow time for the drug to be delivered to the tumor while the BBB is open. Immediately after the mannitol infusion, nonionic contrast dye is administered intravenously. Following completion of chemotherapy, the patient undergoes a CT brain scan.²² Contrast enhancement in the disrupted territory of the brain is compared to that seen in the nondisrupted territory. The degree of disruption is graded as nil, moderate, good, and excellent, according to the scale published by Roman-Goldstein, et al.⁷⁵ (Fig. 2 shows examples of a good disruption).

At OHSU, two different chemotherapy regimens for PCNSL have been used in the past. Protocol I, which was used from 1982 until 1993,⁵⁸ consisted of intraarterial administration of 2.5 g/day methotrexate and intravenous administration of 15 mg/kg cyclophosphamide on each of

FIG. 2. Head CT scans obtained in a patient after BBB disruption of the left ICA (left) and VA (right) distributions. The degree of disruption was graded as good, as evidenced by bright contrast enhancement in each arterial distribution.

TABLE 1
Literature review of patient characteristics and treatment outcomes in clinical studies
for PCNSL in which chemotherapy was used alone or in conjunction with WBRT*

Authors & Year	Tx Modality	No. of Patients	Median Age (yrs)	Median KPS	Overall RR (%)	Median OS (mos)	5-Yr OS (%)
Abrey, et al., 1998	chemo + WBRT	31	59	NR	87.1†	42‡	22.3
Blay, et al., 1998	chemo, WBRT, combined chemo + WBRT	226	61	NR	NR	16	19
Brada, et al., 1998	chemo + WBRT	31	51	60	67†	23	34
Sandor, et al., 1998	chemo	14	57	NR	100	nr	68.8§
McAllister, et al., 2000	chemo + BBBD	74	NR	NR	65†	40.7	42
DeAngelis, et al., 2002	chemo + WBRT	102	56.5	80	94	36.9	32
Batchelor, et al., 2003	chemo	25	60	80	74	≥22.8	NR
Pels, et al., 2003	chemo	65	62	70	71	50	43
Poortmans, et al., 2003	chemo + WBRT	52	51	70	81	46	58**

* BBBD = BBB disruption; chemo = chemotherapy; nr = not reached; NR = not reported; OS = overall survival; RR = response rate; Tx = treatment.

† Complete remissions only.

‡ Median cause-specific survival.

§ At 57 months.

|| In this cohort, 36 patients (49%) were younger than 60, and 38 (51%) were 60 years of age or older. The KPS score was greater than 70 in 46 patients (62%), and 70 or less in 26 patients (35%).

** This value represents the 3-year survival.

2 consecutive days, followed by 14 days of 100 mg/day orally administered procarbazine given monthly for 1 year. In 1993, Protocol II eliminated procarbazine to permit the use of granulocyte colony-stimulating factor. Protocol II consisted of intraarterial administration of 2.5 g/day methotrexate, 150 mg/m²/day intravenous or intraarterial etoposide phosphate, and 500 mg/m²/day intravenous cyclophosphamide on each of 2 consecutive days, repeated monthly for 1 year. Protocol II used 5 mg/kg granulocyte colony-stimulating factor for 7 to 10 days, starting on Day 3. Protocol II was used until 2005, when two new PCNSL treatment protocols, one for first-line treatment and one for relapsing disease, opened for enrollment. Both protocols use rituximab and carboplatin, two drugs that have shown promising results in the treatment of extracerebral lymphoma. For newly diagnosed PCNSL, 2.5 g/day methotrexate and 200 mg/m²/day carboplatin (total dose 400 mg/m²) are given with BBB disruption on each of 2 consecutive days. Additionally, 375 mg/m² rituximab is administered intravenously within 24 hours prior to the first BBB disruption of each monthly course. In the protocol for relapsed disease, rituximab and carboplatin are administered as in the first-line protocol. In addition, 330 mg/m²/day cyclophosphamide (total dose 660 mg/m²) and 200 mg/m²/day etoposide/etoposide phosphate (total dose 400 mg/m²) are given intravenously. In all regimens in which carboplatin is used, patients receive high-dose sodium thiosulfate to protect against carboplatin-induced ototoxicity.²⁰

The Importance of Drug Delivery in the Treatment of PCNSL

Combination chemotherapy for systemic aggressive non-Hodgkin lymphoma results in probable cure for a considerable number of patients; however, it has only very modest efficacy in treating PCNSL when administered intravenously.⁸⁶ It was already suggested in the early 1980s that the BBB interferes with drug delivery to the CNS. This was hypothesized when two patients with systemic

lymphoma in remission experienced CNS involvement while undergoing treatment with multidrug chemotherapy.³⁰ The BBB normally excludes ionized, water-soluble drugs with molecular weights greater than 180 D from entering the brain, and most chemotherapeutic agents weigh between 200 and 1200 D.⁴⁵ Furthermore, the intact BBB is even less permeable to antibodies than to drugs. In a study in which the CSF penetration of the monoclonal antibody trastuzumab was investigated in six patients with breast cancer and brain metastases, the serum/CSF ratio of trastuzumab was 420:1, indicating poor penetration of the agent. However, with impaired integrity of the BBB and blood-CSF barrier, smaller ratios (76:1 in two patients after WBRT and 49:1 in two patients with meningeal carcinomatosis) were observed.⁸⁵

In PCNSL, administration of high-dose intravenous methotrexate has been performed in an attempt to improve delivery across the BBB and blood-CSF barrier. The CSF penetration of intravenously administered methotrexate in humans is dose dependent, as was demonstrated in several studies.^{10,12,28,41,42,87} Nevertheless, cytotoxic CSF levels (> 10⁻⁶ M) were not achieved with a dose of 0.5 g/m², and only 44% of patients treated with 2.5 g/m² showed evidence of cytotoxic CSF concentrations.⁸⁷ A dose of 5 g/m² in children resulted in cytotoxic levels in 66 and 81% of patients in two separate studies.^{41,42} However, it is unclear whether CSF levels after intravenous administration of methotrexate correspond to intratumoral concentrations and levels in brain, brain around tumor, and normal brain. Scarce clinical pharmacokinetic data are available regarding these compartments, which are important in highly infiltrative brain tumors such as PCNSL. Researchers in a recent study used microdialysis to evaluate drug levels in extracellular fluid in four patients with high-grade glioma after intravenous administration of methotrexate.⁶³ Patients in whom the probe was located in contrast-enhancing tumor had higher methotrexate peak concentrations in extracellular fluid compared with patients in whom the probe was placed in nonenhancing tissue next to the enhancing

FIG. 3. Kaplan–Meier plot showing overall survival rates in 74 patients with PCNSL treated with BBB disruption-enhanced delivery of methotrexate. Results confirm the importance of dose intensity in the treatment of CNS neoplasms. In this study, the number of disruptions was used as a surrogate marker for dose intensity. The values on the y axis represent the relative survival. Obs = observations. Reprinted with permission from 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* 48: 1033-1040, 2001.

tumor ($189 \pm 6 \mu\text{M}$ compared with $10.4 \pm 0.4 \mu\text{M}$). These observations indicate that intracerebral methotrexate distribution is associated with BBB integrity as assessed by measuring contrast enhancement on MR images and CT scans. As the tumor shrinks in response to chemotherapy, the BBB integrity is restored. This often results in only a transient tumor response, due to remaining viable tumor cells. In two patients with PCNSL who were initially treated with chemotherapy, significant changes in permeability in the region of the tumor were observed. Within 5 weeks of the start of treatment, permeability values as determined by the washout rate of the radioactive tracer ^{68}Ga -ethylenediaminetetraacetic acid reached the levels found in normal brain.⁶⁵

Intraventricular and intrathecal methotrexate administration lead to therapeutic CSF levels,³¹ but concentrations are inhomogeneous.⁸² Furthermore, intrathecal administration can only achieve therapeutic levels in the superficial 2 to 3 mm of CNS parenchyma located beyond the subarachnoid space.^{6,34} Increased rates of neurotoxicity in patients treated with intravenous methotrexate or WBRT were reported when additional intrathecal methotrexate was given.⁹

Clinical studies in which researchers have documented the delivery of methotrexate and its efficacy when used in conjunction with osmotic BBB modification date back to the late 1970s and early 1980s.^{50,52} Thirty consecutive patients with PCNSL who received the same BBB disruption chemotherapy regimen (Protocol I, using intraarterial methotrexate plus BBB disruption; see earlier description) between 1982 and 1989 and were followed until 1990 were divided into two groups. The 13 patients in Group 1 had received first-line treatment with WBRT and were

enrolled in Protocol I after tumor progression or recurrence. The 17 patients in Group 2 were enrolled in Protocol I immediately and received WBRT only in the case of an incomplete tumor response or disease progression. The difference in median survival duration (17.8 months for Group 1 and 44.5 months for Group 2) was statistically significant ($p = 0.039$). Furthermore, a plateau in survival curves and manageable toxicity in the patients in Group 2 suggested that BBB disruption plus chemotherapy can produce long-term remission with improved survival, acceptable rates of morbidity and mortality, and preservation of cognitive function by sparing these patients WBRT with its associated risk of neurocognitive decline.⁵⁸

These favorable results were confirmed in studies with a larger number of patients and longer follow-up period.^{14,39} In the study by McAllister, et al.,³⁹ consecutive patients with PCNSL whose first-line treatments were Protocols I and II between 1982 and 1997 (see earlier descriptions) were evaluated for tumor response and neurocognitive sequelae. The median follow-up duration after the first BBB disruption treatment was 6.2 years (range 6 months–16.3 years). The complete response rate was 65% (48 patients), and after 1 year the disease in 36 (75%) of these patients continued to be in complete remission. An estimated 5-year survival rate of 42% and a median survival duration of 40.7 months were reported. These outcomes compare well with those reported by other investigators (Table 1) and substantiate the claim of a favorable outcome in patients with PCNSL treated with BBB disruption-enhanced chemotherapy.

The Importance of Dose Intensity in the Treatment of PCNSL

A relationship between the degree of BBB opening and drug delivery and the association of increased dose intensity with improved survival has been demonstrated in preclinical studies by our group and others.^{4,37,74} The importance of dose intensity in the clinical setting has remained an open question in the treatment of both systemic solid tumors and primary brain tumors. It has been hypothesized that dose and the use of combination chemotherapies, that is, the concept of “summation dose intensity,” influences the outcomes of malignant diseases.²⁶ Until recently, however, dose intensity (for example, the concept of high-dose chemotherapy with autologous stem cell support) had proven to be effective in several hematological malignancies but not in solid tumors, especially brain tumors.⁸⁰ We believe that the key to successful treatment of brain tumors is drug delivery to the lesion-infiltrated brain around the tumor, with a relatively intact BBB.⁸⁹ Therefore, the demonstration of a statistically significant connection between dose intensity and improved survival in an infiltrative and chemoresponsive primary brain tumor like PCNSL with variable integrity of the BBB, accomplished using BBB disruption-enhanced chemotherapy delivery, was important for establishing new paradigms in the management of infiltrative primary brain tumors (Fig. 3).³⁵ In this study, in which 74 patients with PCNSL who underwent first-line treatment with BBB disruption-enhanced chemotherapy (the clinical characteristics of this cohort are described in McAllister, et al.³⁹) were assessed, either the number of intraarterial infusions

in conjunction with BBB disruption or a cumulative score derived from the quality of the BBB disruption visualized using cerebral CT scans was used as surrogate markers for dose intensity. In multivariate analysis, the number of disruptions was significantly associated with prolonged survival ($p = 0.04$), and the cumulative disruption score approached statistical significance ($p = 0.066$). Survival bias does not fully explain these associations because increased dose intensity also resulted in improved survival in patients with complete tumor response.

Preservation and Evaluation of Neurocognitive Function in Patients Treated With BBB Disruption–Enhanced Chemotherapy

Treatments for PCNSL in which WBRT is used can prolong survival; however, there is extensive evidence that a substantial risk of neurocognitive decline is associated with WBRT, and it increases with advanced age and prolonged disease-free survival.^{2,33} In many clinical trials of PCNSL treatments, investigators have reported high rates of therapy-related neurotoxicity,^{1,67} which seems especially pronounced with combined-modality therapy (WBRT plus chemotherapy). In one study in which chemotherapy and WBRT were used in newly diagnosed PCNSL, 12 patients (15%) experienced severe delayed neurotoxicity, and eight of them died.¹⁶ Regarding treatment-related neurotoxicity, neurological sequelae usually develop months or years after therapy.³³ Cognitive function can only be accurately evaluated in the absence of tumor because it is impossible to differentiate treatment-related neurotoxicity from the tumor's effects on cognition.¹⁵ As a result, the incidence of neurocognitive decline is directly proportional to the percentage of patients with disease-free survival.¹⁷ With new treatment approaches, long-term disease-free survival in PCNSL has increased, and thus thorough assessment and preservation of neurocognitive function is a very important aspect in the care of patients with PCNSL.

In clinical trials published by investigators in the BBB Program so far, the results indicate preservation of neurocognitive function during and after PCNSL treatment.^{14,39,58} As part of clinical trials within the BBB Program, patients undergo extensive neuropsychological evaluation. The rationale for the choice of specific neuropsychological tests and their appropriate use in cognitive assessment of patients with brain tumors have been reported previously.¹³ In the largest study in which long-term results have been evaluated in patients with PCNSL treated with BBB disruption,³⁹ detailed neuropsychological data were available in 86% of the 36 patients who were in continued, complete remission 1 year posttreatment. None of the patients demonstrated cognitive loss on neuropsychological tests and clinical examinations. Patients either remained at their baseline cognitive level or showed improvement. This series includes eight patients older than 60 years of age in whom stability in neurocognitive function was documented after completion of treatment. These results are in contrast to those reported in elderly patients treated with combined-modality therapy.⁷ In one series,¹ no patient older than 60 years of age at diagnosis remained free of dementia for more than 48 months. In summary, the results of the PCNSL studies published by

investigators in the BBB Program demonstrate that the combination of favorable survival data and preservation of neurocognitive function is a key feature of BBB disruption–enhanced chemotherapy delivery.

Much controversy has surrounded the question whether changes on MR imaging correlate with neurocognitive function in patients with brain tumors. Unfortunately, studies comparing imaging changes and cognitive function over a long period of survival are rare.⁴⁰ In one study, Roman-Goldstein, et al.,⁷⁶ evaluated 15 patients with brain tumors (among them nine with PCNSLs) who had a complete tumor response after undergoing BBB disruption with intraarterial chemotherapy. In some patients, new abnormalities were found to have developed on MR imaging. These patients, however, maintained the same level of cognitive and neurological functioning, and it was concluded that MR imaging findings do not correlate with the results of neurocognitive testing.

Similarly, 16 patients with PCNSL were followed after complete remission of disease was achieved using a methotrexate-based treatment regimen in conjunction with BBB disruption.⁵⁶ Cognitive testing and MR images or CT scans were done for each patient before treatment and at its completion after 12 months. Thereafter, the same studies were done in nine of the 16 patients with complete remission, who were followed for a median of 55 months. Although a significant association ($p < 0.028$) was found between neurocognitive data and abnormalities on T₂-weighted MR images or low-attenuation areas on CT scans before chemotherapy, there was no correlation at completion of the treatment. In seven patients a new abnormality developed by the end of treatment, and was revealed on T₂-weighted MR images or as a low-attenuation area on CT scans, whereas in 15 patients stable, decreased, or resolved baseline imaging abnormalities were demonstrated. Although cognitive impairment was common before they started therapy, all patients' cognitive function had improved significantly ($p < 0.005$) by the end of treatment. The data from this study confirm that neither BBB disruption before delivery of chemotherapy nor changes on imaging studies are associated with a decrease in cognitive function in patients with PCNSL in complete remission. This is in accordance with data in children that also support the suggestion that imaging changes are not associated with neurological dysfunction, and that these changes are frequently overinterpreted in relation to their clinical significance.⁸

Toxicity of BBB Disruption–Enhanced Chemotherapy

In Table 2 we report the toxicities leading to adverse events recorded for 74 patients with PCNSL who were treated with intraarterial chemotherapy in conjunction with BBB disruption.³⁹ All four deaths within 30 days after the procedure were related to infections, and three of them occurred before the routine use of granulocyte colony-stimulating factor. Procedure-related toxicities include those specifically related to angiography (such as strokes and arterial injuries) and events related to BBB disruption procedures or the drugs used to induce general anesthesia. Seizures were the most common BBB disruption–related neurological adverse events and occurred during 6 to 8% of the procedures. They were not associat-

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TABLE 2

*Toxicity of methotrexate-based intraarterially delivered chemotherapy in conjunction with BBB disruption in 74 patients with PCNSL**

Toxicity	No. of Events (no. of patients)	Incidence (% of procedures)
neurological		
reversible neurological deficit (> 48 hrs)	11 (7)	1
asymptomatic extracranial arterial injury	10 (8)	0.9
stroke	5 (5)	0.5
status epilepticus	1 (1)	0.1
cerebral herniation	1 (1)	0.1
death w/in 48 hrs	0	0
death w/in 30 days	4	0.4
nonneurological		
neutropenic fever	21	3.6
deep venous thrombosis	17	1.8
thrombocytopenia or anemia requiring transfusions of platelets or packed redblood cells	14	1.6
pneumonia	3	1.3
septicemia (nonneutropenic)	9 (8)	0.8
dysrhythmia	3	0.3
pulmonary embolism	2	0.3
femoral arterial thrombosis	1	0.2
respiratory arrest	1	0.1
pneumothorax	1	0.1
urethral tear w/ bleeding	1	0.1
femoral artery injury	1	0.1
dose reductions	7	
late complications		
orthopedic (long bone fractures, avascular necrosis)	8 (7)	
ophthalmological†	12 (12)	
acute myelogenous leukemia	1 (1)	
dementia	0	

* Adapted from McAllister, et al. (1074 procedures).

† Includes more than 50% decrease in visual acuity (four cases), asymptomatic macular degeneration (two cases), and new cataracts (two cases).

ed with permanent neurological deficits. Three of five patients who suffered strokes after BBB disruption procedures showed no evidence of long-term cognitive deficits on neuropsychological tests. Transient neurological deficits were generally associated with good to excellent grades of BBB disruption, and the patients ultimately returned to baseline status. One patient experienced tonsillar herniation after a BBB disruption procedure, and that individual remains alive, with a KPS of 100%. The nonneurological toxicities associated with this treatment were primarily related to administration of chemotherapy and corticosteroid drugs. These toxicities included neutropenic fever, anemia requiring transfusions, septicemia, and pneumonia.

New MR Imaging Agents for the Differential Diagnosis of Brain Lesions

The differential diagnosis of PCNSL can be difficult because this tumor can present with radiological, clinical, and laboratory similarities to other brain lesions, especially MS. The prognosis for both PCNSL and MS relies to a significant extent on the patient's neurological condition prior to the start of therapy; early diagnosis is the key for

successful treatment. Thus, MR imaging agents that aid in the differential diagnosis of PCNSL can potentially reduce the interval from symptom presentation to diagnosis and may therefore improve clinical outcomes.

So-called ultrasmall superparamagnetic iron oxide nanoparticles have shown potential in the imaging of brain tumors.^{38,57,59,88} Gadolinium has a short plasma half-life of approximately 90 minutes, but ferumoxtran-10, one of the new investigational nanoparticles being assessed for use as MR imaging contrast agents, has a long half-life of 24 to 30 hours. In contrast to gadolinium, the ferumoxtran-10 molecule is much larger; it is the size of a small virus. Moreover, as opposed to gadolinium, ferumoxtran-10 is endocytosed by phagocytes like macrophages and by glial cells,^{57,88} whereas gadolinium does not enter cells. This cell-specific uptake difference may allow lesion enhancement even with a small area of BBB leakage if the BBB opening permits iron oxide particles to cross slowly, because of the long plasma half-life of ferumoxtran-10.

In a recently published study,³⁸ 23 patients with various CNS lesions (among them five patients with PCNSL and seven with MS) underwent brain MR imaging with and without gadolinium, which was followed at a mean interval of 10 days by a ferumoxtran-10 scan. The ferumoxtran-10 showed different enhancement patterns in a variety of CNS lesions. In PCNSL, ferumoxtran-10 may show a larger extent of the tumor or even additional lesions that are not shown with gadolinium (Fig. 4). Most important for the differential diagnosis of PCNSL, patients with MS demonstrated less or no enhancement with ferumoxtran-10 compared to gadolinium. Thus, the differential diagnosis of PCNSL versus MS (and possibly other inflammatory CNS lesions) can be improved, thereby possibly shortening the interval from the onset of symptoms to the establishment of a diagnosis.

Summary and Future Directions

Tumor recurrence remains common after initial therapy for PCNSL, underscoring the need for novel treatment strategies to induce durable complete remissions. Over the past 25 years, it has been demonstrated in various studies that BBB disruption–enhanced methotrexate-based chemotherapy is an effective treatment for PCNSL that obviates or at least postpones the need for WBRT for a substantial number of patients. Moreover, the potential for long-term survival with preserved cognitive function and the importance of dose intensity has been demonstrated. Although comparisons of published case series are subject to potential biases, in most studies reported so far in which either chemotherapy alone or combined-modality therapy was used, researchers applied patient inclusion criteria similar to those used for the BBB group's studies (Table 1). The 5-year survival rate of 42% and the median survival duration of 40.7 months reported by McAllister, et al.,³⁹ compare well with other recent benchmark studies. In one study in which high-dose methotrexate-based chemotherapy followed by WBRT was used, a median survival duration of 36.9 months with a 5-year survival rate of 32% was reported; however, a high rate of neurotoxicity was observed (Table 1).¹⁶ In the second study, in which high-dose methotrexate-based multiagent chemotherapy

FIG. 4. Neuroimages used for comparison of gadolinium- and ferumoxtran-10-enhanced MR imaging in a patient with PCNSL. A: A T₁-weighted MR image obtained without contrast. No tumor is visible. B: A T₁-weighted, gadolinium-enhanced MR image revealing tumor. C: A T₁-weighted MR image obtained 24 hours after ferumoxtran-10 infusion. The lesion in panel C appears larger and changed in quality compared with the lesion in Panel B.

was used alone, a median survival duration of 50 months and a 5-year survival rate of 43% were achieved. However, there were six treatment-related deaths (9%), and five of these were a result of myelosuppression (Table 1).⁶⁶ Toxicity in patients treated with intraarterial methotrexate-based chemotherapy in conjunction with BBB disruption was generally manageable, and there were no procedure-related deaths. No cases of dementia were recorded. Otherwise, the incidence of toxicities not related to the BBB disruption procedure is comparable to that reported in other trials on PCNSL in which chemotherapy was used alone or as part of the treatment regimen.^{1,5,7,16,66,67}

The aggressive nature of PCNSL compares more to advanced-stage systemic aggressive non-Hodgkin lymphoma than to Stage I_E lymphoma. Patients with advanced-stage systemic lymphoma are currently treated with aggressive chemotherapy, and their survival is not improved with radiotherapy. Moreover, there is no clear relationship between dose and disease outcome for radiotherapy. Furthermore, aggressive systemic non-Hodgkin lymphoma is almost invariably treated with highly active combination chemotherapy in conjunction with first-line rituximab.²⁴ In contrast, for the aggressive neoplasm PCNSL, the mainstay of treatment is methotrexate, a drug with only moderate efficacy in lymphoma.

The use of novel approaches like monoclonal antibodies (for example, rituximab) or radioimmunotherapy (for example, ⁹⁰Y ibritumomab tiuxetan⁶⁰) is hampered in PCNSL because of the impermeability of the BBB to these high-molecular-weight agents. It seems reasonable that clinical trials for new PCNSL therapies should incorporate lessons learned from the treatment of systemic aggressive non-Hodgkin lymphoma. The BBB disruption-enhanced delivery offers the opportunity to translate monoclonal antibody-based approaches and combination treatments into clinical practice for PCNSL. Monoclonal antibody-based treatment regimens and radioimmuno-

therapy will be tested in the rat PCNSL model recently developed by our group. Two treatment protocols containing rituximab are currently open for enrollment at the BBB Program, one for newly diagnosed and another for relapsing PCNSL. A treatment protocol using ⁹⁰Y ibritumomab tiuxetan for relapsing PCNSL is currently in preparation by the BBB Group. Future plans include the establishment of a rat neurotoxicity model that should prove useful in the investigation of various factors contributing to the development of neurotoxicity as well as preventive and therapeutic strategies against this effect. The usefulness of iron oxide-based nanoparticles in the differential diagnosis of PCNSL compared with other brain lesions will be further investigated in both preclinical and clinical settings.

Further progress in the treatment of PCNSL will result from better understanding of its biological features and the performance of clinical trials. A Phase III study in which BBB disruption is compared with intravenously administered chemotherapy is desirable. However, even with committed collaboration among investigators, a randomized trial would be difficult to perform because of the rarity of PCNSL. In addition to the usual end points of survival and response rates, cognitive function and quality of life should be routine end points in all future PCNSL trials.

Disclosure

Leslie L. Muldoon (OHSU), Portland Veterans Affairs Medical Center (PVAMC), and the Department of Veterans Affairs have a significant financial interest in Adherex, a company that may have a commercial interest in the results of this research and technology. This potential conflict of interest was reviewed and managed by the OHSU Integrity Program Oversight Council and the

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PVAMC Conflict of Interest in Research Committee.

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