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Title: AIDS-related Primary Central Nervous System Lymphoma: A Phase II Pilot Study of High-Dose Intravenous Methotrexate with Rituximab, Leucovorin Rescue and Highly Active Antiretroviral Therapy

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PRÉCIS

Background: AIDS-related primary central nervous system lymphoma (AR-PCNSL) is an Epstein-Barr virus (EBV)-driven lymphoproliferative process that typically results in death within a few months. Essentially all of the cases are immunoblastic CD20+ tumors, and occur once the CD4+ cells have fallen to below 50 cells/mm^3. Highly active antiretroviral therapy (HAART) can result in immune reconstitution that decreases the risk of AR-PCNSL. However, a subset of HIV-infected patients still develops AR-PCNSL, often because they are unaware that they are HIV infected, or they do not take HAART. Treatment options for such patients are limited. In the non-AIDS setting, chemotherapy has become the standard of care for primary central nervous system lymphoma (PCNSL) and late neurocognitive decline consequent to radiotherapy can be avoided by such approaches. In the pre-HAART era, AR-PCNSL was generally treated with whole brain radiotherapy, however death due to recurrent lymphoma or to other AIDS complications occurred prior to the potential manifestations of late occurring radiation-related neurotoxicity. Radiation-sparing approaches have not been studied in AR-PCNSL in the HAART era, where advances in antiretroviral therapy have made curative intent chemotherapy feasible for most patients with HIV infection.

Objectives: The primary objective of this study is to estimate the fraction of patients with AR-PCNSL receiving experimental treatment consisting of HAART, combined with rituximab, high-dose methotrexate and leucovorin who are alive and without recurrent lymphoma or severe cognitive problems at two years.

Eligibility: HIV-infected, age 18 years or older, AR-PCSNL that has not previously been treated, and be able to give informed consent or have a durable power of attorney who can provide informed consent, HIV profile that makes them likely to respond to HAART. There are a number of other specific inclusion and exclusion criteria, in part to exclude patients who would be unlikely to tolerate the therapy.

Design: Phase II pilot study investigating high-dose methotrexate-rituximab given with leucovorin rescue and HAART as a treatment for AR-PCNSL. Evaluation will include quantitative measurement of lymphocyte subsets, quantitative polymerase chain reaction (PCR) of HIV and EBV viral loads (including both blood and cerebrospinal fluid in the case of EBV) to assess immune response and anti-viral effects. Tumor evaluation with brain magnetic resonance imaging (MRI) and brain fluoro-2-deoxy-d-glucose positron emission tomography (FDG-PET scans) will be used for staging and response assessment. Longitudinal neuropsychologic testing after complete responses are documented will serve to evaluate neurocognitive parameters post therapy. Chronic administration of HAART and longer-term evaluation of immune system parameters will be studied as part of an overall assessment of the therapy. Patients with AR-PCNSL who cannot receive high-dose methotrexate due to poor renal or cardiac function will receive HAART and “Radiation-Sparing Best Available Care”, generally consisting of dose-dense rituximab, and will be evaluated as a separate cohort for additional secondary endpoints.

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1.1.1 Primary

1.1.1.1 The primary objective of this study is to preliminarily estimate the fraction of patients with primary brain lymphoma receiving experimental treatment consisting of HAART with R-HD-MTX who are alive and without recurrent lymphoma or severe cognitive problems (as defined by Neurological Function Class 3 in Appendix 2) at two years. It will also be important to determine if this fraction may be sufficiently high for further investigation.

1.1.2 Secondary

1.1.2.1 To describe the acute toxicities associated with administering a regimen of high-dose methotrexate with leucovorin and rituximab with HAART in patients with AR-PCNSL

1.1.2.2 To estimate the complete response rate, progression-free, disease-free, and overall survivals to high-dose methotrexate with leucovorin and rituximab given with HAART in patients with AR-PCNSL

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1.1.2.6 To assess overall survival and long-term neurocognitive outcomes in patients with AR-PCNSL who receive "Radiation Sparing Best Available Care", consisting of HAART and any additional first or second line radiation-sparing approaches

1.2 BACKGROUND AND RATIONALE

1.2.1 Biology, Epidemiology and Scope of Problem

AIDS-related primary central nervous system lymphoma (AR-PCNSL) is a rare but lethal complication of advanced HIV disease[1-4]. Essentially 100% of AR-PCNSL is Epstein-Barr virus (EBV)-associated B-cell tumors. They occur almost exclusively in persons with CD4+ cells that are depleted below 50 cells/mm^3. Histologically, these tumors are overwhelmingly represented by diffuse large B-cell lymphomas (DLBCL), typically the immunoblastic variant with pathobiological markers suggesting histogenic derivation from post germinal center B-cells at late stages of maturation[5-7]. Thus, most cases show absence of bcl-6 protein while MUM1and CD138/syndecan-1,

and bcl-2 are expressed[8, 9]. While essentially all cases are EBV-associated, the EBV expression pattern in AR-PCNSL can differ. Moreover, the histological and pathobiological characteristics of AR-PCNSL appear to segregate according to EBV expression patterns. In cases that are negative for LMP-1 expression, bcl-6 protein is expressed, whereas bcl-2 is not[9]. This pattern is invariably associated with a large non-cleaved morphology similar to HIV-negative cases, and suggests germinal center B-cell histogenic derivation. In contrast to AR-PCNLS in which a proportion of tumors appear to derive from non-germinal center B-cells, in HIV-unrelated PCNSL in which EBV is almost never found, the pattern of biologic markers is essentially always most consistent with germinal center histogenic derivation[9, 10]. Treatment outcome and prognosis in AR-PCNSL may be heterogeneous and in part related to these biologic characteristics [11] as has been shown in systemic HIV-unrelated NHL[12-14]. In general, a more favorable prognosis in peripheral NHL is seen in tumors that derive from germinal center-like B-cells. While this appears also to be the case in PCNSL[11], there is uncertainty because of other factors involved in AIDS that affect prognosis. Determination of tumor biology in AR-PCNSL is important toward understanding treatment outcomes, and forms part of the rationale for tumor acquisition on this study.

Prior to the availability of highly active antiretroviral therapy (HAART), nearly 20% of all lymphomas in AIDS patients were AR-PCNSLs[15, 16]. The relative risk of developing PCNSL in AIDS was 1000-fold that of the non-AIDS population[17]. As a consequence of better preserved immune function with HAART[18], the incidence of AR-PCNSL has fallen substantially. In the pre-HAART era the incidence was more than 300% higher than currently seen [3, 19]. Similarly, a much smaller proportion of AIDS-related lymphomas (ARL) are PCNSL, because the decrease in incidence of this tumor is greater than for the other ARLs [19]. HAART has also favorably affected survival in patients with ARL, including AR-PCNSL. Before HAART availability, the median survival after diagnosis of AR-PCNSL was 2 to 3 months. However, six-fold or greater increases in survival are reported when HAART is given compared to cases not treated with HAART [16, 20], though this is not a consistent finding[21]. While AR-PCNSL currently affects relatively few individuals, this may not be a lasting phenomenon as the AIDS epidemic evolves. The number of HIV-infected individuals in the United States continues to increase. At present, estimates suggest at least 950,000 Americans are infected, a quarter of whom do not know their seropositive status[22]. These people often do not receive antiretroviral therapy until after they are confronted with opportunistic disease, including AR-PCNSL[22-26]. As a consequence, AR-PCNSL may continue to be a serious medical problem, especially in medically underserved demographic groups most affected by the HIV epidemic. In such resource poor settings where treatment access is often delayed and HAART is not readily available, AR-PCNSL continues to be a rapidly lethal complication of AIDS[4]. In addition to the changing demographics of persons with AIDS, there has been more than a 50% increase in the prevalence of persons living with AIDS because of the increased longevity consequent to HAART[27]. Since 1996, when HAART was introduced, the number of persons with AIDS in the Unites states increased from approximately 200,000 to nearly 400,000[22]. There is thus an expanding at-risk

population for malignant complications of AIDS, now the major form of HIV-related morbidity and mortality in resource-rich societies where HAART is commonly used[27-29]. Additionally, in Western Europe, the proportion of primary brain lymphomas as an AIDS-defining condition in HIV-infected patients has remained relatively steady in the period 1988-2000[30]. Therefore, even though this tumor is currently rare, it is quite conceivable that the absolute numbers of cases of AR-PCNSL will increase consequent to a number of issues. With increased longevity there may be an overall increase in lifetime risk of malignant complications of HIV disease[31]. As the AIDS epidemic continues to shift to medically underserved populations, individuals may be more likely to first present with complications related to advanced immune depletion, such as AR-PCNSL.

1.2.2 Radiation Related Neurotoxicity and Beyond

In the non-AIDS setting, whole brain radiotherapy (WBRT) has been supplanted by chemotherapy using high-dose methotrexate usually combined with other agents because this approach appears associated with superior survival and avoids the high risk of late occurring neurotoxicity associated with WBRT, especially in high-risk patients[32, 33]. The risk of neurotoxicity increases with age. In those older than 60 years, virtually all long-term survivors after a combined treatment of radiation and methotrexate will develop severe delayed neurotoxicity with devastating consequences in terms of reduced quality of life[34]. In those younger than 60 years, little is known about the exact incidence of this complication. Generally, cognitive dysfunction is less severe in younger patients, and may not be recognized unless formally assessed. Harder et al. investigated a series of 19 consecutive young patients treated in a prospective trail for PCNSL with combined modality treatment with systemic and intrathecal high-dose methotrexate-based chemotherapy followed by WBRT and found cognitive impairments in 63%, including 21% with severe impairment[35]. This contrasts sharply with studies reporting absence of neurocognitive decline in patients treated with high dose methotrexate and not radiotherapy[36, 37].

AIDS patients often have greater than anticipated radiation related toxicity, at least in mucocutaneous sites[38]. Excessive neurotoxicity in ARL has also been reported[39]. In a phase II study of dose-adjusted EPOCH chemotherapy for peripheral AIDS-related lymphomas, death due to the neurotoxic effects of combined chemotherapy and cranial radiotherapy for CNS involvement was not uncommon[39], underscoring the generally accepted principle that AIDS patients are at increased vulnerability to the toxic effects of radiotherapy[38, 40, 41].

The response to WBRT or to large-volume radiotherapy has been divided into three categories based on the timing of onset for neurological symptoms[42]. In general, the early effects are reversible, whereas late onset effects are not. The acutely occurring effects within the first weeks of fractionated radiotherapy are generally characterized by drowsiness, headache, nausea, emesis, and worsening of pre-existing focal symptoms. It is thought that increased cerebral edema causes the symptoms, and so

dexamethasone is commonly used and generally results in symptomatic improvement. It is important to note, however, than in advanced HIV disease, high-dose steroid administration can be associated with increased risk of opportunistic illnesses, and is therefore avoided whenever possible. At 1 to 6 months after completion of radiotherapy, early delayed or subacute encephalopathy typically presents, and is also reversible. Symptoms include headache, somnolence, fatigability, and deterioration in pre-existing deficits[43]. Subacute encephalopathy is believed to be secondary to diffuse demyelination[44]. Although corticosteroids are useful in diminishing the symptoms, spontaneous resolution generally occurs within several months after presentation and so the role of steroids is uncertain. The generally irreversible and progressive late delayed neurologic effects of radiotherapy typically appear more than 6 months after completion of radiotherapy [45]. Injury is often localized to the white matter, and is thought to be a consequence of vascular injury, demyelination, and ultimately brain tissue necrosis. Late effects can be further characterized as focal radiation-related necrosis, diffuse white matter injury, and combined chemotherapy-radiotherapy leukoencephalopathy. Symptoms vary depending on type and location of injury. In focal radiation necrosis, symptoms include seizures, signs of increased intracranial pressure, and neuroanatomic-specific effects, such as functional cord transection[46]. MRI reveals an area of irregular enhancement often with surrounding edema. Focal radiation necrosis is often difficult to distinguish from recurrent or progressive tumor and can thus represent a treatment and diagnostic dilemma[46]. Although positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) may help differentiate the two, tissue biopsy is currently required to make a definitive diagnosis. Symptoms of diffuse white matter injury range from mild lassitude to significant memory loss and severe dementia [47]. Combined therapy leukoencephalopathy can be similar to diffuse white matter injury, but it can also include ataxia, confusion, dysarthria, seizures, and ultimately incapacitating dementia or death[48]. Imaging findings are similar to that of diffuse white matter injury, but they also may include dystrophic calcification of the basal ganglia and gray–white matter interface[47]. Since HIV-infected patients may be more vulnerable to the toxic effects of radiation-induce injury, and to the corticosteroids used to manage the complications, this is a critical consideration for AR-PCNSL[15, 46].

The standard of care in AR-PCNSL remains whole brain radiotherapy (WBRT) to 40-50 Gy. This therapy can successfully induce complete responses in the majority of AR-PCNSL cases[15]. Recurrent lymphoma or other fatal AIDS-related complications severely limits survival, and in practice, the therapeutic intent has generally been palliative. Yet, the treatment advances now standard in non-AIDS PCNSL have not been widely applied in AIDS. Reasons for this vary, but in part it is because of the general perception that such patients cannot tolerate aggressive chemotherapy, and the notion that disease-related death will occur prior to the occurrence of radiation-related neurotoxicity. However, improved tolerance to antineoplastic therapy with HAART has rendered reflexive palliative approaches inappropriate for potentially curable AIDS-related tumors, and improved survival prospects raise concerns regarding neurotoxicity risks [28, 49-51]. Treatment of peripheral ARL has in particular guided this paradigm shift in cancer therapy for patients with AIDS. With HAART,

patients with ARL have improved overall survival[52], equivalent to their HIV-not infected counterparts for certain NHL subtypes [39, 53]. Longer survival and PCNSL remission in the HAART era is dependent in general on reduced AIDS complications consequent to immune reconstitution and also appears related in part to improved immunosurveillance against EBV-related proteins that may help to reduce the risk of PCNSL recurrence[54]. The latter concept is reinforced by case reports that have documented radiographic improvements in AR-PCNSL with HAART alone[55], consistent with the known effects of immunomodulation on EBV-driven lymphoproliferations in the transplant literature[56]. Thus, improved survival prospects for these patients raises the specter that late occurring toxic effects associated with WBRT will diminish the therapeutic benefits. These effects can seriously affect both quality of life and limit survival[37], potentially annulling any life-prolonging benefit from HAART and tumor eradication that patients may otherwise enjoy. Therefore, development of effective non-neurotoxic therapy for AR-PCNSL would advance the field of AIDS oncology. The purpose of this study is to investigate relatively non-immunosuppressive systemic therapy in AR-PCNSL and to simultaneously promote CD4+ cell recovery in order to preserve brain function through tumor control and avoidance of late radiotherapy-related neurotoxic complications.

1.2.3 Methotrexate-based Systemic Therapy in PCNSL

Because up to 80% or more of high-risk patients treated with WBRT develop severe radiation induced neurological injury[37], clinical research for non-HIV-related PCNSL has focused on the development of strategies to reduce this risk. Reduction of WBRT doses to 30 Gy or exposure focus by stereotactic administration of radiotherapy following high-dose methotrexate has been one approach that has appeared to be useful in some patients [57]. However, in AR-PCNSL, treatment to doses higher than 30Gy have been associated with higher response rates [16]. Often, AR-PCNSL is not amendable to stereotactic administration of radiotherapy because of the numerous mass lesions throughout the brain. Also there has been interest in treating PCNSL with systemic therapy, deferring radiation whenever possible. This has led to the use of high-dose methotrexate as a major focus of systemic chemotherapy regimens [58, 59]. Studies have shown that when brain radiotherapy is avoided, that neurocognitive function is much better preserved[37], though as reviewed below, findings are mixed in terms of overall survival between the two general approaches. In non-HIV-related cases, median survival with WBRT typically ranges from 12-18 months[60]. The addition of chemotherapy to the treatment (combined modality therapy) has been associated with increases in survival to more than 40 months in some studies[59]. However, the risk of neurotoxicity is increased with the combined modality therapy, so that as a result of severely impaired cognition there is no overall improvement in the desired outcome (alive without brain dysfunction)[37]. Equivalent data for patients with AR-PCNSL is not available.

Although a substantial body of literature has evolved supporting the use of chemotherapy and combined modality therapy with limited radiotherapy in PCNSL, no large prospective trials have been conducted to inform comparisons of

chemotherapy alone or combined chemo-radiotherapy with radiotherapy alone. Consistent with basic established principles in lymphoma therapy, most (but not all) studies have avoided monotherapy approaches in favor of combination chemotherapy approaches. Although there is not yet a regimen that can be proclaimed as the standard of care in PCNSL, one element from the research to date is quite clear: regimens used for treatment of systemic non-Hodgkin's lymphomas (NHL), such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) combined with WBRT are toxic and do not confer advantage over WBRT alone[61]. Thus, it has been emphasized that CHOP or similar regimens have no role in the treatment of PCNSL[62]. However, this finding does not undermine the basic principle that combined chemotherapy approaches are superior to monotherapy in NHL.

As mentioned above, high-dose methotrexate has been the major focus of clinical investigation in PCNSL. Methotrexate was originally chosen for PCNSL because of its ability to penetrate the blood-brain barrier, and its known activity against lymphoma. Doses of methotrexate for PCNSL have ranged from 1 gram/m^2 to over 8 grams/m^2, with doses of 3 grams/m^2 penetrating into the CNS more reliably than lower doses [63]. Some evidence suggests a positive dose-response relationship to antitumor potential in favor of the higher methotrexate doses[64]. High-dose methotrexate is considered to be the single most important agent for the treatment of PCNSL in immunocompetent patients, but as monotherapy there are mixed results as it compares to WBRT alone or when given along with additional agents. Indeed, as mentioned above, monotherapy for aggressive non-Hodgkin's lymphoma is contrary to the treatment principles established over the past three decades. Thus while high-dose methotrexate monotherapy is not of current interest in clinical investigation, information from previous investigations in the HIV unrelated setting are informative. For example, overall survival correlates favorably with high-dose methotrexate as part of a combined high-dose methotrexate and WBRT regimen, providing encouraging phase II data that high-dose methotrexate has an important role in this disease [65, 66]. A multicenter phase II trial conducted by the New Approaches to Brain Tumor Therapy CNS Consortium reported a 52% complete response rate, and an overall response of 74% with high-dose methotrexate at 8 grams/m^2 every 2 weeks, an outcome comparable to multiagent, generally more toxic regimens[65]. However, phase II trials combining high-dose methotrexate with vincristine and procarbazine before WBRT have resulted in median survivals of 33 to 60 months, an apparent improvement over WBRT alone[33]. The use of high-dose methotrexate with procarbazine and vincristine without WBRT has resulted in similar outcomes, again pointing out the feasibility of avoiding radiotherapy in this disease [33]. Similarly, multiagent regimens designed on a backbone of high-dose methotrexate and also using agents such as carmustine, teniposide, cytarabine, ifosfamide, cyclophosphamide, vinca alkaloids and other agents have been explored[58, 59]. In a report of 15 patients treated with high-dose methotrexate of 8.4 g/m^2 and leucovorin rescue, thiotepa, vincristine, dexamethasone, intrathecal cytarabine and methotrexate, the complete response rate was 79%, and the median progression-free survival 16.5 months[67]. Seven patients in this study had formal neuropsychometric testing, and five had no evidence of neurocognitive decline. Two patients had severe neurocognitive

and neuromotor deterioration after therapy, though one of these had evidence of prior multi-infract brain pathology. In general, preservation of neurocognitive function appears feasible with systemic chemotherapy. However, the acute myelosuppression and lymphocytotoxicity associated with these regimens is substantial. This feature of the therapy may be counterproductive to overall outcome in AR-PCNSL owing to the clear relationship between immune status and tumor development. Given that more than 12 months is required for lymphocyte recovery to baseline levels following the completion of lymphocytotoxic chemotherapy for ARL[39], regimen components associated with these toxicities may best be avoided in this setting. A successful treatment strategy in this disease may require an emphasis on early CD4+ lymphocyte restoration.

1.2.4 CD4+ Lymphocyte Sparing Therapy for AR-PCNSL

1.2.4.1 High-dose Methotrexate and Leucovorin

Attempts to promote more rapid CD4+ lymphocyte reconstitution requires use of relatively T-lymphocyte sparing regimens, and this may be a more suitable approach in the AIDS population. Given with leucovorin rescue, high-dose methotrexate itself is relatively non-myelotoxic and relatively non-injurious to the immune system[68, 69]. There is no specific data to unequivocally determine the best dose for high-dose methotrexate in this setting. However, considering higher doses appear to be associated with better outcome and that doses less than 3 gram/m^2 may be insufficient for optimal tumor penetration, combined with the mono-cytotoxic approach in this clinical trial, a relatively high dose within the spectrum of that typically used appears logical. The strategy in this study therefore will be to use high-dose methotrexate at 6 grams/m^2 with leucovorin rescue in combination with agents not associated with particular T-lymphocyte depletion.

Although use of high-dose methotrexate in AR-PCNSL has not been extensively studied, there is some prior experience with its use in this setting. Pre-HAART, a small study suggested that the toxicity was substantial but that some patients did benefit from the approach[70]. High-dose methotrexate in combination with HAART has been feasible in certain systemic AIDS-related lymphomas associated with advanced immune depletion[69]. There is substantial precedent for high-dose chemotherapy in the HAART era for patients with AIDS and cancer. High-dose chemotherapy and both autologous and allogeneic bone-marrow transplant has been successfully undertaken in AIDS patients[51, 71-74]. Thus a treatment strategy for primary brain lymphoma that utilizes high-dose methotrexate with leucovorin rescue, similar to the non-AIDS setting, is worth investigating in AIDS. By combining HAART with the therapy, it may be possible to promote earlier control of HIV infection and initiate immune recovery.

1.2.4.2 Highly Active Antiretroviral Therapy

Immunologic recovery is essential toward preventing the substantial risk of death due to other AIDS complications, and may also help to prevent lymphoma recurrence, as mentioned above. There are several features of AR-PCNSL that highlight the importance of immune recovery as a component of therapy in this disease: 100% of AR-PCNSL are EBV-associated lymphoproliferations; AR-PCNSL rarely occurs until the CD4+ lymphocyte subsets are depleted to less than 50 cells/mm^3; and a number of case reports have stressed that complete tumor regressions occasionally occur in parallel with immune recovery on HAART alone [55]. In immunologically normal individuals, activated T-cells traffic through normal brain tissue[75]. These cells may perform an important function in tumor immunosurveillance against viral and/or tumor-associated antigens and this concept is consistent with the constant finding that profound immune depletion is directly related to AR-PCNSL risk. Restoration of this immunosurveillance may hence be important to the outcome in therapy for this disease. Therefore, HAART should be considered as part of the oncologic therapy for purposes of this clinical trial.

1.2.4.3 Rituximab

In devising an overall T-cell sparing therapeutic regimen for AR-PCNSL to augment the high-dose methotrexate (and HAART), it is worth noting that AR-PCNSL are virtually all B-cell immunoblastic tumors associated with latent EBV infection showing only latent expression patterns[6]. The brain lymphoma cells typically express the CD20+ antigen in both AIDS-related and non-AIDS PCNSL, whereas normal neurons or glia do not. This observation has informed development of small studies that have investigated the use of the monoclonal anti-CD20 antibody, rituximab, in non-AIDS PCNSL[76-79]. There are a number of issues that on the surface may cast doubt on the capacity of this therapy to work as effectively in PCNSL as it does in peripheral lymphomas. However, closer examination of the data appears to in fact support the use of rituximab in PCNSL. For example, following infusion of the standard dose of rituximab (375 mg/m^2), serum rituximab levels typically range from 107 to 962 mcg/mL[80]. Pharmacokinetic studies have shown that the cerebrospinal fluid (CSF) levels of rituximab are approximately 0.1 to 1% of serum levels associated with therapeutic activity in patients with systemic NHL, a finding not surprising given its high molecular weight[81, 82]. Although this may at first seem to represent insufficient CSF penetration, it ranges from 1 to nearly 10 times the amount of rituximab shown to saturate 90% of cell surface CD20 antigen[83] and thus in fact provides more than sufficient rituximab exposure to have the desired biologic effect. It is important to recognize that CSF drug levels do not adequately reflect parenchymal brain tumor exposure to agents, as log-fold greater drug concentrations can be found within brain tumors compared to the CSF[84]. On the other hand, there has been no data to suggest that radiolabeled rituximab concentrates in PCNSL preferentially. Again, close examination of this finding reveals it to be not fully relevant to the use of unlabeled rituximab for CNS lymphoma. A case report of single photon emission computed tomography (SPECT) imaging of non-AIDS PCNSL using idodine-123 labeled rituximab failed to demonstrate rituximab

uptake in PCNSL in three of four patients, suggesting that systemic rituximab-based radiotherapy approaches would not be feasible for this tumor[85]. The strength of these observations is weak. For example, a similar technique in peripheral lymphoma (for which rituximab is approved by the US Food and Drug Administration), delineated tumor in only 14 of 37 patients studied, because sites in proximity to blood vessels could not be adequately defined[86]. Thus, this technique, though informative, has limitations that must be taken into account, particularly as relates to the case studies that failed to show increased uptake in the few cases of PCNSL studied. An additional consideration may be that the standard pre-infusion with unlabeled rituximab that is first administered in such studies could potentially have the effect of competing with the radiolabeled product for antigen and thus underestimating rituximab penetration into the tumor. Also, the extent to which these results inform the use of biotherapy should be placed into the context of toxicity related to radiolabeled rituximab. There is organ specific toxicity that limits repeated dosing of radiolabeled rituximab. Therefore, if the tumor-specific exposure is not sufficient for adequate therapeutic effect over one or two dosing administrations, radiolabeled rituximab is not a feasible treatment because repetitive dosing becomes too toxic. Thus, while the results of the case-control report cast real doubt on the use of radiolabeled rituximab for PCNSL, it does not necessarily address the use of unlabeled rituximab. In contrast to radiolabeled rituximab, unlabeled rituximab can be safely administered over multiple doses, so that even if there is only a moderate tumor-drug interaction per dose, repeated doses can be safely used to treat the tumor.

Encouraging data on the utility of unlabeled rituximab in AIDS-unrelated PCNSL has been reported in small studies. Since CNS lymphoma has a predisposition for perivascular infiltration that disrupts the blood brain barrier[87], greater anticancer agent penetration into areas of the brain affected by tumor are seen when compared to surrounding normal brain, as shown in biopsy and autopsy specimen in studies addressing this[88]. Indeed, there is mounting clinical experience that rituximab can be useful in primary brain lymphoma when administered intravenously [76, 77, 89, 90]. In small studies of refractory PCNSL, responses have been seen in the majority of treated patients who received rituximab monotherapy or rituximab in combination with cytotoxic agents. Although reports that rituximab monotherapy in previously treated refractory-relapsed PCNSL results in clinical and radiographic responses suggests that rituximab can penetrate into areas of bulky disease in the brain[91], there is insufficient data to know with certainty what the overall contribution of rituximab is in the combination chemotherapy setting. However, PCNSL experts have supported its clinical trials development[90, 92-94]. The Cancer and Acute Leukemia Group B trial 50202 entitled “Intensive Chemotherapy and Immunotherapy in Patients with Newly Diagnosed Primary CNS Lymphoma" is a multi-institutional study further investigating rituximab combining it with high-dose methotrexate and temozolomide. Thus, there is well-established precedent for investigating the use of rituximab in PCNSL in the HIV unrelated setting. Likewise, rituximab may be a promising agent in AIDS-related PCNSL as

articulated above. The core regimen in this protocol will thus investigate the use of leucovorin protected high-dose methotrexate with rituximab and HAART.

Rituximab will be omitted from cycles 7 and 8. The rationale for this is that only patients who have achieved a complete response will receive the additional two cycles. Therefore, it is assumed that rituximab penetration into the area previously affected by obvious tumor will become insufficient due to improved blood-brain barrier integrity. However, it may be beneficial to administer additional high-dose methotrexate cycles with increased dose-intervals in order to help prevent early tumor recurrence while allowing for additional time for greater immunologic recovery.

It should be noted that rituximab is not considered at present to be a standard of care in HIV-associated lymphomas, and it appears to be associated with excess morbidity and mortality when used with CHOP chemotherapy for peripheral ARL, especially for patients with advanced CD4+ depletion[95]. The NCI sponsored AIDS Malignancy consortium has reported published results of a large multicenter trial of ARL in which all patients received HAART and CHOP and were randomized to receive rituximab or not[95]. Treatment related deaths occurred in 14% on the rituximab arm compared to 2% of those not receiving rituximab. Moreover, the complete response rate was not different between the two arms, and survival was not improved. Thus, there was excess treatment-related mortality without sufficient evidence of benefit in this trial. Subset analysis suggested that there may have been a subset of patients for whom a benefit could be realized, but the study was not designed to assess this with scientific validity. In contrast, positive benefit of rituximab in ARL has been reported in pooled results of three phase 2 trials of R-iCDE (rituximab with infusional cyclophosphamide, doxorubicin, and etoposide). The complete response rate of 70% was quite high (substantially higher than that previously seen with CDE alone), but again there was evidence of excess treatment-related deaths with approximately 20% of patients having non-fatal infectious complications[96]. For these reasons, some experts have now recommended that rituximab not be used in ARL as standard practice, but that additional investigational studies are needed to better understand the role of rituximab in AIDS [97] (Yarchoan et al, Nat. Onc. in press) [95, 96]. Experience from the transplant setting is also in evaluating rituximab in immunocompromised patients. High-dose rituximab of up to 1000 mg/m^2 has been reported to be potentially more effective than standard dose in high-risk lymphoma patients being treated with bone marrow transplant, a finding that also emphasizes the relative safety of rituximab in immunosuppressed patients. Transplant-related EBV lymphoproliferative disease has been successfully forestalled and treated with rituximab[98, 99]. Additionally, there is increasing evidence supporting the use of rituximab for primary CNS post- transplant lymphoproliferative disorder. In a retrospective multicenter review of 71 cases, 48% received rituximab as part of their treatment, including 8 patients who received rituximab alone. Patients receiving rituximab had improved 3-year progression free survival (40% vs. 22%, p= 0.02) and a trend towards a significant improvement in 3 year overall survival

(43% vs. 22%, p=0.06).[100] In a review of 9 cases of AR-PCNSL treated by HAMB investigators with HAART, rituximab, and various chemotherapies, the estimated overall survival at one year and beyond is 77.8%. This series included one patient treated with HAART and rituximab alone due to significant cardiac comorbidity and poor performance status, who has achieved a complete response to therapy with 21 months follow up, has evidence of immune reconstitution on HAART, improvement in performance status, and no evidence of AR-PCNSL recurrence.[101]

Thus, although there is clinical data that emphasizes the need for cautionary use of rituximab in immunosuppressed patients, there is also data supporting its use, particularly in poor prognosis aggressive lymphoma. The present protocol has been designed to avoid multiple lymphocytotoxic agents in an attempt to reduce the infectious risks associated with rituximab when combined with CHOP in ARL. Additionally, other approaches that can be considered for AR-PCNSL (e.g. published combination chemotherapy regimens used in AIDS-unrelated PCNSL) are likely to be substantially more toxic than rituximab, even as reported in advanced AIDS when combined with CHOP. Safety may also be improved this with filgrastim, shown to rapidly reverse the late-onset leucopenic effects of rituximab[102, 103]. It is predicted that since rituximab as a single agent is very well tolerated[80], it is unlikely to show excessive toxicity when given with high-dose methotrexate and leucovorin with HAART. Careful assessment of this is one of the study objectives, and the data generated on the study will inform as to the outcome in this regard. Vigilant surveillance and monitoring will be required in this regard.

1.2.5 Novel Tumor Assessment and Psychometric Testing

In addition to the antitumor therapy, this protocol will in a preliminary manner assess novel measures of response and predictors of disease recurrence based on important recent advances in the diagnosis of AR-PCNSL. Based on the finding that essentially all AR-PCNSL are EBV associated[104-106], RT-PCR detection of EBV in the CSF has been found to be a sensitive and specific marker for lymphoma in HIV-infected patients with focal brain lesions[106, 107]. In combination with SPECT thallium or fluoro-2-deoxy-d-glucose positron emission tomography (FDG-PET) scanning of the brain, a minimally invasive diagnostic procedure for AR-PCNSL appears feasible[106, 108]. The combination of positive EBV PCR in the CSF and positive SPECT thallium has been shown to have 100% positive predictive value for lymphoma in AIDS patients with cerebral mass lesions[106, 108] Likewise, if both tests are negative, the negative predictive value for lymphoma is 100%. Discordant findings require biopsy or persistent growth in the presence of anti-toxoplasmosis therapy in order for an empiric lymphoma diagnosis to be made. While minimally invasive approaches to defining focal brain lesions has gained acceptance and is considered an acceptable standard of care for initiation of specific therapy in AR-PCNSL in certain settings[51], the use of these studies have not been studied in terms of tumor response to therapy or in the relapsed setting. Also, the peripheral blood EBV loads have not been well correlated with AR-PCNSL, though there is evidence that the peripheral EBV load is

elevated in the setting of AR-PCNSL[109]. Thus, study of EBV in the spinal fluid and plasma before, during and after treatment may provide preliminary information that can be useful for developing additional measures to assess tumor response (e.g. relatively non-invasive measures of minimal residual disease) and could provide a means toward predicting durability of response. Along with the more standard response assessments utilizing MRI and FDG-PET used in brain lymphoma, serial EBV DNA quantitation by PCR of the CSF and plasma will be used to provisionally assess response in correlation with the other standard outcome measures.

Neurocognitive preservation is central to the goals of this clinical investigation, and serial neuropsychometric testing will be performed on patients. Since at the time of presentation patients are expected to have tumor related cognitive impairment, baseline neuropsychometric testing will be performed within the first two months of the time of complete tumor response. Repeat testing will be performed every 3 to 6 months up to 2 years following end of treatment, and then again at 3 years. Such measures have been found to be useful in PCNSL patients for assessing neurocognitive functioning over time[110].

The protocol will also assess patients from the standpoint of the underlying HIV infection. Since justification for pursuing non-radiation-based therapy is that HAART may improve survival such that patients live long enough to experience radiation-induced brain injury, patients with multidrug resistant HIV not amenable to suppression will not be eligible for the study, because a meaningful lasting immune recovery will not be feasible. Thus, immune parameters such as the CD4+ and CD8+ cell counts, HIV viral loads, HIV resistance mutations, and HAART adherence will be recorded. Patients who fail to maintain low HIV viral loads and as a consequence experience other HIV-related complications that can affect neurocognitive function will be censored as soon as this becomes evident, and such patients can be replaced, as discussed in the statistical section of the protocol.

1.2.6 Summary of Study Direction

The intent of this protocol is to provide data regarding the feasibility and effectiveness of high-dose methotrexate with leucovorin and rituximab given with HAART from a small number of patients with AR-PCNSL who have a favorable prognosis from the standpoint of their HIV disease. A major focus is to determine if a majority are alive without substantial brain dysfunction at two years after diagnosis. If the trial is favorable, then the approach may be of interest for further development in the extramural multicenter setting. The NCI-sponsored AIDS Malignancy Consortium will be apprised of the results as the trial progresses, as this is the group in the United States most likely to be interested in pursuing further research in the area.

This protocol was originally conducted in conjunction with a protocol being conducted by NIAID investigators in collaboration with our group focused on diagnosis of CNS brain masses (Protocol Number 05-CC-0246, Principal Investigator

Leatha Healey, MD) Patients diagnosed with AR-PCNSL were followed up to 24 months on the NIAID study. The NIAID investigators on that study are also associate investigators on this study. Although 05-CC-0246 has since closed, we continue to collaborate with NIAID investigators, and patients may be concurrently enrolled in 06-C-0051 and appropriate NIAID protocols. Continued collaboration comprises a comprehensive diagnostic and treatment research effort that is intended to help foster further collaborative research and improved ability to study this disease in the extramural NIAID and NCI communities.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Positive HIV serology (previous records acceptable)

2.1.1.2 Diagnosis of Primary Central Nervous System Lymphoma

2.1.1.3 Confirmed histopathologic diagnosis by NCI Laboratory of Pathology

2.1.1.4 If tissue diagnosis is not feasible for any reason, such as undue risk to the patient to acquire tissue diagnosis, the following will be accepted as confirmed AR-PCNSL diagnosis:

- Positive brain FDG-PET and
- EBV detected in the CSF using PCR

2.1.1.5 Age 18 years or greater

2.1.1.6 ECOG performance 0-4

2.1.1.7 Ability to understand and willing to provide informed consent

2.1.1.7.1 If patient unable to understand informed consent, a previously designated durable power of attorney for healthcare or an individual with legal authority may substitute in this capacity

2.1.1.7.2 Assignment of a durable power of attorney for healthcare if not already done

2.1.2 Exclusion Criteria

2.1.2.1 Prior therapy for CNS lymphoma

2.1.2.1.1 Steroids not an exclusion

2.1.2.2 Evidence of lymphoma outside of the central nervous system

2.1.2.2.1 Ocular involvement will not exclude

2.1.2.3 Multidrug resistant HIV not amenable to long-term suppression based on either or both:

2.1.2.3.1 Clinical history of poor adherence to multiple antiretroviral drugs deemed sufficient to render effective HIV control unattainable;

2.1.2.3.2 HIV mutational analysis (genotyping and/or phenotyping) that reveals high-level resistance such that a combination regimen comprised of agents from at least two drug classes cannot be devised to suppress HIV long-term.

2.1.2.3.3 Refusal to adhere to HAART

2.1.2.4 Concurrent malignancy other than Kaposi sarcoma, resectable squamous cell or

basal cell skin cancer, or T1 anal cancer amenable to surgical resection.

2.1.2.5 Heart failure, Class IV by New York Heart Association criteria

2.1.2.6 Chronic Liver Disease, Child-Pugh class B or C

2.1.2.7 Pregnancy

2.1.2.8 Refusal to practice contraception during chemotherapy.

2.1.2.9 Any condition or set of circumstances that the Principal Investigator (PI) or Lead Associate Investigator (LAI) interprets as creating undue risk to the patient by participating on this study or would make the patient unlikely to comply with the study.

2.2 RESEARCH ELIGIBILITY EVALUATION

2.2.1 Screening Studies

All radiographic studies and EKG must be completed within 3 weeks prior to study entry; all standard laboratory tests must be done within 72 hours prior to entry; tumor diagnostic studies and viral studies may be done at any time prior to entry)

2.2.1.1 History and physical examination with documentation of abnormal findings

2.2.1.1.1 Evaluation of ECOG status

2.2.1.1.2 Detailed history of antiretroviral therapy and assessment for potential multidrug resistant HIV

2.2.1.1.3 HIV genotyping and/or phenotyping if needed to assess for HIV resistance (previous outside reports adequate if done within prior 6 months)

2.2.1.2 Brain Imaging Studies

2.2.1.2.1 MRI scan

2.2.1.2.2 FDG-PET scan

2.2.1.3 Computed tomography scans of the chest abdomen and pelvis to assess for peripheral lymphoma

2.2.1.4 Cerebrospinal fluid studies (if previously performed on sister NIAID protocol, not necessary to repeat)

2.2.1.4.1 Cytopathological examination

2.2.1.4.2 Flow cytometric examination

2.2.1.4.3 Measurement of cell count with differential, glucose and protein

2.2.1.4.4 Quantitative EBV PCR (CC Microbiology)

2.2.1.4.5 Ig PCR and EBV PCR (Molecular Pathology, Dr. Mark Raffeld, 2N116-A)

2.2.1.5 Brain Biopsy at site of tumor

2.2.1.5.1 May be omitted if unsafe and if FDG-PET and EBV studies are positive

2.2.1.6 Serum creatinine

2.2.1.6.1 Calculated and/or measured creatinine clearance

2.2.1.7 Complete blood count and differential

2.2.1.8 PT and PTT

2.2.1.9 Biochemical assessment

2.2.1.9.1 Serum electrolytes

2.2.1.9.2 Hepatic panel
2.2.1.9.3 Mineral panel

2.2.1.10 EKG

2.2.1.11 HIV serology if previous records not available

2.2.1.12 Obtaining consent from a patient without capacity

When a subject does not have the capacity to give informed consent to enroll in this protocol, consent may be obtained from the subject's legal guardian or the subject's durable power of attorney for health care. In these cases, assent will be obtained if the subject is capable of providing it, and the subject's dissent will be respected.

Typically, the surrogate must be present to give informed consent. In exceptional cases, when it is not feasible for the surrogate to come to the Clinical Center, surrogate consent may be obtained by telephone. When this approach is used, a copy of the consent form will be provided to the surrogate ahead of time. In addition, a copy of legal guardianship papers, patient's durable power of attorney for health care, or documentation that the individual has legal authority will be placed in the patient's chart.

At the beginning of the call, the surrogate will be asked to identify themselves and confirm that they are the subject's legal guardian or hold the subject's durable power of attorney, and that they have the written consent form with them. The study, including the purpose, risks, potential benefits, and alternatives will be explained by a member of the Clinical Investigator from the research team who is authorized to obtain informed consent for the study. The surrogate will be offered the opportunity to ask any questions, and to deliberate and discuss with other parties before making a decision. If the surrogate wants more time, a second call will be arranged.

The conversation will be monitored by a third party at the Clinical Center who is not a member of the research team (e.g. nurse, social worker, bioethics). The surrogate will be asked to sign the consent form, retain a copy, and mail the original to the research team. An explanation of the circumstances and manner of obtaining the surrogate's consent, the names of the person providing consent, the person obtaining consent, and the monitor, will be documented in a progress note in the subject's medical record, and signed and dated.

When surrogate consent is obtained by telephone, the research team will establish a reliable means of communicating with the surrogate regarding future decisions, and will consider the possibility of having someone present at the Clinical Center to make day-to-day decisions on the patient's behalf. At the time of continuing review, the research team will provide the IRB with a summary of the instances in which telephone consent was used, and the justifications for using it.

2.3 REGISTRATION PROCEDURES

Each patient will be discussed with the Principal Investigator or Lead Associate Investigator, or other designated Associate Investigator. Once it has been determined that a patient qualifies, informed consent will be obtained from the patient, as documented by a signed statement of informed consent approved by the NCI-IRB.

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 TREATMENT ASSIGNMENT

Cohorts

Number	Name	Description
1	Single Cohort	HIV-infected, adult patients with AIDS-related primary central nervous system lymphoma

Arms

Number	Name	Description
1	Single arm	Induction treatment cycles with rituximab, high-dose methotrexate and leucovorin will be administered every 2 weeks for 6 cycles. Two additional consolidation cycles of high-dose methotrexate without rituximab will be administered at 4 weeks and 8 weeks following completion of the combined therapy.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The primary objective of this study is to estimate the fraction of patients with AR-PCNSL receiving experimental treatment consisting of HAART and rituximab combined with high-dose methotrexate and leucovorin who are alive and without recurrent lymphoma or severe cognitive problems at two years.

3.1.1 Additional exclusion criteria that would prohibit use of high dose methotrexate

In order to receive the primary protocol therapy, patients must not have any of these additional exclusion criteria that would prohibit the use of high dose methotrexate:

3.1.1.1 ECOG Performance Status = 4

3.1.1.2 Laboratory abnormalities that make the protocol therapy unsafe to administer:

3.1.1.2.1 Platelet count <50,000/mm^3

3.1.1.2.2 Inability to obtain an absolute neutrophil count >1000 cells/mm^3 despite use of filgrastim for at least 5 days (see section 3.3.5.6)

3.1.1.2.3 ALT and/or AST >2 X upper limit of normal unless due antiretroviral therapy or other necessary medications, in which case ALT and AST exclusion cut-off is >5X upper limit of normal

3.1.1.2.4 Bilirubin >2 X upper limit of normal unless due to protease inhibitor therapy

3.1.1.2.5 Creatinine >1.5 mg/dL, unless estimated or measured creatinine clearance is ≥60 mL/min.

- If creatinine clearance estimated with the method of Cockcroft and Gault is <100 mL/min, a measured creatinine clearance should be obtained.

3.1.1.3 Patients with Class III or higher New York Heart Association Classification of Congestive Heart Failure unresponsive to treatment that would make hydration infeasible.

3.1.1.4 Evidence of other opportunistic diseases or complications of HIV infection that would make the protocol therapy infeasible to administer. Examples include, but are not limited to:

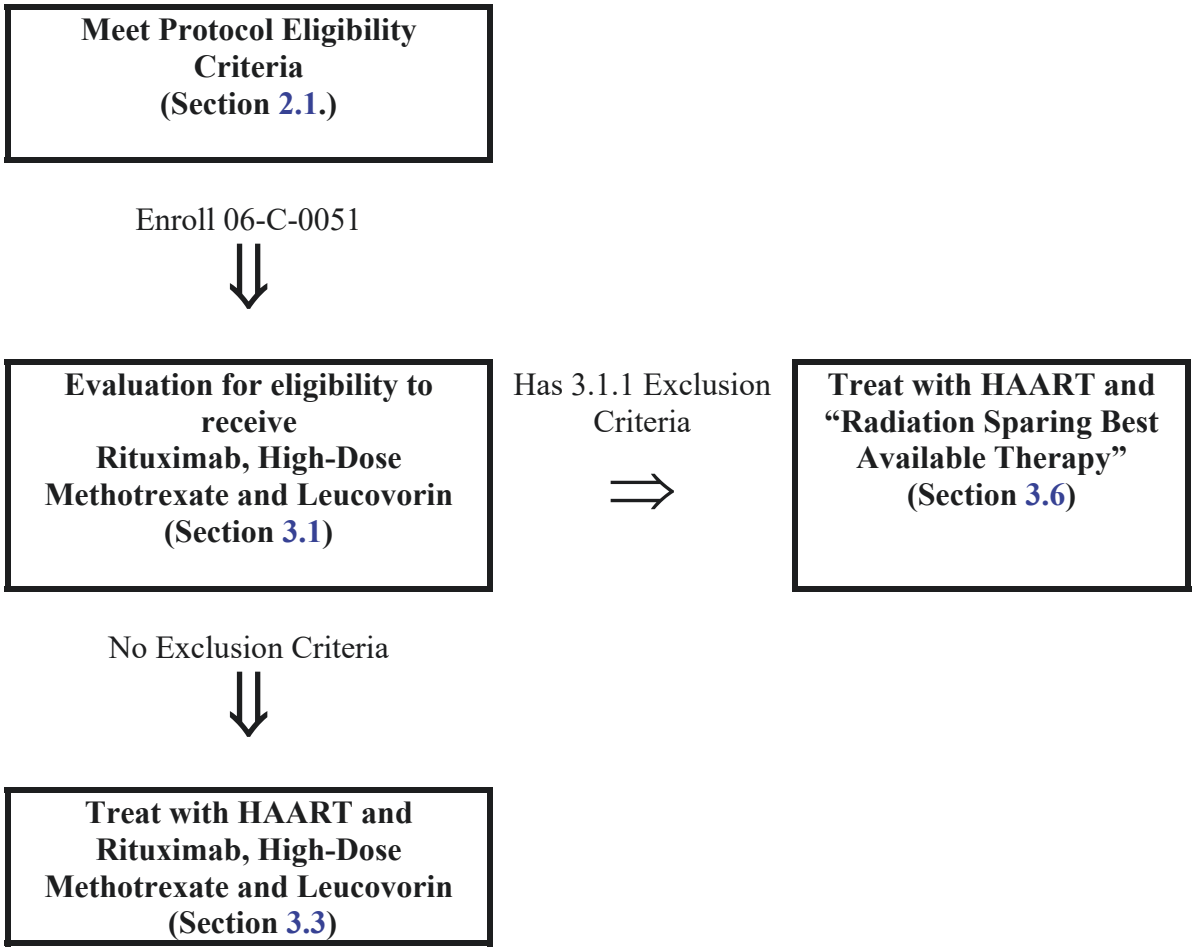
3.1.1.4.1 Aggressive symptomatic Kaposi's sarcoma requiring systemic therapy

3.1.1.4.2 Uncontrolled systemic and/or CNS-invasive fungal infection

3.1.1.5 Hypersensitivity to murine proteins or any component of rituximab

Patients who are ineligible to receive rituximab, high-dose methotrexate and leucovorin will be treated with "Radiation-sparing Best Available Care", as outlined in Section 3.6.

Figure 1: Study Schema: Eligibility Evaluation and Initial Therapy



3.2 PHASE II STUDY DESIGN FOR HIGH-DOSE METHOTREXATE WITH LEUCOVORIN RESCUE AND RITUXIMAB

Induction treatment cycles with rituximab, high-dose methotrexate and leucovorin will be administered every 2 weeks for 6 cycles. Two additional consolidation cycles of high-dose methotrexate without rituximab will be administered at 4 weeks and 8 weeks following completion of the combined therapy.

MRI will be repeated at the end of cycles 2, 4, 6, and 8, and additionally as clinically indicated. At the time of first evidence for complete tumor response by MRI criteria, FDG-PET scan and EBV DNA assessments of the blood and CSF will be repeated. If there is not a complete response by end of cycle 6, patients will be categorized as treatment failures and will not receive the two additional high-dose methotrexate doses. At end of treatment, restaging by MRI will be performed once every 2 months x 3; then once every 3 months x 6; then once every 6 months x 2; then as clinically indicated or annually up to year 5. After this period, clinical evaluations may continue and restaging

performed if clinical suspicion of CNS pathology. Additionally, FDG-PET scan will be repeated as outlined in section 3.8.4.2. (See also Appendix 3).

3.3 DRUG ADMINISTRATION: HIGH-DOSE METHOTREXATE WITH LEUCOVORIN RESCUE AND RITUXIMAB, AND HAART

3.3.1 Review all concomitant medications prior to day 1 of each cycle. Discontinue any drug that may potentially interact with methotrexate elimination.

3.3.2 Rituximab

3.3.2.1 Rituximab 375 mg/m^2 IV day 1 of each cycle prior to administration of high-dose methotrexate.

3.3.2.2 Administer the first dose at an initial rate of 50 mg/hr; if hypersensitivity reactions and/or infusion-related events do not occur, the infusion rate may be increased in increments of 50 mg/hour every 30 minutes to a maximum infusion rate of 400 mg/hour. Subsequent rituximab infusions may be administered at an initial infusion rate of 100 mg/hour; the rate of infusion may be increased every 30 minutes in increments of 100 mg/hour as tolerated to a maximum infusion rate of 400 mg/hour. Rituximab must be diluted prior to injection (See Section 9.1). No other drugs should be added or administered in the same IV line as rituximab.

3.3.2.3 Administer acetaminophen 650 mg PO 30-60 minutes prior to starting rituximab administration (May hold if a patient is unable to swallow oral medications).

3.3.2.4 Administer diphenhydramine 25 – 50 mg IV 10- 30 minutes prior to starting rituximab administration.

3.3.2.5 Consider holding antihypertensive medication during the 12-hour period prior to dosing rituximab, and on the day of rituximab administration.

3.3.3 Intravenous fluid and urine alkalization

3.3.3.1 On Day 1, 12 – 18 hours before the anticipated start of methotrexate, start IV hydration containing sodium bicarbonate ($NaHCO_3$) to alkalinize a patient's urine to pH ≥7.0, but ≤ 8.0 at a rate that achieves a urine output ≥100 mL/hr (recommended start by 6PM local time on day 1). See Table below for options recommended to initially produce an alkaline urine.

3.3.3.1.1 Order q 6-hour urine pH measurements (e.g., 0000, 0600, 1200, 1800) and strict urine output monitoring to start with initiation of IV hydration. Record urine output hourly until urine output is > 100 cc/hour, then at least every four hours.

3.3.3.1.2 Nursing staff should notify a medically responsible individual (investigator or staff on-call) for urine output <100 mL/hour or urine pH <7 or >8.

3.3.3.2 Urine alkalinization to pH ≥7.0 to ≤8.0 and urine output ≥100 mL/hour often initially requires more $NaHCO_3$ and more rapid hydration rates than will be required to maintain either or both parameters after they are achieved.

3.3.3.2.1 The amount of sodium bicarbonate added to intravenously administered fluids should produce a solution with sodium content not greater than the concentration of sodium in 0.9% Sodium Chloride Injection (≤154 mEq/L).

3.3.3.2.2 Recommendations for initially achieving urine alkalinization to pH ≥7.0 to ≤8.0 and urine output ≥100 mL/hour:

Fluid	Administration Rate	Sodium Bicarbonate Content (per 1,000 mL)
0.45%NS	150 – 250 mL/h	50 – 75 mEq
0.2%NS		100 – 125 mEq
D5W/0.45%NS		50 – 75 mEq
D5W/0.2%NS		100 – 125 mEq
D5W		150 mEq

D5W: 5% Dextrose Injection, USP

NS: Sodium Chloride Injection, USP

3.3.3.3 In cases where the investigators feel that a patient can reliably maintain adequate hydration orally, intravenous hydration may be discontinued after the serum methotrexate level is <0.5 mcmol/L.

3.3.3.3.1 Continue to monitor urine output and pH

3.3.3.3.2 Hydration should be resumed if needed to maintain urine pH ≥7.0 to ≤8.0 and urine output >100 mL/hour

3.3.4 High-Dose Methotrexate with sodium bicarbonate followed by leucovorin rescue

3.3.4.1 Prior to HIGH-DOSE METHOTREXATE infusion, administer anti-emetic medication. Recommended: ondansetron 24 mg orally 1 hour prior to methotrexate, followed by ondansetron 8 mg orally every 8 – 12 hours for 3 days.

3.3.4.2 On day 2, after urine pH ≥7.0 and urine output >100 mL/hour are confirmed, administer methotrexate 6000 mg/m^2 IV over 4 hours (goal start time for methotrexate administration is 9AM – 10AM):

3.3.4.2.1 Administer methotrexate 6000 mg/m^2 x 1 bottle with $NaHCO_3$ 25-75 mEq diluted in 5% Dextrose/0.45% Sodium Chloride Injection (qs.) 1000 mL at 250 mL/hour (i.e., over 4 hours).

3.3.4.2.2 Order daily serum methotrexate levels timed to start 24 hours after methotrexate administration begins and continue daily measurements until the serum methotrexate level is ≤0.05 mcmol/L.

3.3.4.2.3 Continue IV or oral hydration with urine alkalinization (pH ≥7.0 to ≤8.0) until serum methotrexate level is ≤0.05 mcmol/L (see sections 3.3.3.3 and 3.3.4.2)

3.3.4.3 **Leucovorin rescue**, starting 24 hours after methotrexate administration began (generally on day 3):

3.3.4.3.1 Administer leucovorin calcium 100 mg/m2 per dose IV (preferred) or levoleucovorin calcium 50 mg/m2 per dose IV every 6 hours until the serum methotrexate level is <0.5 mcmol/L.

3.3.4.3.2 When the methotrexate level is <0.5 mcmol/L, parenterally administered leucovorin or levoleucovorin may be replaced with leucovorin administered

orally (10 mg/m2, or a fixed 25-mg dose for patients with BSA <2.5 m2), until the methotrexate level is ≤0.05 mcmol/L.

3.3.4.3.3 For patients with delayed methotrexate clearance, see sections 3.4.3 and 3.4.4.

3.3.5 Filgrastim

3.3.5.1 Routine use of filgrastim is not required for this therapy

3.3.5.2 Do not administer filgrastim within 24 hours before administering methotrexate

3.3.5.2.1 If filgrastim has already been administered, delay methotrexate until >24 hours have passed since filgrastim administration

3.3.5.2.2 Do not administer filgrastim after methotrexate administration until methotrexate levels have decreased to <0.05 mcmol/L.

3.3.5.3 Pegfilgrastim may not be used.

3.3.5.4 Indications for filgrastim use for patients on this study:

3.3.5.4.1 HIV-related neutropenia

- Includes neutropenia related to antiretroviral therapy, or other HIV-disease-related therapy

3.3.5.4.2 Methotrexate-related neutropenia

3.3.5.4.3 Rituximab-related neutropenia

- Includes delayed-onset neutropenia

3.3.5.5 Filgrastim doses will be individually titrated according to the clinical scenario.

3.3.5.6 Filgrastim 300-480 mcg/day subcutaneously may be used as needed to maintain an absolute neutrophil count ≥1000 cells/mm3. Filgrastim will not be administered more frequently than once daily. Intravenous administration may be utilized.

3.3.6 Highly Active Antiretroviral Therapy (HAART)

3.3.6.1 Whenever possible, patients should be taking HAART as soon as is feasible

3.3.6.2 HAART interruptions for toxicity and tolerance are allowed

3.3.6.3 Refusal to adhere to HAART is not permitted, as it is considered part of the oncology therapy for this protocol

3.3.6.4 A regimen of highly active anti-retroviral therapy will be initiated as soon as feasible in all patients. Treatment will in general be based on Department of Health and Human Services Antiretroviral Guidelines for Adults and Adolescents found at http://www.aidsinfo.nih.gov/guidelines/ . Particular events may require treatment suspension, modification and will be considered individually for best medical care. Therapy may be managed by NCI and or NIAID co-investigators on this protocol or on the NIAID sister protocol.

3.3.6.5 Order HLA B*5701 Pharmacogenetic test at baseline to evaluate for possible use of abacavir. Recommend a tenofovir-free regimen to avoid potential nephrotoxicity and potential deleterious effect on methotrexate renal excretion.

3.4 TREATMENT MODIFICATIONS: HIGH-DOSE METHOTREXATE WITH LEUCOVORIN RESCUE AND RITUXIMAB

3.4.1 Rituximab

3.4.1.1 For infusion related events or hypersensitivity reactions, the infusion rate should be slowed (e.g. for moderate flu-like symptoms of fever, chills, rigors; other infusion related reactions such as nausea, urticaria, pruritus, rash, fatigue, headache) or infusion of the drug should be interrupted or discontinued as clinically indicated (e.g. for reactions such as angioedema, hypotension, bronchospasm); following appropriate treatment and/or resolution of the reaction(s), administration of rituximab may be reinitiated at a slower infusion rate (one-half the previous infusion rate), and the rate of infusion may be incrementally increased as tolerated.

3.4.2 High-dose methotrexate

3.4.2.1 The creatinine clearance (CrCl) will be calculated by method of Cockcroft-Gault prior to each cycle (see Appendix 4). If CrCl is ≥100 mL/min, then no dose adjustment is necessary. If CrCl is <100 mL/min, then the methotrexate dose is adjusted by the fraction of CrCl less than 100 mL/min (e.g., methotrexate dose = 6000 mg/m^2 x CrCl/100).

3.4.2.1.1 For estimated creatinine clearance <100 mL/min, a 24-hour urine collection should be obtained *plus* a serum creatinine at the end of the collection period to determine a measured creatinine clearance.

3.4.2.2 Delayed methotrexate serum clearance (>3 days after methotrexate infusion) may occur and mandates accelerated rescue and continued hydration and alkalinization. The minimum interval between achieving a serum methotrexate level ≤0.05 mcmol/L and the start of a subsequent treatment cycle is 7 days.

3.4.2.3 For creatinine clearance <60 mL/min, no methotrexate will be administered. Administer rituximab without methotrexate after discussion with a medically responsible investigator if renal function cannot be expected to be optimized (CrCl ≥60 mL/min) within 3 days.

3.4.2.4 For transient ALT and/or AST elevations ≥10X the upper limit of normal, withhold methotrexate until both ALT and AST are ≤5 X the upper limit of normal, then permanently reduce methotrexate dosage by 25% for all subsequent cycles beginning with the next cycle.

3.4.2.5 For ANC <1000 cells/mm^3, delay methotrexate until ANC recovers to ≥1000/mm^3.

3.4.2.6 For platelet counts <50,000/mm^3, delay methotrexate until platelet count recovers to ≥50,000/mm^3.

3.4.2.7 For any other Grade 3 or greater toxicities related to methotrexate, with the exception of hematologic toxicity, hold methotrexate until toxicity abates to Grade ≤2.

3.4.2.8 For pulmonary toxicity related to methotrexate, permanently discontinue methotrexate.

3.4.2.9 Note, if toxicity attributed to methotrexate prevents subsequent methotrexate treatment prior to the time of complete response, patient will be considered a treatment failure. Failure of toxicity to resolve and thus creating dose delays in excess of 15 days will result in methotrexate discontinuation.

3.4.3 Leucovorin

3.4.3.1 In the case of delayed early methotrexate elimination (see Table 1 below) and/or evidence of acute renal injury, administer leucovorin calcium according to Table 1 until the serum methotrexate level is ≤0.05 mcmol/L.

3.4.3.2 If leucovorin is not available (e.g., due to a drug shortage), replace leucovorin with levoleucovorin calcium every 6 hours until serum methotrexate level is ≤0.5 mcmol/L, see Table 1 for dosing.

3.4.3.3 Alternatively, glucarpidase (formerly "carboxypeptidase G_2") may be used (see section 3.4.4 below)

3.4.3.3.1 Evidence of acute renal injury includes a 100% or greater increase in serum creatinine level at 24 hours after methotrexate administration (e.g., an increase from 0.5 mg/dL to a level ≥1 mg/dL).

Table 1: Leucovorin and Levoleucovorin dosing for delayed methotrexate clearance

Methotrexate Level	Leucovorin Dose	Levoleucovorin Dose
24 hours: >10 mcmol/L	100 mg/m^2 IV q 6 hours	50 mg/m^2 IV q 6 hours
48 hours: >1 mcmol/L	100 mg/m^2 IV q 3 hours	50 mg/m^2 IV q 3 hours
60 hours: >2 mcmol/L	200 mg/m^2 IV q 3 hours	100 mg/m^2 IV q 3 hours
72 hours: >0.1 mcmol/L	100 mg/m^2 IV q 6 hours	50 mg/m^2 IV q 6 hours

When the methotrexate level is ≤0.5 mcmol/L, leucovorin/levoleucovorin may be administered as oral leucovorin calcium until serum methotrexate level is ≤0.05 mcmol/L (see Section 3.3.4.3.2).

3.4.3.4 In addition to appropriate leucovorin/levoleucovorin therapy, these patients require continuing hydration and urinary alkalinization, and close monitoring of fluid and electrolyte status, until the serum methotrexate level has fallen to <0.05 mcmol/L and renal failure has resolved.

3.4.3.5 Serum methotrexate monitoring levels are now performed at Children's Hospital 7 days per week at 2PM. Specimens collected for methotrexate monitoring must be delivered to the NIH CC Department of Laboratory Medicine's Chemistry Service during morning hours so they can be delivered to Washington's Children's Hospital and processed in a timely manner. This process is designed to obtain same day results for patients on 06-C-0051 so the clinical team can make protocol-defined treatment decisions, such as stopping leucovorin or IV fluids, or implementing treatment for delayed methotrexate clearance with glucarpidase (Voraxaze®)

3.4.3.5.1 Methotrexate Levels need to be **drawn at scheduled times**

3.4.4 Glucarpidase Rescue of Methotrexate toxicity and Acute Renal Failure

3.4.4.1 Glucarpidase may be utilized in addition to leucovorin or levoleucovorin for methotrexate toxicity in the setting of renal toxicity and delayed methotrexate clearance.

3.4.4.2 Glucarpidase **may ONLY be administered** with the approval of a medically responsible study investigator.

3.4.4.3 Clinical prerequisites:

3.4.4.3.1 Serum methotrexate concentration of >10 mcmol/L at 24-48 hours after methotrexate infusion,

3.4.4.3.2 Urine output decreases (less than 50% excretion of the input hydration) or a serum creatinine >1.5-times the upper limit of normal, and

3.4.4.4 Documented increase of serum creatinine during the infusion period.

3.4.4.5 Glucarpidase administration:

3.4.4.5.1 Glucarpidase 50 Units/kg of body weight is administered as a single IV injection over 5 minutes.

3.4.4.5.2 **Do not administer leucovorin or levoleucovorin within 2 hours either before or after a dose of glucarpidase** because reduced folates such as leucovorin and levoleucovorin are substrates for degradation by glucarpidase.

3.4.4.5.3 For the first 48 hours after glucarpidase, administer leucovorin or levoleucovorin at the same dose and schedule as were given prior to administering glucarpidase. At times >48 hours after glucarpidase, administer leucovorin or levoleucovorin based on measured methotrexate concentrations.

3.4.4.5.4 Do not discontinue leucovorin or levoleucovorin based on the determination of a single methotrexate concentration <0.05 mcmol/L. Leucovorin or levoleucovorin should be continued until the methotrexate concentration has been maintained <0.05 mcmol/L for a minimum of 3 days.

3.4.4.5.5 Continue hydration and alkalinization of urine as indicated per protocol, or as directed by a medically responsible study investigator.

3.5 Criteria for Discontinuing Rituximab and High-Dose Methotrexate with Leucovorin Rescue

3.5.1 Completion of therapy

3.5.2 Delayed methotrexate clearance due to impaired renal function, requiring glucarpidase

3.5.3 Creatinine clearance <60 mL/min based on a 24-hour urine collection that cannot be corrected within 2 weeks

3.5.4 Any creatinine clearance <30 mL/min based on 24-hour urine collection.

3.5.5 Inability to tolerate required intravenous hydration (minimum 60 hours of intravenous fluid at a rate ≥100 mL/hour)

3.5.6 Dose delays in excess of 15 days

3.5.7 Withdrawal of consent.

3.5.8 Inability to administer to HAART or other oncologic therapies.

3.5.9 Progressive neurologic symptoms attributed to progressive AR-PCNSL

3.5.10 Untreatable concurrent illness

3.5.11 Methotrexate with leucovorin rescue may be discontinued, if in the opinion of the PI or LAI, the risk of continued therapy outweighs the potential benefit of continued therapy, or if patient non-adherence interferes with study objectives.

3.5.12 Patients who discontinue Rituximab and methotrexate with leucovorin rescue may continue with "Radiation-sparing Best Available Therapy", including dose-dense Rituximab, if clinically indicated.

3.6 "Radiation-sparing Best Available Therapy" for patients not eligible for High-Dose Methotrexate in the Phase II cohort

Patients with EBV+ AR-PCNSL that have co-morbidities prohibiting administration of high dose methotrexate will receive HAART combined with dose-dense rituximab[111] unless there is a clinical reason that this should not be used or would not be considered best therapy, such as hypersensitivity to murine proteins.

Dose dense administration of rituximab increases serum concentration[112], which would be expected to allow for better distribution to the CNS. Patients will receive one 4-week cycle, consisting of a total of 6 doses of rituximab administered on Days 1, 3, 5, 8, 15, and 21.

Patients who are hypersensitive to murine proteins or any component of rituximab, or other clinical contraindications to using the dose-dense rituximab regimen, will receive HAART (See Section 3.6.2). If clinically indicated, additional chemotherapy or radiotherapy may be employed in such patients (See Section 3.6.3).

3.6.1 Rituximab

3.6.1.1 Rituximab 375 mg/m^2 IV days 1, 3, 5, 8, 15, and 21

3.6.1.2 Administer the first dose at an initial rate of 50 mg/hr; if hypersensitivity reactions and/or infusion-related events do not occur, the infusion rate may be increased in increments of 50 mg/hour every 30 minutes to a maximum infusion rate of 400 mg/hour. Subsequently, rituximab infusions may be administered at an initial infusion rate of 100 mg/hour; the rate of infusion may be increased every 30 minutes in increments of 100 mg/hour as tolerated to a maximum infusion rate of 400 mg/hour. Rituximab must be diluted prior to injection (See Section 9.1). No other drugs should be added or administered in the same IV line as rituximab.

3.6.1.3 Administer acetaminophen 650 mg PO 30-60 minutes prior to starting rituximab administration (May be held if a patient is unable to swallow oral medications).

3.6.1.4 Administer diphenhydramine 25 – 50 mg IV 10-30 minutes prior to starting rituximab administration.

3.6.1.5 Consider holding antihypertensive medication during the 12-hour period prior to dosing rituximab, and on the day of administration, especially if systolic blood pressure is <105 mm Hg. If antihypertensive medications are held, patients should continue to have blood pressure monitored at least every 8 hours. Antihypertensive medication should generally be continued or resumed if systolic blood pressure is >160 mm Hg or diastolic blood pressure is >100 mm Hg

3.6.2 Highly Active Antiretroviral Therapy (HAART)

3.6.2.1 Whenever possible, patients should be taking HAART as soon as is feasible

3.6.2.2 HAART interruptions for toxicity and tolerance are allowed

3.6.2.3 Refusal to adhere to HAART is not permitted, as it is considered part of the oncology therapy for this protocol

3.6.2.4 A regimen of highly active anti-retroviral therapy will be initiated as soon as feasible in all patients. Treatment will in general be based on Department of Health and Human Services Antiretroviral Guidelines for Adults and Adolescents found at http://www.aidsinfo.nih.gov/guidelines/. Particular events may require treatment suspension, modification and will be considered individually for best medical care. Therapy may be managed by NCI and or

NIAID co-investigators on this protocol or on NIAID protocols. Recommend tenofovir-free regimen to avoid potential nephrotoxicity and potential effect on methotrexate renal excretion.

3.6.3 Treatment modification: "Radiation-sparing Best Available Therapy"

3.6.3.1 Chemotherapy

Alternative or additional chemotherapy may be employed in patients who:

- Cannot receive the methotrexate or the dose-intense rituximab regimens
- Have stable disease or partial response as a best response to therapy
- Have disease recurrence after either regimen

Choice of additional chemotherapeutic agents will be individualized, and agents and regimens used in treating PCNSL in immunocompetent hosts. Patients treated with second-line chemotherapy can be followed on study as long as they remain on HAART and are evaluable for long-term neurocognitive outcomes.

3.6.3.2 Whole Brain Radiation Therapy

Following the Guidelines in Section **3.10** patients may also, or as an alternative, receive whole brain radiation therapy if indicated. As noted in Section **3.12.2**, patients treated with radiotherapy can be followed on study as long as they remain on HAART and are evaluable for long-term neurocognitive outcomes.

3.6.4 Supportive Care

The following supportive care measures may be individualized to patients' symptoms and laboratory abnormalities:

3.6.4.1 Filgrastim

3.6.4.1.1 Routine use of filgrastim are not routinely required for this therapy

3.6.4.1.2 Indications for use on this study:

- HIV-related neutropenia
  - Includes neutropenia related to antiretroviral therapy, or other HIV-disease-related therapy
- Rituximab related neutropenia
  - Includes delayed onset neutropenia

3.6.4.1.3 Filgrastim dose will be individually titrated according to the clinical scenario.

3.6.4.1.4 Filgrastim 300-480 mcg/day subcutaneously may be used as needed to maintain the absolute neutrophil count ≥1000 cells/mm3. Filgrastim will not be administered more than once daily. Intravenous administration may be utilized.

3.6.4.2 Steroids, systemically administered

3.6.4.2.1 Doses of steroids up to dexamethasone 4 mg every 6 hours or equivalent may be used for patients with neurologic symptoms attributed to lymphoma

3.6.4.2.2 Duration of steroids will be generally not be greater than 5 days, in same cases tapering of steroids will be required

3.6.4.2.3 Courses of steroid treatment may be repeated if needed

3.7 SECOND-LINE THERAPY

Second-line chemotherapy AND/OR radiotherapy assessment and treatment may be included as part of the protocol when necessary for best patient care; in such a case, patient can remain on-study if they are evaluable for disease specific and overall survival endpoints. Importantly, such patients will be assessed for long-term neurocognitive outcomes using protocol defined neuropsychometric testing. This will be to assess in an observational manner late term neurotoxicity should the patient survive long enough to manifest this complication. Patients receiving radiation therapy will be evaluated separately in regards to neuropsychometric testing. As noted in section 3.6 and 5.3.6, patients requiring radiotherapy will be considered treatment failures for statistical purposes of the protocol. If patient is unable or unwilling to participate in neuropsychometric testing, patient will remain on study until completion of second-line therapy at NCI or until referral to another facility, whichever is first.

3.8 ON STUDY EVALUATION

3.8.1 Research Specimens

3.8.1.1 Baseline

3.8.1.1.1 Cerebrospinal fluid (not necessary to repeat if previously acquired within 21 days before starting therapy)

- Ig PCR and EBV PCR (Molecular Pathology, Dr. Mark Raffeld, 2N116-A)
- Cytopathology
- Flow Cytometry (Room B1B58)
- Quantitative EBV PCR (CC Microbiology)

3.8.1.1.2 Blood (not necessary to repeat if previously acquired within 14 days before starting therapy)

- Quantitative EBV PCR (CC Microbiology)
- HIV viral load
- T-cell subsets (Randy Stevens, Leidos Biomedical Research, Inc., Frederick)
- Storage: 1 red top for serum; 1 yellow top for plasma; 1 green top for cells

3.8.1.2 Sequence based HLA B for HLA B*5701 Pharmacogenetic testing. This may be excluded if HLA type has been previously documented.

3.8.1.3 Every odd-numbered cycle

3.8.1.3.1 Blood

- Quantitative EBV PCR (CC Microbiology)
- HIV viral load

- T-cell subsets (Randy Stevens, Leidos Biomedical Research, Inc., Frederick)
- Storage: 1 red top for serum; 1 yellow top for plasma; 1 green top for cells

3.8.1.4 End of therapy (see Appendix 3)

3.8.1.4.1 Blood: days 29 (+/- 7 days) then monthly for 2 months, then every 2 months x3, then every 3 months x 6, then every 6 months x 2, then every 12 months x 2 (for patients co-enrolled in 01-C-0038 and followed clinically beyond year 3). Also obtain at time of lymphoma recurrence in concert with CSF studies outlined in section 3.8.5.4.2

- Quantitative EBV PCR (CC Microbiology)
- HIV viral load
- T-cell subsets (Randy Stevens, Leidos Biomedical Research, Inc., Frederick)
- Storage: 1 red top for serum; 1 yellow top for plasma; 1 green top for cells

3.8.2 Clinical and laboratory assessments

3.8.2.1 Baseline assessment prior to treatment (within 72 hours before therapy)

3.8.2.1.1 Physical examination with particular emphasis on neurological examination)

3.8.2.1.2 Folstein Mini Mental Status Exam (MMSE). See Appendix 1.

3.8.2.1.3 Assignment of Neurologic Function Class (see Appendix 2)

3.8.2.1.4 CBC with differential (CBC with differential drawn up to 24 hours prior to starting therapy acceptable for patients receiving filgrastim to manage neutropenia)

3.8.2.1.5 Electrolytes

3.8.2.1.5.1 Serum creatinine

- calculated and/or measured creatinine clearance
- Whenever the calculated creatinine clearance is <100 mL/min, a measured creatinine clearance should be obtained.

3.8.2.1.5.2 Hepatic panel

3.8.2.1.5.3 Mineral panel

3.8.2.1.6 Ophthalmologic examination to assess for ocular involvement by lymphoma

3.8.2.1.7 Viral hepatitis studies (not necessary to repeat previously documented positive studies; results not required prior to treatment):

- Hepatitis A
- anti-HAV IgG
- Hepatitis B
- anti-HBsAg
- HBsAg
- Anti-HB core
- Hepatitis C
- anti-HCV

3.8.2.1.8 Serum anti-toxoplasmosis antibody titers (not necessary to repeat if already obtained on NIAID protocol)
3.8.2.1.9 Syphilis serology (not necessary to repeat if already available within 6 months prior to study entry)
3.8.2.1.10 HIV viral load if not available within 14 days prior to enrollment
3.8.2.1.11 T-cell subsets if not available within 14 days prior to enrollment
3.8.2.1.12 HIV genotype or Virtual Phenotype® to assess for resistant HIV if not previously done

3.8.3 Day 1 each cycle; then monthly for 2 months, then every 2 months x3, then every 3 months x6, then every 6 months x 2, then every 12 months x 2 (for patients co-enrolled in 01-C-0038 and followed clinically beyond year 3).

For "Radiation-Sparing Best Available Care" Cohort, scheduled evaluations on Days 1, 8, 15, 21, 28 then monthly for 2 months, then every 2 months for 3 times, then every 3 months of total of 36 months, then yearly for 2 years (for patients followed clinically beyond year 3).

3.8.3.1 Physical examination with documentation of abnormalities

3.8.3.2 Particular emphasis on neurological examination

3.8.3.3 Assign neurologic function class (see Appendix 2)

3.8.3.4 CBC with differential (CBC with differential drawn up to 24 hours prior to starting therapy acceptable for patients receiving filgrastim to manage neutropenia)

3.8.3.5 Electrolytes

3.8.3.5.1 Serum creatinine

- calculated and/or measured creatinine clearance
- Whenever the calculated creatinine clearance is <100 mL/min, a measured creatinine clearance should be obtained.

3.8.3.5.2 Hepatic panel
3.8.3.5.3 Mineral panel

3.8.3.6 During treatment cycles until serum methotrexate level ≤0.05 mcmol/L:

3.8.3.6.1 Blood specimens for methotrexate levels must be drawn at scheduled times
3.8.3.6.2 Hepatic panel every other day
3.8.3.6.3 CBC with differential daily
3.8.3.6.4 Electrolytes, creatinine, BUN daily

3.8.4 Baseline Staging

3.8.4.1 MRI: if done within 2 weeks before study entry, repeat only if interval clinical deterioration has occurred

3.8.4.2 FDG-PET: not necessary to repeat if previously done within 3 weeks of study entry

3.8.5 Restaging

3.8.5.1 MRI

3.8.5.1.1 End of cycles 2, 4, 6 and 8, then every 2 months x3, then every 3 months x 6, then every 6 months x 2, then as clinically indicated, or annually up to year 5

(for patients followed clinically beyond year 3). For "Radiation-Sparing Best Available Care" Cohort, Scheduled evaluations on Week 4, Week 8, Week 12, then every 2 months x3, then every 3 months x 5, then every 6 months x 2, then as clinically indicated, or annually up to year 5 (for patients followed clinically beyond year 3).

3.8.5.2 Ophthalmologic

3.8.5.2.1 If ocular involvement, restage same schedule as with MRI

3.8.5.2.2 If no ocular involvement, examine for lymphoma if clinically indicated

3.8.5.3 FDG-PET

3.8.5.3.1 End of cycle 1

3.8.5.3.2 At the time of complete response (or CRu) by MRI criteria

- Due to scheduling, may be done at following cycle

3.8.5.3.3 End of cycle 6 if best response less than CRu, otherwise, end of cycle 8

3.8.5.3.4 At month 12 at time of MRI

3.8.5.3.5 At the time of suspected disease recurrence/progression by MRI criteria following complete response and previous negative FDG-PET

3.8.5.4 Cerebrospinal Fluid

3.8.5.4.1 At the time of complete response (or CRu) by MRI criteria

- Due to scheduling, may be done at following cycle

3.8.5.4.2 At the time of suspected disease recurrence/progression by MRI criteria

- Ig PCR and EBV PCR (Molecular Pathology, Dr. Mark Raffeld, 2N116-A)
- Flow cytometry (Maryalice Stetler-Stevenson, Room B1 B58)
- Cytopathology
- Quantitative EBV PCR (CC Microbiology)
- Cell count with differential, glucose, protein

3.8.5.4.3 At end of treatment and at 6 months post treatment:

- Ig PCR and EBV PCR (Mark Raffeld)
- Quantitative EBV PCR (CC Microbiology)
- Cytopathology
- Flow cytometry (Maryalice Stetler-Stevenson, Room B1 B58)
- Cell count with differential, glucose, protein

3.8.6 Neuropsychometric testing

3.8.6.1 Folstein Mini-Mental Status Examination will be done for comparative purposes prior to therapy, after cycle 1, and again at completion of treatment course.

3.8.6.2 Baseline formal neuropsychologic assessment will be administered 1-2 months after completion of chemotherapy treatment course.

3.8.6.3 Follow-up assessments will be administered at 6, 12, 18, 24, and 36 months (± 3 months) after completion of therapy. Comprehensive assessment will be performed at the post-therapy baseline and annually thereafter whereas monitoring assessments will be administered at the half-year evaluations (see Appendix 5 for the comprehensive and monitoring neuropsychologic test

batteries).

3.8.7 Adherence Assessment

3.8.7.1 At 1-2 months after completion of therapy, patients will complete measures assessing barriers to adherence, knowledge of HIV, and perceived social support (see Appendix 6 and Appendix 7).

3.8.7.2 A follow-up knowledge and social support assessment will be administered at 6 months. Follow-up adherence assessments (via self-report) will be conducted every 3 months, beginning 3 months post-treatment through month 24. Results from these data will guide interventions aimed at identifying patterns of non-adherence and specific barriers identified by the patient as being problematic.

3.8.8 Optional Blood Draws

Up to 50 cc of blood may optionally be drawn for immunological testing, establishment of cell lines, evaluation of hematologic parameters, or other studies that become clinically important during conduct of the trial. However, this should not be drawn if it brings the total amount of blood drawn to more than 450 mL during a 6-week period. Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document or are of minimal risk. If new and significant risks are associated with the research (e.g. analysis of germ line genetic mutations) the principal investigator must obtain IRB approval. Handling of optional biospecimens is outlined in Section 7.5.

3.9 CONCURRENT THERAPIES

3.9.1 Anti-retroviral therapy

See section 3.6.2

3.9.2 Contraception

All females of childbearing potential will be given contraception or provided with barrier contraceptive devices.

3.9.3 Pneumocystis jiroveci prophylaxis (PJP/PCP)

All patients will receive PCP prophylaxis throughout the treatment period and until the CD4+ cells are sustained over 200 cells/mm^3 for a 3-month or longer period of time.

If cotrimoxazole (trimethoprim+sulfamethoxazole; Bactrim®) is used, it should be withheld during methotrexate treatment until completion of leucovorin rescue. Alternatives include:

- Dapsone 50 – 100 mg PO daily or 100 mg PO 2x/week
- Aerosolized pentamidine 300 mg by inhalation, monthly
- Atovaquone 750 mg PO BID

3.9.4 MAC prophylaxis

Essentially all patients on study will require prophylaxis since AR-PCNSL occurs in those with CD4+ cells <50/mm^3.
Azithromycin 1200 mg orally once weekly is recommended, but other agents are

acceptable.

3.9.5 Antibacterial Prophylaxis

For patients with anticipated severe neutropenia (ANC <500 cells/mm^3) related to cyclic chemotherapy, consider levofloxacin 500 mg PO daily for up to seven consecutive days, timed to coincide with periods of severe neutropenia that cannot be managed with filgrastim.

3.9.6 Hepatitis B antigenemia

For patients with serologic evidence of chronic persistent/active HBV infection, lamivudine 150 mg PO daily, tenofovir 300 mg PO daily, or other agents with demonstrated anti-HBV activity should be given continually throughout treatment cycles until a medically-responsible investigator advises discontinuation. Since chemotherapy in general and rituximab in particular are associated with HBV reactivation, this should also be considered in patients with anti-HB core-alone positivity.

3.9.7 Contraindicated therapies

Trimethoprim/sulfamethoxazole should not be given within 48 hours prior to methotrexate administration and until methotrexate concentration is <0.05 mcmol/L.

Methotrexate should not be given concurrently with any of the following medications without the approval of a medically responsible study investigator:

1. Asparaginase
2. Aspirin
3. Acidic drugs
4. Amoxicillin
5. Cephalosporins
6. Chloramphenicol
7. Cholestyramine
8. Cotrimoxazole
9. Cyclosporine
10. Diclofenac
11. Ibuprofen
12. Indomethacin
13. Ketoprofen
14. Mercaptopurine
15. Mezlocillin
16. Naproxen
17. Non-steroidal anti-inflammatory drugs
18. Omeprazole
19. Penicillins
20. Piperacillin (±tazobactam)
21. Probenecid
22. Proton pump inhibitors (e.g., dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole)
23. Pyrimethamine
24. Salicylates
25. Sulfonamides
26. Sulindac
27. Tetracyclines
28. Tolmetin
29. Trimethoprim
30. Trimethoprim/sulfamethoxazole
31. Vaccines, Live (viral and bacterial)

Generally, these agents should be withheld 48 hours prior to the initiation of methotrexate and until methotrexate concentration is <0.05 mcmol/L.

3.10 RADIATION THERAPY GUIDELINES

3.10.1 Radiation therapy to the brain will be deferred in all patients unless there is progression of disease with high-dose methotrexate- rituximab, or radiation-sparing best therapy, stable disease or partial response as best response to high-dose methotrexate- rituximab or radiation-sparing best therapy, or recurrence following apparent complete response with high-dose methotrexate- rituximab or

radiation-sparing best therapy, and further chemotherapy approaches by themselves are not assessed by the treatment team to be clinically appropriate. Patients requiring radiotherapy (or any other additional therapy for AR-PCNSL) will be considered treatment failures for this study. Patients requiring such therapy will be returned to the care of the referring physicians when possible. If possible, continuation of neuropsychometric testing outlined in the study will continue, to describe the cognitive outcome.

3.10.2 Radiation following high-dose methotrexate may confer additional risk of neurotoxicity. For this reason, when feasible, salvage chemotherapy strategies will be considered as appropriate.

3.10.3 Progressive disease

3.10.3.1 Alternative chemotherapy or WBRT to achieve best tumor control.

3.10.4 Partial Response

3.10.4.1 Limited volume RT (if possible) to achieve best tumor control and limit CNS exposure and late term radiation-related neurotoxicity.

3.10.5 Early Recurrence

3.10.5.1 Minimal disease will be treated with limited volume RT if possible, to achieve best tumor control and limit CNS exposure and late term radiation-related neurotoxicity. WBRT will be utilized if indicated.

3.10.6 Diffuse recurrence may be treated with WBRT if indicated to achieve best tumor control

3.11 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.11.1 Criteria for removal from protocol therapy

- Completion of therapy
- Dose delays in excess of 15 days
- Withdrawal of consent
- Inability to tolerate HAART or other oncologic therapies

See Section **3.5** for additional criteria for discontinuing rituximab and high-dose methotrexate with leucovorin rescue.

3.11.2 Off Study Criteria

- Refusal to adhere with HAART
- Refusal of oncologic therapy
- Patient or patient advocate (if patient not competent) voluntary withdrawal
- Progression of disease and either refusing or not eligible to receive second-line chemotherapy AND/OR radiotherapy
- Progressive, irreversible neurocognitive deficits, not attributed to AIDS-PCNSL or treatable infection, occurring greater than 90 days after the completion of

radiation therapy that would be scored as grade 3 or higher by the RTOG/EORTC Late Radiation Morbidity Scoring Scheme

- Completion of 5 years follow-up with no recurrent AR-PCNSL

3.11.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

3.12 POST-STUDY EVALUATION

3.12.1 Patients will be followed on study for 3 years on protocol (see Appendix 3). After the end of the three-year period, patients may be followed for clinical assessment on an annual basis for up to a total of 5 years. Specific additional assessments will be determined according to clinical findings at that time, and in concert with referring physicians.

3.12.2 Patients followed in the "Radiation-sparing Best Available Care" cohort will be followed using the same schedule as those receiving protocol defined therapy.

4 SUPPORTIVE CARE

4.1 PNEUMOCYSTIS JIROVECI PNEUMONIA

Patients who develop PCP will receive standard of care including trimethoprim-sulfamethoxazole, steroids when appropriate and alternative standard agents such as IV pentamidine, atovaquone, or trimetrexate in combination with leucovorin when trimethoprim-sulfamethoxazole cannot be used. Trimethoprim-sulfamethozole must be held during administration of high dose –methotrexate and leucovorin rescue, as outlined in Section _Contraindicated_therapies**3.9.7.**

4.2 CYTOMEGALOVIRUS (CMV)

Periodic ophthalmologic evaluations will be conducted on all patients, as this subset of AIDS patients are at particular risk of CMV retinitis. Baseline examination will be conducted as soon as possible at entry, and then approximately every 1-3 months until the CD4+ cells are over 75 cells/mm^3. Ganciclovir ocular implants will in general be the initial treatment choice. Intravenous ganciclovir or oral valganciclovir can be considered, but due to its hematological toxicity, it should not be used without consulting the PI or LAI. Both cidofovir and foscarnet are effective in CMV, but are nephrotoxic, and will not be used during high-dose methotrexate. Urinalysis for proteinuria and creatinine clearance estimates or measurements made if these agents are to be used. All cases of CMV infection must be discussed with the PI or LAI.

4.3 FUNGAL INFECTIONS

4.3.1 Oral Candidiasis – if asymptomatic, recommend clotrimazole troches. If symptomatic, recommend oral fluconazole.

4.3.2 Esophageal Candidiasis – recommend oral fluconazole, but if no response,

consider itraconazole, amphotericin B.

4.3.3 Aspergillosis, scedosporiosis and other invasive fungal infections infection may present as meningitis, pneumonia, or fungemia. Treatment decisions will be made in concert with infectious disease consultants, and may include the use of agents such as voriconazole, liposomal amphotericin B, or other novel agents as the clinical presentation requires.

4.4 MYCOBACTERIAL DISEASES

4.4.1 Mycobacterium tuberculosis – Inactive versus active infection must be distinguished. In patients with low CD4+ counts, 2/3's present with extrapulmonary disease. Diagnosis may require biopsies in addition to culture. There is a high incidence of drug resistant strains. Consultation with the infectious disease service is mandatory for these patients. Combination therapy with isoniazid, rifampin, pyrazinamide, ethambutol and pyridoxine (vitamin B6) is usually initiated. These drugs may cause hepatitis, bone marrow suppression, optic neuritis and peripheral neuropathy. Careful monitoring of toxicities is required if these drugs are given concomitantly with HAART and/or with high-dose methotrexate.

4.4.2 Mycobacterium avium complex usually occurs in patients with CD4+ count $<100/mm^3$. Disseminated infection presents with fever, weight loss, night sweats, diarrhea, anemia, neutropenia, and thus may be difficult to differentiate from "B" symptoms. Sputum and blood cultures should be obtained. Clarithromycin or azithromycin in combination with clofazimine or rifampin are recommended.

4.5 TOXOPLASMOSIS

Patients with concurrent toxoplasmosis will be treated in concert with infectious disease consultation. In patients receiving high-dose methotrexate, sulfadiazine should not be given 48 hours prior to administration of methotrexate administration through completion of leucovorin rescue due its effect on renal excretion of methotrexate, (see Section 3.7) therefore sulfadiazine-sparing regimens are preferred.

4.6 PROPHYLAXIS FOR FEBRILE NEUTROPENIA

See Sections 3.6.4.1 and 3.9.5 for guidelines on use of filgrastim and antibiotics.

4.7 FEBRILE NEUTROPENIA

A life-threatening complication requiring hospitalization and urgent broad-spectrum antibiotics.

4.8 ANEMIA

If symptomatic or if the hemoglobin falls below 8 mg/dL, erythropoietin may be considered. Red blood cell transfusions are sometimes associated with increased HIV replication and opportunistic infections, but if necessary, red blood cell transfusion support should be utilized.

4.9 THROMBOCYTOPENIA

Should be treated conservatively. In the absence of bleeding or a planned invasive

procedure, platelet transfusions should be given for a platelet count below 10,000/mm^3. If invasive procedures (including lumbar puncture) are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count >50,000/mm^3.

4.10 CENTRAL VENOUS ACCESS

If peripheral access not feasible for a patient, temporary lines are preferred (removed after completion of each infusion) and include: internal jugular line; PICC lines via the brachial vein. Other lines include semi-permanent HICKMAN, GROSHONG catheters or medi-port implanted devices. All devices will have nursing supervision to include patient self-care and cleaning/flushing of the devices.

4.11 IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS)

IRIS will be defined using AIDS Clinical Trial Group (ACTG) 2009 Criteria, using the following criteria:

4.11.1 Initiation, reintroduction or change in antiretroviral therapy/regimen or therapy for opportunistic infections (OI).

AND

4.11.2 Evidence of:

4.11.2.1 An increase in CD4+ cell count as defined by ≥50 cells/mm3 or a ≥2- fold rise in CD4+ cell count, and/or

4.11.2.2 Decrease in the HIV-1 viral load of >0.5 log10 and/or

4.11.2.3 Weight gain or other investigator-defined signs of clinical improvement in response to initiation, reintroduction or change of either antiretroviral therapy/regimen or OI therapy.

AND

4.11.2.4 Symptoms and/or signs that are consistent with an infectious or inflammatory condition.

AND

4.11.2.5 These symptoms and/or signs cannot be explained by a newly acquired infection, the expected clinical course of a previously recognized infectious agent, or the side effects of medications.

AND

4.11.2.6 The infectious/inflammatory condition must be attributable to a specific pathogen or condition.

Although ACTG IRIS criteria does not explicitly include CNS IRIS due to EBV-associated PCNSL, it is biologically likely that a reconstituted immune system would react to EBV associated antigens in AIDS-PCNSL. As such, patients with neurologic deterioration on therapy who do not meet criteria for progressive lymphoma and have evidence of CNS edema on brain MRI or CT imaging and T-cells in the central nervous system will be managed for CNS IRIS.

All patients with IRIS will be managed with appropriate antibiotics, and steroids. For CNS IRIS, patients can receive up to 1gram of solumedrol (or equivalent) daily for a minimum of 4 days, followed by tapering of steroids. Protocol therapy for AR-PCNSL will not be discontinued due to CNS IRIS, although treatment may be delayed to perform diagnostic studies or in cases with Grade 4 Neurotoxicity at least possibly attributable to CNS IRIS if the PI or LAI determines that treatment delay is clinically indicated to diagnose and treat CNS IRIS.

4.12 NUTRITIONAL ASSESSMENT AND PSYCHOLOGICAL SUPPORT

AR-PCNSL is commonly complicated by malnutrition. Patients with weight loss or evidence of wasting syndrome should have a nutritional consult and nutritional intake should be optimized by either enteral or parental means. All patients on the study will be informed of and encouraged to see a NIH Social Worker for evaluation and support.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The members of the Retroviral Diseases Section Research Team will collect data.

The PI will be responsible for overseeing entry of data into C3D, an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for 30 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

5.2 EXCEPTIONS TO REPORTING

5.2.1 Grade 4 events related to AR-PCNSL or HIV and not to therapy will not require reporting. Examples include:

5.2.1.1 Grade 4 lymphocytopenia

5.2.1.2 Grade 4 CPK

5.2.1.3 Grade 4 hyperuricemia

5.2.1.4 Grade 4 hyperamylasemia without physiologic correlates of non-pancreatic origin.

5.2.1.5 Since lymphocytopenia is a hallmark of HIV infection and this is highly likely in this population of patients, Adverse Event Reporting will not be required for any grade lymphocytopenia.

5.2.2 Grade 4 treatment-related events not requiring reporting:

5.2.2.1 Leucopenia

5.2.2.2 Neutropenia

5.2.2.3 Uncomplicated (no microbiologic confirmation of infection) treatment-related febrile neutropenia requiring hospitalization.

5.2.3 Unexpected drug toxicities will be reported to the FDA via MedWatch.

5.3 RESPONSE CRITERIA

Response criteria are based on published standards for phase II studies of supratentorial malignant glioma[113] and a modification of the response assessment of aggressive non-Hodgkin's lymphoma by integrated international workshop criteria and fluorine-18 – fluorodeoxyglucose positron emission tomography[114], and an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma [115]. Cross-sectional measurements of tumors will be used to assess baseline and responses.

5.3.1 Complete Response - disappearance of all enhancing lesions on MRI of the brain, negative FDG-PET scan persisting for at least 4 weeks in patients who were off corticosteroids, negative CSF cytology and neurologically improved from baseline.

5.3.2 Complete Response Unconfirmed (CRu) – either a CRu, PR or SD by MRI criteria with a completely negative FDG-PET off steroids, and negative CSF cytology. If there is no evidence of progression while off steroids for 4 months, response will be reclassified as CR.

5.3.3 Partial Response

5.3.3.1 A reduction of enhancing tumor volume by more than 50% maintained for at least 4 weeks, steroids stable or reduced, and neurologically stable or improved with positive FDG-PET, or

5.3.3.2 Normalization of MRI, negative FDG-PET, but with persistent disease by CSF cytology.

5.3.4 Progressive disease - an increase of tumor volume of more than 25% or occurrence of new lesions, or neurologically worse, steroids stable or increased.

5.3.5 Stable disease – not meeting the criteria for complete response, partial response, or progressive disease and with a positive FDG-PET

5.3.6 Treatment failure - progressive or stable disease, relapse after initial response, death, or discontinuation of chemotherapy because of complications or because of patient's or Investigator's decision. Patients who require radiotherapy will be considered treatment failures for purposes of the protocol, regardless of long-term outcome.

5.3.7 Substantial brain dysfunction and cognitive problems (as referred to in sections 1.2.3 and 7.4.4) that will define treatment failure refer to post-treatment recurrent brain lymphoma, treatment related functional brain pathology, and/or neurologic function class 3 or 4 as defined in Appendix 2: Neurological Function Class.

5.4 TOXICITY CRITERIA

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. CTCAE version 3.0 can be downloaded from the CTEP homepage at http://ctep.info.nih.gov/ . All appropriate treatment areas should have access to a copy of CTCAE version 3.0.

6 STATISTICAL CONSIDERATIONS

6.1.1 Racial/gender make-up

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section 2.1. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/ or ineffective treatments on the one hand, and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. As a practical matter, it should be noted as outlined in the background section, that the demographics of the AIDS epidemic are shifting in the United States. Currently, the largest growing population is African-American women. It is difficult to predict how these changing demographics will affect accrual for this particular protocol, but patients from minority populations are disproportionately affected by HIV disease and thus efforts to inform minority communities about this study will be a priority in the recruitment effort.

6.1.2 Age Exclusion

Children under 18 years of age are excluded from the study. AR-PCNSL in children is extremely rare, and comprises less than 1% of all cases of AR-PCNSL. For example,

only 1 child was reported with AR-PCNSL in 1996[116]. The incidence of vertical transmission of HIV in the United States has become extraordinarily low and in 2003, 147 cases of perinatal HIV infection were documented and only 161 cases of HIV/AIDS were diagnosed in those 13 years of age or under[117]. The estimated number of AIDS cases in this age group was only 59 for 2003. The study requires multiple invasive procedures and is not designed to assess the tolerability of the treatments in young children. However, if the preliminary study results are favorable, and pediatric cases with AR-PCNSL otherwise eligible for the study except for age criteria come to our attention, if appropriate, the study may be amended after discussion with the NCI IRB if the risks and benefits appear to be in favor of including children on the study.

6.1.3 Sample Size and Accrual Limits

The primary objective of this study is to preliminarily estimate the magnitude of, and determine if there is potentially an acceptable fraction of, patients with primary brain lymphoma, who will receive the experimental treatment consisting of HAART with high-dose methotrexate-rituximab who are able to survive without recurrent lymphoma or severe cognitive problems (as defined by Neurological Function Class 3 in Appendix 2) for at least two years.

The goal of this limited size pilot trial is to enroll 15 evaluable patients who receive all treatment, including high-dose methotrexate. With conventional treatments, including whole brain irradiation, it would be expected that only 25-30% of all such patients with this disease would be alive without recurrent brain lymphoma and free of cognitive problems at the end of two years (a satisfactory result, but in too small a fraction of patients). We believe it quite possible that by using this particular chemotherapy, as many as 65% would be alive without recurrent lymphoma and without cognitive problems after two years. Sixty five percent is a figure chosen that is within range of the outcome expected in high-risk patients with non-AIDS primary brain lymphoma treated with high-dose methotrexate based strategies. With 15 patients, using a one-sided 0.1 alpha level exact test of binomial proportions, there is 88% power to rule out 30% with a satisfactory result after two years in favor of 65% with a satisfactory result. Should the results appear at all promising, even if not to this degree, the strategy may be taken to the AIDS Malignancy Consortium to see if they are interested in pursuing the idea in a larger, more definitive fashion. The results of this study will be weighed against the best available data from other studies to make a reasonable determination as to how to proceed with or modify this approach for further development.

Additionally, up to 10 additional patients who are not eligible to receive high-dose methotrexate may be accrued in the “Radiation-sparing Best Available Care” Cohort. The patients enrolled in this limited size cohort will be evaluated for the same endpoints as in the main cohort receiving high-dose methotrexate, but given the limited number of patients who will enroll into this cohort, all analyses in this cohort will be considered hypothesis generating and exploratory. Accrual to this cohort will close when 15 evaluable patients have been enrolled in the main cohort.

All secondary endpoints will be evaluated using explorative techniques, and will be reported as exploratory, since the study is not designed to address any of these points with more than minimal power.

Since part of the treatment strategy is aimed at HIV suppression and immune reconstitution, up to 3 patients who are non-adherent with HAART within the first 9 months of therapy may be replaced. It can often take up to 9 months to adjust medications for purposes of optimizing medication tolerance and adherence to antiretroviral therapy. Patients who are clearly non-adherent and thus are unable to maintain an HIV viral load of <5000 copies HIV mRNA/mL plasma and do not have an increment in the CD4+ cells to above 75/mm^3 by the end of 9 months may be replaced. Patients who maintain lower HIV viral loads but do not have adequate immune recovery will not be replaced, and will be considered treatment failures in the event of tumor recurrence. Informal comparison will be made between those who are HAART naive at entry and those who are antiretroviral experienced.

This is a rare condition, and it is expected to take between 6 and 10 years to enroll 15 patients onto this trial in the main cohort and up to 10 in the cohort not receiving high-dose methotrexate. Up to three patients may be replaced if during the first 9 months they are clearly non-adherent with HAART as described above. Thus an accrual ceiling of 28 patients will be used.

7 HUMAN SUBJECTS PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION

7.1.1 AR-PCNSL is a rare disease. HIV-infected individuals are at increased risk of this lymphoma. The purpose of the study is to investigate therapy that avoids late-occurring neurocognitive toxicities associated with WBRT. Selection of individuals with favorable survival prospects from the standpoint of the underlying HIV disease is essential in order to study late occurring treatment toxicities for this disease. Therefore, patients who have resistant HIV that cannot be suppressed and would therefore not be able to have immunologic recovery (thought essential to maintaining the lymphoma in remission), will not be appropriate subjects for this trial. Additionally, up to 3 patients who do not adhere to HAART and have high HIV viral loads (see section 6.1.3) without immune recovery and have a lymphoma relapse within the first 9 months may be replaced as subjects.

7.1.2 Strategies for recruitment will include announcements on the World Wide Web (see Appendix 8) as well as letters to referring physicians and AIDS treatment bulletins.

7.1.3 Pregnancy is excluded on the basis of the overall toxicity expected from the treatment, which would likely threaten fetal viability and potentially increase the risk to the mother and the potential teratogenicity of high-dose methotrexate.

7.1.4 Exclusion of patients less than 18 years of age is based on the extreme rarity of this disease in children. Inclusion of children on the study would result in such an

insufficient number that it would not be possible to evaluate the therapy relative to the purpose of the study.

7.2 PARTICIPATION OF CHILDREN

See section **7.1.4** above. The standard of care for AR-PCNSL is currently WBRT. The treatments in this protocol have uncertain efficacy, and the risk to very young children may be more than minimal while the estimates of benefit are not fully known. If the therapy appears beneficial, a risk/benefit assessment will be made for the potential enrollment of children, and if it appears reasonable to do so, consideration for amending the protocol for enrollment of children will be made.

7.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults who are unable to provide initial informed consent are eligible for enrolling in this protocol as noted in Section **2.2.1.12**. There is also a possibility, though unlikely, that subjects who provide initial consent could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **7.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

7.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

7.4.1 The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the patient or the patient's surrogate, and a signed informed consent document will be obtained.

7.4.2 The potential benefit is that the protocol therapy may result in specific control of AR-PCNSL in those patients to whom it is administered and potentially avoid the late occurring neurotoxic effects associated with WBRT.

7.4.3 The potential risk is that the protocol therapy may be toxic, and/or be ineffective. It is not known whether in AIDS, high-dose methotrexate will cause CNS damage, though the limited available data suggests that it does not cause excess neurological damage. The treatment requires up to 6 or more in-patient admissions that are likely to last 7 days or longer on each admission. Additionally, the staging and restaging requirements of the study requires frequent, sometimes invasive, evaluations. Thus, the time commitment and potential morbidity associated with tumor biopsy represents a potential risk and discomfort to study participants.

7.4.4 Discussion of risks to subjects in relation to anticipated benefits and the

importance of the knowledge that may reasonably be expected from the results: as outlined in the introduction, the standard of care for AR-PCNSL is predicted to cause serious brain dysfunction in patients who survive long enough for late-occurring radiation neurotoxic effects to manifest. The median time to cognitive decline following WBRT in high risk populations is 12-18 months. This study is aimed at developing a strategy toward long-term remission of AR-PCNSL in part through novel relatively non-lymphocytotoxic chemotherapeutic approaches and in part through efforts at immune reconstitution.

7.5 SPECIMEN HANDLING GUIDELINES

7.5.1 It is understood that per the NCI policy regarding the Requirements for the Research Use of Stored Human Specimens and Data, prospective NIH IRB approval and continuing IRB oversight must be obtained for research involving identified or coded samples or data where investigators can identify the source. This policy applies to research protocols where the remaining research activities are limited to data analysis and to the subsequent research use of specimens or data previously collected under a now terminated protocol. The following guidelines describe how these principles apply to this specific protocol.

7.5.2 Many samples on this study will be processed and stored in the AIDS Monitoring Laboratory (AML) run by Leidos Biomedical Research, Inc. in the NCI-Frederick facility located with Fort Detrick. The samples are stored under code, and the information linking these unique codes to the patients is kept on the AML database. The laboratory informatics system conforms to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. All laboratory staff is trained to adhere to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. Computers used to access inventory systems require username and password for login. The laboratory database is housed in a secure, protected environment and backups are performed routinely. Access to specimen information, clinical data, and stored specimens is limited to approved laboratory staff and the investigator in charge of the study (or individuals authorized by the investigator).

AIDS Monitoring Laboratory
Leidos Biomedical Research, Inc.
Frederick National Laboratory for Cancer Research
Frederick, MD 21702-1201
Phone: 301-846-5217

7.5.3 The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The research nurse on the study will be in charge of tracking this information for the protocol team.

7.5.4 Some of the specimens are sent to the laboratory of Dr. Denise Whitby, also in the Leidos Biomedical Research, Inc. NCI-Frederick facility located with Fort

Detrick. The samples sent are coded by the protocol research team and have no patient identifiers. They are logged in by Dr. Whitby's laboratory and kept in a locked facility. They are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

7.5.5 Denise Whitby, PhD
Leidos Biomedical Research, Inc.
50 Boyles St, Building 535, Room 428A
Frederick, MD
301-846-5828

7.5.6 A limited number of samples are sent to Dr. Yarchoan's laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized.

7.5.7 Robert Yarchoan, MD
Center Drive, Room 5A-25
Bethesda, MD 20892
301-402-3630

7.5.8 Clinical testing of all samples will be one in accordance to the protocol. The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The research nurse on the study will be in charge of tracking this information for the protocol team.

7.5.9 Many routine samples and a sample of the biopsy specimens are sent to the Clinical Pathology or Pathology Departments of the NIH Clinical Center. These samples will be handled according to the procedures of these departments.

7.5.10 If patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples may also be tested under the specifications of that study. Similarly, if patients have co-enrolled on other studies approved by the NCI IRB that call for maintaining and testing the samples, then they may be transferred to those studies.

7.5.11 At the termination of the study, if patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples will be transferred to that study unless the patients request that this not occur. Also, if patients have co-enrolled on other studies approved by the NCI IRB that call for maintaining the samples, then they will be maintained on those protocols. Otherwise, the unused samples will be destroyed.

7.5.12 The PI will report any loss or destruction of the samples to the IRB, and any new use of the samples, specimens, or data will require prospective IRB approval.

7.6 CONSENT PROCEDURES AND DOCUMENTS

All patients or the appropriate power of attorney for health care will read and sign the informed consent document prior to enrollment. Members of the protocol team will

describe the protocol, alternative therapies, and the risks and benefits of each to the individual signing the consent.

7.6.1 Telephone Consent

Telephone consent may obtained via the following procedure: The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator.

7.6.2 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12 and 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long-translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

Adverse events while on-treatment and for 30-days after the last dose of therapy will be reported to the NCI IRB according to the guidelines below. All serious adverse events occurring while on study will be reported as outlined in section 8.2.1. Since all therapy will be standard, commercially available therapy, data will not be reported to other organizations. The Principal Investigator will be responsible for data safety monitoring.

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

8.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

8.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient

or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

8.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

8.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

8.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

8.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

8.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

As noted in section **8**, this reporting applies to events while on-treatment and for 30-days after the last dose of therapy. Reports must be received within 7 days of PI awareness via iRIS.

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported. Also, as noted in section **8**, reporting is required only for events while on-treatment and for 30-days after the last dose of therapy.

9 PHARMACEUTICAL INFORMATION

9.1 RITUXIMAB

9.1.1 Source: rituximab will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.1.2 Toxicity:

9.1.2.1 Hypersensitivity Reaction

9.1.2.2 Angioedema

9.1.2.3 Bronchospasm

9.1.2.4 Hypotension

9.1.2.5 Cardiac arrhythmia

9.1.2.6 Rash

9.1.2.7 Headache

9.1.2.8 Pruritus

9.1.2.9 Fatigue

9.1.2.10 Severe infusion reaction

9.1.2.11 Fatal reactions within 24 hours after administration

9.1.2.12 80% of fatal infusion reactions occurred in association with the first infusion

9.1.2.13 After an infusion reaction complex including 1 or more:

9.1.2.14 Hypoxia

9.1.2.15 Pulmonary infiltrates
9.1.2.16 Acute respiratory distress syndrome
9.1.2.17 Myocardial infarction
9.1.2.18 Ventricular fibrillation
9.1.2.19 Cardiogenic shock
9.1.2.20 Severe Mucocutaneous Reactions, some with fatal outcome
9.1.2.21 Constitutional symptoms
9.1.2.22 Fever
9.1.2.23 Chills
9.1.2.24 Infection
9.1.2.25 Asthenia
9.1.2.26 Headache
9.1.2.27 Abdominal Pain
9.1.2.28 Pain
9.1.2.29 Back Pain
9.1.2.30 Throat Irritation
9.1.2.31 Flushing
9.1.2.32 Cardiovascular System
9.1.2.33 Hypotension
9.1.2.34 Hypertension
9.1.2.35 Digestive System
9.1.2.36 Nausea
9.1.2.37 Diarrhea
9.1.2.38 Vomiting
9.1.2.39 Hematologic and Lymphatic System
9.1.2.40 Lymphopenia
9.1.2.41 Leukopenia
9.1.2.42 Neutropenia
9.1.2.43 Thrombocytopenia
9.1.2.44 Anemia
9.1.2.45 Metabolic and Nutritional Disorders
9.1.2.46 Angioedema
9.1.2.47 Hyperglycemia
9.1.2.48 Peripheral Edema
9.1.2.49 LDH Increase
9.1.2.50 Musculoskeletal System
9.1.2.51 Myalgia
9.1.2.52 Arthralgia

9.1.2.53 Nervous System

9.1.2.54 Dizziness

9.1.2.55 Anxiety

9.1.2.56 Respiratory System

9.1.2.57 Increased Cough

9.1.2.58 Rhinitis

9.1.2.59 Bronchospasm

9.1.2.60 Dyspnea

9.1.2.61 Sinusitis

9.1.2.62 Skin and Appendages

9.1.2.63 Night Sweats

9.1.2.64 Rash

9.1.2.65 Pruritus

9.1.2.66 Urticaria

9.1.3 Formulation and Preparation

9.1.3.1 How Supplied

9.1.3.1.1 RITUXAN® (Rituximab) is supplied as 100 mg and 500 mg of sterile, preservative-free, single-use vials.

9.1.3.1.2 Single unit 100 mg carton: Contains one 10 mL vial of RITUXAN (10 mg/mL). (NDC 50242-051-21)

9.1.3.1.3 Single unit 500 mg carton: Contains one 50 mL vial of RITUXAN (10 mg/mL). (NDC 50242-053-06)

9.1.3.2 Mixing and Dilution:

9.1.3.2.1 Use appropriate aseptic technique. Withdraw an amount of rituximab appropriate for a patient's dose and dilute it to a final concentration of 2 mg/mL in an infusion bag containing either 0.9% Sodium Chloride Injection, USP, or 5% Dextrose Injection, USP. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

9.1.4 Stability and Storage

9.1.4.1 Rituximab solutions for infusion may be stored at 2°—8°C (35.6°—46.4°F) for 24 hours. Rituximab solutions for infusion have been shown to be stable for an additional 24 hours at room temperature after storage for up to 24 hours under refrigeration. However, since rituximab solutions do not contain a preservative, diluted solutions should be stored refrigerated (2°—8°C). No incompatibilities between rituximab and polyvinylchloride or polyethylene bags have been observed.

9.1.4.2 Intact vials should be stored at 2°—8°C (36°—46°F). Do not use beyond the labeled expiration date. Rituximab vials should be stored in the original

packaging carton, protected from direct sunlight.

9.1.5 Administration Procedures

9.1.5.1 DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

9.1.5.2 Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding antihypertensive medications 12 hours prior to rituximab infusion, and on the day of rituximab administration.

9.1.5.3 First Infusion

9.1.5.3.1 The rituximab solution for infusion should be administered intravenously at an initial rate of 50 mg/h (25 mL/h). Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion reactions do not occur, escalate the infusion rate in 50 mg/h (25 mL/h) increments every 30 minutes, to a maximum of 400 mg/h (200 mL/h). If hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of patient symptoms."

9.1.5.4 Subsequent Infusions

9.1.5.4.1 If the patient tolerated the first infusion well, subsequent rituximab infusions can be administered at an initial rate of 100 mg/h (50 mL/h), and increased by 100 mg/h (50 mL/h) increments at 30-minute intervals, to a maximum of 400 mg/h (200 mL/h) as tolerated. If the patient did not tolerate the first infusion well, follow the guidelines under First Infusion.

9.2 METHOTREXATE

9.2.1 Source: Preservative-free methotrexate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.2.2 Formulation: Methotrexate Injection, USP, is available in single-dose vials containing 50, 100, 200, 250, or 1000 mg of sterile, isotonic, preservative-free methotrexate sodium for parenteral administration.

9.2.3 Preparation: Dilute an amount of preservative-free methotrexate appropriate for a patient's dose in (qs.) 1000 mL 5% Dextrose/0.45% Sodium Chloride Injection, USP, with an amount of sodium bicarbonate equivalent to what is given over 4 hours in hydration to maintain a patient's urine pH within the range of 7-8.

9.2.4 Stability: When prepared as described above and stored at room temperature or under refrigeration, methotrexate is physically and chemically stable for at least 24 hours.

9.2.5 Storage: Store intact vials containing preservative-free methotrexate at controlled room temperature 15°–30°C (59°–86°F), and protect methotrexate-containing solutions from light. The extent of photodegradation correlates inversely with methotrexate concentration and is exacerbated by admixture with sodium bicarbonate.

9.2.6 Administration:

9.2.6.1 Pre-hydration: Patients will receive IV hydration with a suitable parenteral fluid containing sodium bicarbonate (e.g., 5% Dextrose with 0.45% Sodium Chloride Injection + 50-150 mEq sodium bicarbonate) to alkalinize the urine to a pH ≥7, but ≤8. Intravenous hydration should commence on Day 1 and continue at least until serum methotrexate levels are less than 0.5 mcmol/L. (See Sections 3.3.3.2.2, 3.3.3.3 and 3.4.3.4.)

9.2.6.2 Methotrexate 6000 mg/m^2 will be administered by intravenous infusion over 4 hours after confirming that the recipient patient's urine pH is within the range ≥7 to ≤8, and urine output is ≥100 mL/hour.

9.2.7 Adverse Events:

9.2.7.1 Hematologic: Leukopenia, thrombocytopenia, agranulocytosis, eosinophilia, and megaloblastic anemia. Other rarely reported hematologic adverse events, include: aplastic anemia, lymphadenopathy, lymphoproliferative disorders, and hypogammaglobulinemia.

9.2.7.2 Cardiovascular: Pericarditis, pericardial effusion, hypotension, and thromboembolic events (arterial thrombosis, DVT, retinal vein thrombosis, thrombophlebitis, and pulmonary embolism) have occurred with the use of methotrexate.

9.2.7.3 Central Nervous System: Headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis and convulsions. A transient, acute neurologic syndrome was observed 6 days after a second or third weekly treatment with methotrexate 8000–9000 mg/m^2 IV over 4 hours, followed by leucovorin rescue. The most common findings were behavioral abnormalities, focal sensorimotor signs, and abnormal reflexes, and may be accompanied by seizure activity. Leukoencephalopathy has been associated with intravenous administration of methotrexate in patients who received craniospinal irradiation. Demyelinating leukoencephalopathy may occur many months to years after onset of therapy, and is characterized by dementia, ataxia, spasticity, seizures, and coma. Its incidence is further increased in patients who received cranial irradiation therapy plus intrathecal methotrexate therapy and varies directly with the amount of methotrexate administered.

9.2.7.4 Gastrointestinal: Gingivitis, pharyngitis, anorexia, ulcerative stomatitis, diarrhea, hematemesis, melena, and GI bleeding or ulceration. Nausea and vomiting are observed in approximately 25% of patients treated with high-dose therapy. Mucositis, characterized by mouth soreness, stomatitis, or diarrhea has been reported with methotrexate therapy. Mucositis usually occurs 7–14 days after therapy and lasts 4–7 days.

9.2.7.5 Genitourinary: Defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction, vaginal discharge, gynecomastia, abortion, fetal defects, fetal death and infertility. Nephrotoxicity occurs when the concentration of excreted methotrexate and its metabolites exceed their solubility in the renal tubules. Methotrexate precipitation in the renal tubules produces obstructive renal failure. The concentration threshold at which methotrexate precipitates is approximately 1 mg/mL at a urine pH of 5.7 and 10 mg/mL at a urine pH of 6.9. Therefore, patients will receive IV hydration containing sodium bicarbonate to

alkalinize the urine to a pH ≥7.0. Methotrexate therapy has been associated with azotemia, cystitis, severe nephropathy, and hematuria. Increased BUN and serum creatinine have also been reported with methotrexate and may indicate nephrotoxicity

9.2.7.6 Hepatic: Increased serum transaminases, LDH, alkaline phosphatase, and bilirubin may indicate drug induced liver dysfunction. Hepatitis from high dose therapy is uncommon and is associated with prolonged and high serum methotrexate concentrations.

9.2.7.7 Dermatologic: Erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis, erythema multiforme, toxic epidermal necrolysis, Stevens-Johnson syndrome, skin necrosis, and exfoliative dermatitis, acral erythema, nail hyperpigmentation and acute paronychia, methotrexate-induced solar and thermal burn reactivation, radiation recall dermatitis, toxic epidermal necrolysis have been reported.

9.2.7.8 Hypersensitivity: Anaphylactic reactions with rash, periorbital edema and pruritus have been reported with both initial and repeated exposure to methotrexate

9.2.8 Methotrexate is categorized as a HAZARDOUS DRUG at the NIH Clinical Center.

9.2.9 Drug Interactions: Methotrexate should not be given concurrently with non-steroidal anti-inflammatory drugs, or with penicillins, cephalosporins, salicylates, sulfonamides, and other acidic drugs that compete with methotrexate for renal excretion. Other folate inhibitors (e.g., trimethoprim, pyrimethamine) may interfere with assays for methotrexate in body fluids and should be avoided when possible. Methotrexate toxicity has been reported in several patients taking a variety of penicillins (amoxicillin, mezlocillin, piperacillin, penicillin). See Section 3.9.7 for a list of potentially interacting drugs that should be avoided during administration of high-dose methotrexate and leucovorin rescue. Methotrexate is eliminated by the kidney through both tubular secretion and glomerular filtration. Consequently, penicillins decrease methotrexate renal clearance due to competition for tubular secretion and potentially may enhance methotrexate toxicity. Tissue culture and animal studies have demonstrated that the administration of asparaginase immediately prior to or concurrently with methotrexate can diminish or abolish methotrexate antineoplastic activity. The effect persists as long as plasma asparagine levels are suppressed. Concomitant aspirin and methotrexate therapy has been reported to result in increased methotrexate toxicity (leukopenia, thrombocytopenia, anemia, nephrotoxicity, mucosal ulcerations). Methotrexate, its 7-hydroxy metabolite, and salicylates compete for renal tubular secretion. The effect of aspirin is to decrease the rate of methotrexate clearance, prolonging systemic exposure to toxic concentrations of the drug and its metabolites. Patients receiving methotrexate should not receive salicylates. Cholestyramine may enhance the nonrenal elimination of methotrexate by binding methotrexate in the gut, thus decreasing the amount available for reabsorption; i.e., interrupting its enterohepatic circulation. Oral antibiotics such as chloramphenicol may decrease intestinal absorption of methotrexate or interfere with enterohepatic circulation by inhibiting bowel flora

and suppressing metabolism of the drug by enteric bacteria Coadministered cotrimoxazole (trimethoprim/sulfamethoxazole) may increase methotrexate toxicity, often manifesting as myelotoxicity and pancytopenia. The mechanism of this interaction is thought to be additive inhibition of dihydrofolate reductase by methotrexate and trimethoprim. In addition, sulfamethoxazole may increase free serum levels of methotrexate by displacement of methotrexate from plasma protein binding sites or decreased renal tubular elimination. Concomitant administration of methotrexate and cyclosporine may interfere with the elimination of both drugs, resulting in increased blood concentrations of both drugs and increased toxicity. Use of NSAIDs (including diclofenac, ibuprofen, indomethacin, ketoprofen, naproxen, sulindac, tolmetin), with methotrexate has been shown to delay methotrexate elimination and increase the potential for methotrexate toxicities (leukopenia, thrombocytopenia, anemia, nephrotoxicity, mucosal ulcerations). In case reports, deaths have resulted from severe hematologic and gastrointestinal toxicity. Preexisting renal dysfunction and NSAID-induced renal dysfunction also increases the risk of adverse reactions. Live virus vaccines (e.g., vaccinia, rotavirus) and bacterial vaccines should not be administered to patients whose immunity is suppressed by chemotherapeutic agents. Administration to immune compromised patients has resulted in severe and fatal infections. In patients whose immunity is suppressed only by chemotherapy treatment, at least three months should elapse between the discontinuation of chemotherapy and vaccination with a live vaccine. Concomitant administration of oral mercaptopurine 75 mg/m^2 and oral methotrexate 20 mg/m^2 resulted in significantly increased mercaptopurine AUC and peak serum levels (31% and 26%, respectively). One case report describes a patient who experienced elevated methotrexate levels for several days. Methotrexate levels rapidly declined after omeprazole was discontinued, which suggests that omeprazole may inhibit H+-, K+-ATPase in the kidney, thereby blocking active methotrexate secretion in the kidney. Omeprazole may have to be temporarily discontinued after methotrexate administration to avoid the potential for methotrexate toxicity. Increased phenytoin elimination has been reported with high-dose methotrexate. In addition, methotrexate is partially bound to serum albumin, and methotrexate toxicity (myelotoxicity, pancytopenia, megaloblastic anemia) may be increased because of displacement by phenytoin. Concomitant use of methotrexate and probenecid reduces methotrexate renal clearance by interfering with tubular secretion in the proximal tubule and greatly increases serum methotrexate levels, thus increasing the risk of toxicities from methotrexate (leukopenia, thrombocytopenia, anemia, nephrotoxicity, mucosal ulcerations). Coadministration of pyrimethamine with methotrexate may increase the risk of bone marrow suppression. In addition, pyrimethamine has been shown to cross-react with methotrexate in the TDX (FPIA) assay used by the Clinical Pathology Dept. Sulfonamides may increase free serum levels of methotrexate by displacing of methotrexate from plasma protein binding sites or decreasing methotrexate excretion by the renal tubules. Oral antibiotics such as tetracycline may decrease intestinal absorption of methotrexate or interfere with enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by enteric bacteria.

Methotrexate may decrease the clearance of theophylline. A pharmacokinetic study in steroid dependent asthma patients demonstrated that concurrent use of methotrexate intramuscularly and oral theophylline resulted in a 19% decrease in theophylline clearance presumably by decreasing theophylline metabolism in the liver. Trimethoprim: see the statements for "cotrimoxazole" and "pyrimethamine", above.

9.3 LEUCOVORIN CALCIUM

9.3.1 Source: Leucovorin calcium will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.3.2 Formulations

9.3.2.1 Parenteral Leucovorin Calcium for Injection is a sterile product indicated for intramuscular or intravenous administration, and is supplied in vials containing 50 mg, 100 mg, 200 mg, or 350 mg leucovorin calcium. Each milligram of leucovorin calcium contains leucovorin 0.002 mmol and calcium 0.002 mmol (0.004 mEq).

9.3.2.2 Leucovorin calcium oral tablets: Leucovorin Calcium Tablets, USP, contain either 5 mg or 25 mg leucovorin as the calcium salt, equivalent to either 5.40 mg or 27.01 mg of anhydrous leucovorin calcium, respectively. Each tablet also contains the following excipients: microcrystalline cellulose, corn starch, croscarmellose sodium, povidone, colloidal silicon dioxide, and magnesium stearate. The 25-mg tablets also contain D&C yellow #10 (product label dated 08/1999; Roxane Laboratories, Inc., Columbus, OH).

9.3.3 Preparation

9.3.3.1 Parenteral leucovorin calcium: Reconstitute leucovorin calcium with SWFI, as follows:

Amount of Leucovorin Calcium per Vial	Volume of Diluent per Vial	Concentration After Reconstitution
50 mg	5 mL	10 mg/mL
100 mg	10 mL	
200 mg	20 mL	
350 mg	17.5 mL	20 mg/mL

9.3.3.2 An amount of leucovorin appropriate for a patient's dose will be further diluted in 25 mL 0.9%NS or D5W for clinical use.

9.3.4 Stability

9.3.4.1 Parenteral leucovorin calcium: Leucovorin calcium 10 mg/mL in SWFI stored at 4°C and 25°C is stable for 7 days.

9.3.4.2 Leucovorin calcium oral tablets: Bottles containing leucovorin tablets are stable until the manufacturer's expiration date if they are maintained under the

recommended storage conditions.

9.3.5 Storage

9.3.5.1 Parenteral leucovorin calcium: Store at 25°C (77° F), protected from light.

9.3.5.2 Leucovorin calcium oral tablets: Store at 15°–30°C (59°–86°F) and protect the product from light and moisture.

9.3.6 Administration

9.3.6.1 Oral absorption of leucovorin is saturable at doses >25 mg. The apparent oral bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg and 37% for 100 mg administered orally.

9.3.6.2 Leucovorin calcium doses will be administered orally or by short IV infusion over 15 minutes. Because of its calcium content, leucovorin calcium should not be administered intravenously at a rate >160 mg/minute.

9.3.7 Adverse Events: Allergic sensitization, including anaphylactoid reactions and urticaria, has been reported following administration of both oral and parenteral leucovorin. Anaphylactic reactions, including shock, have been reported. Seizures and syncope have been reported rarely in patients with cancer who received leucovorin, usually during concomitant treatment with a fluoropyrimidine.

9.3.8 Drug Interactions: Capecitabine: see the statement for "fluorouracil", below. Leucovorin increases the concentration of fluorouracil and enhances its toxicity. The pharmacodynamics effects of fluorouracil are enhanced by leucovorin coadministration, including a predisposition to increased toxicity (granulocytopenia, anemia, thrombocytopenia, stomatitis, vomiting) Fosphenytoin: see the statement for "phenytoin", below. Leucovorin can counteract the therapeutic effect of folic acid antagonists, including methotrexate. Folic acid (dihydrofolate) may counteract the anticonvulsant effect of phenobarbital resulting in increased seizure frequency. Folic acid (dihydrofolate) may counteract the anticonvulsant effect of phenytoin resulting in decreased phenytoin levels and increased seizure frequency in some patients. Folic acid (dihydrofolate) may counteract the anticonvulsant effect of primidone.

9.4 LEVOLEUCOVORIN CALCIUM PENTAHYDRATE

9.4.1 Source: Levoleucovorin calcium pentahydrate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.4.2 Formulation: Parenteral levoleucovorin calcium pentahydrate for Injection (Brand name Fusilev®, Spectrum Pharmaceuticals) is a sterile product indicated for intravenous administration, and is supplied in vials containing 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

9.4.3 Preparation: Parenteral levoleucovorin calcium pentahydrate : Prior to intravenous injection, the 50 mg vial of Fusilev for Injection is reconstituted with 5.3 mL of 0.9% Sodium Chloride Injection, USP to yield a levoleucovorin concentration of 10 mg per mL. Reconstitution with Sodium Chloride solutions with preservatives

(e.g. benzyl alcohol) has not been studied. The use of solutions other than 0.9% Sodium Chloride Injection, USP is not recommended.

9.4.4 Stability: After reconstitution, levoleucovorin solutions may be further diluted, immediately, to concentrations of 0.5 mg/mL to 5 mg/mL in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Initial reconstitution or further dilution using 0.9% Sodium Chloride Injection, USP may be held at room temperature for not more than a total of 12 hours.

9.4.5 Storage: Store at 25° C (77 °F) in carton until contents are used. Excursions permitted from 15-30° C (59-86 °F). [See USP Controlled Room Temperature]. Protect from light.

9.4.6 Administration: Levoleucovorin calcium doses will be administered by short IV infusion over 15 minutes. Because of its calcium content, leucovorin calcium should be administered intravenously at a rate NOT GREATER THAN 160 mg of levoleucovorin per minute.

9.4.7 Adverse Events: Allergic sensitization, including anaphylactoid reactions and urticaria, has been reported following administration of parenteral leucovorin. Gastrointestinal adverse reactions, including stomatitis, nausea, and vomiting have also been reported.

9.4.8 Drug Interactions:

9.4.8.1 Capecitabine: see the statement for “fluorouracil”, below.

9.4.8.2 Leucovorin increases the concentration of fluorouracil and enhances its toxicity. The pharmacodynamics effects of fluorouracil are enhanced by leucovorin coadministration, including a predisposition to increased toxicity (granulocytopenia, anemia, thrombocytopenia, stomatitis, vomiting)

9.4.8.3 Fosphenytoin: see the statement for “phenytoin”, below.

9.4.8.4 Leucovorin can counteract the therapeutic effect of folic acid antagonists, including methotrexate.

9.4.8.5 Folic acid (dihydrofolate) may counteract the anticonvulsant effect of phenobarbital resulting in increased seizure frequency.

9.4.8.6 Folic acid (dihydrofolate) may counteract the anticonvulsant effect of phenytoin resulting in decreased phenytoin levels and increased seizure frequency in some patients.

9.4.8.7 Folic acid (dihydrofolate) may counteract the anticonvulsant effect of primidone.

9.5 GLUCARPIDASE

9.5.1 Source: On January 17, 2012, glucarpidase (brand name Voraxaze®) was approved by FDA for the treatment of toxic plasma methotrexate concentrations (>1 mcmole per liter) in patients with delayed methotrexate clearance due to impaired renal function. Glucarpidase is commercially available for intravenous use under the brand name, Voraxaze®. Glucarpidase will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.5.2 Formulation: Parenteral glucarpidase for Injection (Brand name Voraxaze®, BTG

International Inc.) is a sterile, preservative-free white lyophilized powder. Each individually packaged vial contains 1,000 Units of glucarpidase, lactose monohydrate (10 mg), Tris-HCl (0.6 mg) and zinc acetate dihydrate (0.002 mg). The individually packaged glass vial is closed with bromo butyl elastomerase stopper and blue flip-off seal. NDC 50633-210-11

9.5.3 Preparation: Prior to intravenous injection, reconstitute the contents of 1 vial glucarpidase 1,000 Units with 1 mL of 0.9% sterile Sodium Chloride Injection, USP. Roll and tilt the vial gently to mix, do not shake. Inspect the vial and discard if the solution is not clear, colorless, and free of particulate matter.

9.5.4 Stability: After reconstitution, use reconstituted glucarpidase immediately or store under refrigeration at 36° to 46°F (2° to 8°C) for up to 4 hours if not used immediately. Glucarpidase contains no preservative and is supplied as a single-use vial. Discard any unused product.

9.5.5 Storage: Store glucarpidase (Voraxaze®) at 36°F to 46°F (2°C to 8°C). Do not freeze. Do not use glucarpidase after the expiration date on the vial.

9.5.6 Administration: Glucarpidase will be administered by a short IV infusion over 5 minutes. Flush intravenous line before and after glucarpidase administration with 0.9% Sodium Chloride Injection. Continue to administer leucovorin or levoleucovorin after glucarpidase. Do not administer leucovorin or levoleucovorin within 2 hours before or after a dose of glucarpidase because leucovorin is a substrate for glucarpidase.

9.5.7 Adverse Events: Allergic sensitization, including anaphylactoid reactions may occur. The most common adverse reactions (incidence >1%) with glucarpidase include paraesthesias, flushing, nausea and/or vomiting, hypotension, and headache.

9.5.8 Drug Interactions: Leucovorin is a substrate for glucarpidase. Do not administer leucovorin within 2 hours before or after a dose of glucarpidase. Beyond 2 hours, no dose adjustment is recommended for continuing a leucovorin regimen, because the leucovorin dose is based on the patient's pre-glucarpidase serum methotrexate concentration. Other potential exogenous substrates of glucarpidase may include reduced folates and folate antimetabolites.

9.6 FILGRASTIM

9.6.1 Source: Filgrastim will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.6.2 Formulation: Filgrastim is a single chain, nonglycosylated, 175-amino acid polypeptide recombinant (human) Granulocyte-Colony Stimulating Factor, expressed by Escherichia coli, marketed as Neupogen® (Amgen, Inc., Thousand Oaks, CA). Specific activity = 1 x 108 U/mg of protein. Fifty Units is defined as the amount required for 50% of maximal stimulation of colony formation by normal marrow cells. The drug product is a clear, colorless, preservative-free solution for parenteral injection in 2 mL capacity single-use vials. Each vial contains 300 mcg/mL or 480 mcg/1.6 mL. Each milliliter also contains acetate

0.59 mg/mL, mannitol 50 mg/mL, TWEEN 80 0.004%, sodium 0.035 mg/mL, and water for injection, USP, qs. 1 mL. Product pH = 4.0.

9.6.3 Stability: When refrigerated (2°–8°C or 35.6°–46.4°C), intact vials are stable for at least two years after manufacture. Avoid shaking the vials. Filgrastim aggregates when frozen (–4°C; 20°F) and when held at temperatures >30°C (>86°F); therefore, it should not be exposed to temperatures exceeding those extremes. When maintained between 9°–30°C (47°–86°F), the drug product is stable and safe to use for up to 24 hours provided that the solution remains clear (not cloudy) and contains no particulate matter.

9.6.4 Storage: Intact vials should be stored under refrigeration. Do not allow the product to freeze.

9.6.5 Administration: Filgrastim is suitable for subcutaneous or direct IV injection. Filgrastim may be administered at doses of 300 – 480 mcg/day subcutaneously starting after completion of leucovorin rescue and continuing until the AGC ≥5000/mm3. Dose adjustments will be allowed as clinically indicated. Patients and/or their caregivers will be instructed how to administer filgrastim subcutaneously.

9.6.6 Adverse Events: The most frequent complication associated with filgrastim use is mild-to-moderate bone pain and musculoskeletal pain that occurs during or after intravenous or subcutaneous administration. In general, patients characterize bone pain as non-specific or specific with distribution to the shoulders, legs, chest, lower back, ribs, lumbar spine, and sternum. Symptoms are often described during a first course of filgrastim, but there is considerable variation in onset; e.g., after the first dose, during growth factor administration, a few hours after completing administration (for infusional delivery), or after patients' granulocyte counts normalize. Once and twice-daily administration schedules produce similar adverse event profiles, including mild to moderate bone pain, headache, and mild fatigue. Bone pain often can be prevented by prophylactic acetaminophen ± diphenhydramine administered prior to filgrastim; however, pain can sometimes be severe requiring opioid analgesics. Acute fever and neutrophilic dermatosis (Sweet syndrome), which is characterized by rapid onset of erythematous and tender plaques, fever, and leukocytosis, neutrophilia, sudden onset of asymmetric, painful skin lesions and oral mucosa with dense dermal infiltrates of mature neutrophils without signs of vasculitis and biopsy-proven cutaneous leukocytoclastic vasculitis that usually follows an increase in ANC, subsides after ANC decreases, does not recur if ANC was kept <800/mm3, and can be managed by reducing filgrastim doses, discontinuing therapy, and use of topical steroids. Skin rash and edema are infrequently observed during filgrastim therapy. Filgrastim may exacerbate and precipitate psoriasis. Other neutrophilic dermatoses associated with filgrastim use include pyoderma gangrenosum, neutrophilic eccrine hidradenitis, and single cases of temporally-related lichenoid cutaneous reaction at injection sites, and a disseminated eruption characterized by the presence of numerous large, atypical histiocytes in the dermis with several mitotic figures, mimicking dermal involvement by a malignant process also have been reported. Conjunctival erythema, iridocyclitis scleritis, and migrainous

episodes have occurred in patients treated with filgrastim. Iridocyclitis was controlled easily with homatropine eye drops. Severe photophobia has been the treatment-limiting toxicity with filgrastim doses of 30,000 and 100,000 mcg/m^2 per day. All symptoms resolve within 24–48 hours after discontinuing filgrastim. Filgrastim has been implicated in exacerbating chemotherapy-induced pulmonary toxicity when used in conjunction with bleomycin and cyclophosphamide ± other antineoplastics. However, randomized controlled studies have failed to show increased pulmonary toxicity when filgrastim accompanied bleomycin therapy and it has been suggested that an increase in pulmonary toxicity may be attributable to the concomitant use of other antineoplastics. Increased serum uric acid has been observed in patients treated with filgrastim, particularly with high doses (30–60 mcg/kg per day). The effect is transient and probably is related to increased leukocyte turnover. Reactivation of various inflammatory disorders have been reported following of granulocyte colony-stimulating factors administration, including rheumatoid arthritis and pseudogout. Allergic-type reactions have been reported in <1 of 4000 patients treated with filgrastim. Reactions tend to occur within the first 30 minutes after administration and appear to be more frequent in patients who receive filgrastim by the intravenous route. Some reactions occurred on initial exposure. Symptoms recurred in more than half the patients who were rechallenged. In most cases, symptoms have rapidly resolved after administering antihistamines, steroids, bronchodilators and/or epinephrine. Fever >38°C occurred in eight of 39 patients (21%) who received intravenous filgrastim to shorten the duration of chemotherapy-induced neutropenia. These patients had no evidence of acute infection. Fever occurred 4–8 days after initiating daily intravenous filgrastim infusions, which commenced three days after intensive chemotherapy. In several patients, fever developed only with filgrastim therapy, and not after chemotherapy alone. Splenic enlargement was observed on magnetic resonance imaging in six patients with cyclic neutropenia during treatment with filgrastim; however, no clinical symptoms were observed, and there were no abnormalities in splenic architecture or blood-cell morphology. Splenic enlargement was also reported in two of five patients who received filgrastim for congenital agranulocytosis. Spontaneous splenic rupture four days after a six-day course of filgrastim was reported in a donor of peripheral blood stem cells. Other side-effects that have been associated with filgrastim use include pain and paresthesias secondary to submental nerve compression; increased leukocyte and serum alkaline phosphatases and LDH; and decreased platelet counts which usually are not clinically significant, anemia, epistaxis, headache, diarrhea, and cutaneous vasculitis.

10 REFERENCES

1. Levine AM. AIDS-related malignancies: the emerging epidemic. J Natl Cancer Inst 1993;85:1382-97.
2. Goplen AK, Dunlop O, Liestol K, Lingjaerde OC, Bruun JN, Maehlen J. The impact of primary central nervous system lymphoma in AIDS patients: a population-based autopsy study from Oslo. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14:351-4.
3. von Gunten CF, Von Roenn JH. Clinical aspects of human immunodeficiency virus-related lymphoma. Curr Opin Oncol 1992;4:894-9.
4. Modi M, Mochan A, Modi G. Management of HIV-associated focal brain lesions in developing countries. Qjm 2004;97:413-21.
5. Morgello S. Epstein-Barr and human immunodeficiency viruses in acquired immunodeficiency syndrome-related primary central nervous system lymphoma. Am J Pathol 1992;141:441-50.
6. Camilleri-Broet S, Davi F, Feuillard J, et al. AIDS-related primary brain lymphomas: histopathologic and immunohistochemical study of 51 cases. The French Study Group for HIV-Associated Tumors. Hum Pathol 1997;28:367-74.
7. Camilleri-Broet S, Davi F, Feuillard J, et al. High expression of latent membrane protein 1 of Epstein-Barr virus and BCL-2 oncoprotein in acquired immunodeficiency syndrome-related primary brain lymphomas. Blood 1995;86:432-5.
8. Carbone A, Gloghini A, Larocca LM, et al. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. Blood 2001;97:744-51.
9. Larocca LM, Capello D, Rinelli A, et al. The molecular and phenotypic profile of primary central nervous system lymphoma identifies distinct categories of the disease and is consistent with histogenetic derivation from germinal center-related B cells. Blood 1998;92:1011-9.
10. Miller DC, Hochberg FH, Harris NL, Gruber ML, Louis DN, Cohen H. Pathology with clinical correlations of primary central nervous system non-Hodgkin's lymphoma. The Massachusetts General Hospital experience 1958-1989. Cancer 1994;74:1383-97.
11. 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.
12. Hermine O, Haioun C, Lepage E, et al. Prognostic significance of bcl-2 protein expression in aggressive non- Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood 1996;87:265-72.
13. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002;346:1937-47.
14. Lossos IS, Czerwinski DK, Alizadeh AA, et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med 2004;350:1828-37.

15. Ling SM, Roach M, 3rd, Larson DA, Wara WM. Radiotherapy of primary central nervous system lymphoma in patients with and without human immunodeficiency virus. Ten years of treatment experience at the University of California San Francisco. Cancer 1994;73:2570-82.
16. Newell ME, Hoy JF, Cooper SG, et al. Human immunodeficiency virus-related primary central nervous system lymphoma: factors influencing survival in 111 patients. Cancer 2004;100:2627-36.
17. Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet 1991;337:805-9.
18. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112-6.
19. Besson C, Goubar A, Gabarre J, et al. Changes in AIDS-related lymphoma since the era of highly active antiretroviral therapy. Blood 2001;98:2339-44.
20. Hoffmann C, Tabrizian S, Wolf E, et al. Survival of AIDS patients with primary central nervous system lymphoma is dramatically improved by HAART-induced immune recovery. Aids 2001;15:2119-27.
21. Dore GJ, Li Y, McDonald A, Ree H, Kaldo JM. Impact of highly active antiretroviral therapy on individual AIDS- defining illness incidence and survival in Australia. J Acquir Immune Defic Syndr 2002;29:388-95.
22. Jaffe H. Public health. Whatever happened to the U.S. AIDS epidemic? Science 2004;305:1243-4.
23. Katz MH, Cunningham WE, Mor V, et al. Prevalence and predictors of unmet need for supportive services among HIV-infected persons: impact of case management. Med Care 2000;38:58-69.
24. Andersen R, Bozzette S, Shapiro M, et al. Access of vulnerable groups to antiretroviral therapy among persons in care for HIV disease in the United States. HCSUS Consortium. HIV Cost and Services Utilization Study. Health Serv Res 2000;35:389-416.
25. Stein MD, Crystal S, Cunningham WE, et al. Delays in seeking HIV care due to competing caregiver responsibilities. Am J Public Health 2000;90:1138-40.
26. Turner BJ, Cunningham WE, Duan N, et al. Delayed medical care after diagnosis in a US national probability sample of persons infected with human immunodeficiency virus. Arch Intern Med 2000;160:2614-22.
27. CDC. HIV/AIDS Surveillance Report. Atlanta: Centers for Disease Control; 2001.
28. Bonnet F, Morlat P, Chene G, et al. Causes of death among HIV-infected patients in the era of highly active antiretroviral therapy, Bordeaux, France, 1998-1999. HIV Med 2002;3:195-9.
29. Bonnet F, Lewden C, May T, et al. Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. Cancer 2004;101:317-24.
30. Dal Maso L, Franceschi S. Epidemiology of non-Hodgkin lymphomas and other haemolymphopoietic neoplasms in people with AIDS. Lancet Oncol 2003;4:110-9.

31. Clifford GM, Polesel J, Rickenbach M, et al. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. J Natl Cancer Inst 2005;97:425-32.
32. Blay JY, Conroy T, Chevreau C, et al. High-dose methotrexate for the treatment of primary cerebral lymphomas: analysis of survival and late neurologic toxicity in a retrospective series. J Clin Oncol 1998;16:864-71.
33. Abrey LE, Yahalom J, DeAngelis LM. Treatment for primary CNS lymphoma: the next step. J Clin Oncol 2000;18:3144-50.
34. Abrey LE, DeAngelis LM, Yahalom J. Long-term survival in primary CNS lymphoma. J Clin Oncol 1998;16:859-63.
35. Harder H, Holtel H, Bromberg JE, et al. Cognitive status and quality of life after treatment for primary CNS lymphoma. Neurology 2004;62:544-7.
36. Fliessbach K, Urbach H, Helmstaedter C, et al. Cognitive performance and magnetic resonance imaging findings after high-dose systemic and intraventricular chemotherapy for primary central nervous system lymphoma. Arch Neurol 2003;60:563-8.
37. Correa DD, DeAngelis LM, Shi W, Thaler H, Glass A, Abrey LE. Cognitive functions in survivors of primary central nervous system lymphoma. Neurology 2004;62:548-55.
38. Smith KJ, Skelton HG, Tuur S, Yeager J, Decker C, Wagner KF. Increased cutaneous toxicity to ionizing radiation in HIV-positive patients. Military Medical Consortium for the Advancement of Retroviral Research (MMCARR). Int J Dermatol 1997;36:779-82.
39. Little RF, Pittaluga S, Grant N, et al. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood 2003;101:4653-9.
40. Chak LY, Gill PS, Levine AM, Meyer PR, Anselmo JA, Petrovich Z. Radiation therapy for acquired immunodeficiency syndrome-related Kaposi's sarcoma. J Clin Oncol 1988;6:863-7.
41. Kirova YM, Belembaogo E, Frikha H, et al. Radiotherapy in the management of epidemic Kaposi's sarcoma: a retrospective study of 643 cases. Radiother Oncol 1998;46:19-22.
42. Sheline GE, Wara WM, Smith V. Therapeutic irradiation and brain injury. Int J Radiat Oncol Biol Phys 1980;6:1215-28.
43. Boldrey E, Sheline G. Delayed transitory clinical manifestations after radiation treatment of intracranial tumors. Acta Radiol Ther Phys Biol 1966;5:5-10.
44. van der Kogel AJ. Radiation-induced damage in the central nervous system: an interpretation of target cell responses. Br J Cancer Suppl 1986;7:207-17.
45. Kramer S. The hazards of therapeutic irradiation of the central nervous system. Clin Neurosurg 1968;15:301-18.
46. Little RF, Gutierrez M, Jaffe ES, Pau A, Horne M, Wilson W. HIV-associated non-Hodgkin lymphoma: incidence, presentation, and prognosis. Jama 2001;285:1880-5.
47. Schultheiss TE, Kun LE, Ang KK, Stephens LC. Radiation response of the central nervous system. Int J Radiat Oncol Biol Phys 1995;31:1093-112.

48. Price RA, Jamieson PA. The central nervous system in childhood leukemia. II. Subacute leukoencephalopathy. Cancer 1975;35:306-18.
49. Antinori A, Cingolani A, Ammassari A, et al. AIDS-Related Focal Brain Lesions in the Era of HAART. In: 6th Conference on Retroviruses and Opportunistic Infections; 1999 January 31 - February 4; Chicago; 1999. p. Abstract 413.
50. Antinori A, Cingolani A, Alba L, et al. Better response to chemotherapy and prolonged survival in AIDS-related lymphomas responding to highly active antiretroviral therapy. Aids 2001;15:1483-91.
51. Yarchoan R, Little R. Immunosuppression-related malignancies. In: DeVita VT, Hellman S, Rosenberg SA, eds. Cancer Principles and Practice of Oncology 7th Edition. 7th ed. Philadelphia: Lippincott; 2005:2247-63.
52. Thirlwell C, Sarker D, Stebbing J, Bower M. Acquired immunodeficiency syndrome-related lymphoma in the era of highly active antiretroviral therapy. Clin Lymphoma 2003;4:86-92.
53. Vaccher E, Spina M, Talamini R, et al. Improvement of systemic human immunodeficiency virus-related non-Hodgkin lymphoma outcome in the era of highly active antiretroviral therapy. Clin Infect Dis 2003;37:1556-64.
54. Gaidano G, Capello D, Carbone A. The molecular basis of acquired immunodeficiency syndrome-related lymphomagenesis. Semin Oncol 2000;27:431-41.
55. Chotmongkol V, Pesee M. AIDS-related primary central nervous system lymphoma: prolonged remission associated with highly active antiretroviral therapy. J Med Assoc Thai 2002;85:634-7.
56. Smets F, Latinne D, Bazin H, et al. Ratio between Epstein-Barr viral load and anti-Epstein-Barr virus specific T-cell response as a predictive marker of posttransplant lymphoproliferative disease. Transplantation 2002;73:1603-10.
57. Watanabe T, Katayama Y, Yoshino A, Komine C, Yokoyama T, Fukushima T. Long-term remission of primary central nervous system lymphoma by intensified methotrexate chemotherapy. J Neurooncol 2003;63:87-95.
58. Poortmans PMP, Kluin-Nelemans HC, Haaxma-Reiche H, et al. High-Dose Methotrexate-Based Chemotherapy Followed by Consolidating Radiotherapy in Non-AIDS-Related Primary Central Nervous System Lymphoma: European Organization for Research and Treatment of Cancer Lymphoma Group Phase II Trial 20962. J Clin Oncol 2003;21:4483-8.
59. Pels H, Schmidt-Wolf IGH, Glasmacher A, et al. Primary Central Nervous System Lymphoma: Results of a Pilot and Phase II Study of Systemic and Intraventricular Chemotherapy With Deferred Radiotherapy. J Clin Oncol 2003;21:4489-95.
60. Deangelis LM. Current management of primary central nervous system lymphoma. Oncology (Huntingt) 1995;9:63-71; discussion , 5-6, 8.
61. Mead GM, Bleehen NM, Gregor A, et al. A medical research council randomized trial in patients with primary cerebral non-Hodgkin lymphoma: cerebral radiotherapy with and without cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. Cancer 2000;89:1359-70.

62. Deangelis LM, Yahalom J. Primary Central Nervous System Lymphoma. In: Vincent T. Devita J, Hellman S, Rosenberg SA, eds. Cancer Principles & Practice of Oncology. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005:2012-20.
63. Bertino JR. Clinical use of methotrexate--with emphasis on use of high doses. Cancer Treat Rep 1981;65 Suppl 1:131-5.
64. Park DM, Abrey LE. Pharmacotherapy of primary CNS lymphoma. Expert Opin Pharmacother 2002;3:39-49.
65. 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.
66. Shenkier TN, Voss N, Chhanabhai M, et al. The treatment of primary central nervous system lymphoma in 122 immunocompetent patients. Cancer 2005.
67. Sandor V, Stark-Vancs V, Pearson D, et al. Phase II trial of chemotherapy alone for primary CNS and intraocular lymphoma. J Clin Oncol 1998;16:3000-6.
68. Flombaum CD, Meyers PA. High-Dose Leucovorin as Sole Therapy for Methotrexate Toxicity. J Clin Oncol 1999;17:1589-.
69. Boulanger E, Daniel MT, Agbalika F, Oksenhendler E. Combined chemotherapy including high-dose methotrexate in KSHV/HHV8-associated primary effusion lymphoma. Am J Hematol 2003;73:143-8.
70. Jacomet C, Girard PM, Lebrette MG, Farese VL, Monfort L, Rozenbaum W. Intravenous methotrexate for primary central nervous system non- Hodgkin's lymphoma in AIDS. Aids 1997;11:1725-30.
71. Little RF, Wilson WH. Update on the Pathogenesis, Diagnosis, and Therapy of AIDS-related Lymphoma. Curr Infect Dis Rep 2003;5:176-84.
72. Little RF. AIDS-related non-Hodgkin's lymphoma: etiology, epidemiology, and impact of highly active antiretroviral therapy. Leuk Lymphoma 2003;44 Suppl 3:S63-8.
73. Kang EM, De Witte M, Malech H, et al. Gene therapy-based treatment for HIV-positive patients with malignancies. J Hematother Stem Cell Res 2002;11:809-16.
74. Kang EM, de Witte M, Malech H, et al. Nonmyeloablative conditioning followed by transplantation of genetically modified HLA-matched peripheral blood progenitor cells for hematologic malignancies in patients with acquired immunodeficiency syndrome. Blood 2002;99:698-701.
75. Hickey WF. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. Brain Pathol 1991;1:97-105.
76. Wong ET, Tishler R, Barron L, Wu JK. Immunochemotherapy with rituximab and temozolomide for central nervous system lymphomas. Cancer 2004;101:139-45.
77. Pels H, Schulz H, Schlegel U, Engert A. Treatment of CNS lymphoma with the anti-CD20 antibody rituximab: experience with two cases and review of the literature. Onkologie 2003;26:351-4.
78. Pels H, Schulz H, Manzke O, Hom E, Thall A, Engert A. Intraventricular and intravenous treatment of a patient with refractory primary CNS lymphoma using rituximab. J Neurooncol 2002;59:213-6.

79. Chang KL, Flaris N, Hickey WF, Johnson RM, Meyer JS, Weiss LM. Brain lymphomas of immunocompetent and immunocompromised patients: study of the association with Epstein-Barr virus. Mod Pathol 1993;6:427-32.
80. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997;90:2188-95.
81. Rubenstein JL, Combs D, Rosenberg J, et al. Rituximab therapy for CNS lymphomas: targeting the leptomeningeal compartment. Blood 2003;101:466-8.
82. Schulz H, Pels H, Schmidt-Wolf I, Zeelen U, Germing U, Engert A. Intraventricular treatment of relapsed central nervous system lymphoma with the anti-CD20 antibody rituximab. Haematologica 2004;89:753-4.
83. Chow KU, Sommerlad WD, Boehrer S, et al. Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro: role of cytokines, complement, and caspases. Haematologica 2002;87:33-43.
84. Fortin D. [The blood-brain barrier should not be underestimated in neuro-oncology]. Rev Neurol (Paris) 2004;160:523-32.
85. Dietlein M, Pels H, Schulz H, et al. Imaging of central nervous system lymphomas with iodine-123 labeled rituximab. Eur J Haematol 2005;74:348-52.
86. Scheidhauer K, Wolf I, Baumgartl HJ, et al. Biodistribution and kinetics of (131)I-labelled anti-CD20 MAB IDEC-C2B8 (rituximab) in relapsed non-Hodgkin's lymphoma. Eur J Nucl Med Mol Imaging 2002;29:1276-82.
87. O'Brien P, Roos D, Pratt G, et al. Phase II multicenter study of brief single-agent methotrexate followed by irradiation in primary CNS lymphoma. J Clin Oncol 2000;18:519-26.
88. Donelli MG, Zucchetti M, D'Incalci M. Do anticancer agents reach the tumor target in the human brain? Cancer Chemother Pharmacol 1992;30:251-60.
89. Hanel M, Fiedler F, Thorns C. Anti-CD20 monoclonal antibody (Rituximab) and Cidofovir as successful treatment of an EBV-associated lymphoma with CNS involvement. Onkologie 2001;24:491-4.
90. Enting RH, Demopoulos A, DeAngelis LM, Abrey LE. Salvage therapy for primary CNS lymphoma with a combination of rituximab and temozolomide. Neurology 2004;63:901-3.
91. Raizer J, Lisa D, Zelenetz A, Lauren A. Activity of Rituximab in Primary Central Nervous System Lymphoma PCNSL. In: 2000 ASCO Annual Meeting 2000 May 20-23; Orlando; 2000. p. Abstract 642.
92. Hoang-Xuan K, Camilleri-Broet S, Soussain C. Recent advances in primary CNS lymphoma. Curr Opin Oncol 2004;16:601-6.
93. Batara JF, Grossman SA. Primary central nervous system lymphomas. Curr Opin Neurol 2003;16:671-5.
94. Deangelis LM, Hormigo A. Treatment of primary central nervous system lymphoma. Semin Oncol 2004;31:684-92.
95. Kaplan LD, Lee JY, Ambinder RF, et al. Rituximab does not improve clinical outcome in a randomized phase III trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin's lymphoma: AIDS-malignancies consortium trial 010. Blood 2005.

96. Spina M, Jaeger U, Sparano JA, et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin lymphoma: pooled results from 3 phase 2 trials. Blood 2005;105:1891-7.
97. Straus DJ. HIV-associated lymphoma: promising new results, but with toxicity. Blood 2005;105:1842.
98. van Esser JWJ, Niesters HGM, van der Holt B, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood 2002;99:4364-9.
99. Horwitz SM, Tsai D, Twist C, et al. Rituximab is Effective Therapy for Post-Transplant Lymphoproliferative Disorder (PTLD) not Responding to Reduction in Immunosuppression: a Prospective Trial in Adults and Children. In: American Society of Clinical Oncology; 2001 May 12-15; San Francisco; 2001. p. Abstract 1134.
100. Evans AM, Choquet S, Kroll A, et al. Primary Central Nervous System (PCNS) Post-Transplant Lymphoproliferative Disease (PTLD): An International Report of 65 Cases in the Modern Era In: 53rd ASH Annual Meeting. San Diego, CA; 2011:Abstract 879.
101. Uldrick TS, Aleman K, Polizzotto MN, et al. AIDS-associated Primary Central Nervous System Lymphoma (AIDS-PCNSL) Treated with HAART and Radiation-sparing Therapy: The NCI HIV and AIDS Malignancy Branch Experience, 2004-2011. In: 19th Conference on Retroviruses and Opportunistic Infections; 2012 Mar 7; Seattle, WA; 2012.
102. Chaiwatanatorn K, Lee N, Grigg A, Filshie R, Firkin F. Delayed-onset neutropenia associated with rituximab therapy. Br J Haematol 2003;121:913-8.
103. Dunleavy K, Hakim F, Kyung Kim H, et al. B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostasis. Blood 2005.
104. DeAngelis LM, Wong E, Rosenblum M, Furneaux H. Epstein-Barr virus in acquired immune deficiency syndrome (AIDS) and non-AIDS primary central nervous system lymphoma. Cancer 1992;70:1607-11.
105. Bashir R, McManus B, Cunningham C, Weisenburger D, Hochberg F. Detection of Eber-1 RNA in primary brain lymphomas in immunocompetent and immunocompromised patients. J Neurooncol 1994;20:47-53.
106. Cingolani A, De Luca A, Larocca LM, et al. Minimally invasive diagnosis of acquired immunodeficiency syndrome-related primary central nervous system lymphoma. J Natl Cancer Inst 1998;90:364-9.
107. Cinque P, Brytting M, Vago L, et al. Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. Lancet 1993;342:398-401.
108. Antinori A, De Rossi G, Ammassari A, et al. Value of combined approach with thallium-201 single-photon emission computed tomography and Epstein-Barr virus DNA polymerase chain reaction in CSF for the diagnosis of AIDS-related primary CNS lymphoma. J Clin Oncol 1999;17:554-60.

109. Bossolasco S, Cinque P, Ponzoni M, et al. Epstein-Barr virus DNA load in cerebrospinal fluid and plasma of patients with AIDS-related lymphoma. J Neurovirol 2002;8:432-8.
110. Gregor A, Cull A, Traynor E, Stewart M, Lander F, Love S. Neuropsychometric evaluation of long-term survivors of adult brain tumours: relationship with tumour and treatment parameters. Radiother Oncol 1996;41:55-9.
111. Patrick A, Wee A, Hedderman A, Wilson D, Weiss J, Govani M. High-dose intravenous rituximab for multifocal, monomorphic primary central nervous system posttransplant lymphoproliferative disorder. J Neurooncol 2010.
112. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001;19:2153-64.
113. Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 1990;8:1277-80.
114. Juweid ME, Wiseman GA, Vose JM, et al. Response Assessment of Aggressive Non-Hodgkin's Lymphoma by Integrated International Workshop Criteria and Fluorine-18-Fluorodeoxyglucose Positron Emission Tomography. J Clin Oncol 2005;23:4652-61.
115. Abrey LE, Batchelor TT, Ferreri AJ, et al. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. J Clin Oncol 2005;23:5034-43.
116. CDC. HIV/AIDS Surveillance Report. Atlanta: Centers for Disease Control and Prevention; 1996 December. Report No.: Volume 8 No. 2.
117. CDC. HIV/AIDS Surveillance Report. Atlanta: Centers for Disease Control and Prevention; 2003. Report No.: Volume 15.

11 APPENDICES

11.1 APPENDIX 1: FOLSTEIN MINI MENTAL STATUS EXAMINATION

Folstein Mini Mental Status Examination			
Task	**Instructions**	**Scoring**	
Date Orientation	"Tell me the date?" Ask for omitted items.	One point each for year, season, date, day of week, and month	5
Place Orientation	"Where are you?" Ask for omitted items.	One point each for state, county, town, building, and floor or room	5
Register 3 Objects	Name three objects slowly and clearly. Ask the patient to repeat them.	One point for each item correctly repeated	3
Serial Sevens	Ask the patient to count backwards from 100 by 7. Stop after five answers. (Or ask them to spell "world" backwards.)	One point for each correct answer (or letter)	5
Recall 3 Objects	Ask the patient to recall the objects mentioned above.	One point for each item correctly remembered	3
Naming	Point to your watch and ask the patient "what is this?" Repeat with a pencil.	One point for each correct answer	2
Repeating a Phrase	Ask the patient to say "no ifs, ands, or buts."	One point if successful on first try	1
Verbal Commands	Give the patient a plain piece of paper and say "Take this paper in your right hand, fold it in half, and put it on the floor."	One point for each correct action	3
Written Commands	Show the patient a piece of paper with "CLOSE YOUR EYES" printed on it.	One point if the patient's eyes close	1
Writing	Ask the patient to write a sentence.	One point if sentence has a subject, a verb, and makes sense	1
Drawing	 Ask the patient to copy a pair of intersecting pentagons onto a piece of paper.	One point if the figure has ten corners and two intersecting lines	1
Scoring	A score of 24 or above is considered normal.		**30**
Adapted from Folstein et al, Mini Mental State, J PSYCH RES 12:196-198 (1975)			

11.2 Appendix 2: Neurological Function Class

1. Able to work and perform normal activities. Neurological findings minor or absent.
2. Able to carry out normal activities with minimal difficulty. Neurological impairment does not require nursing care or hospitalization.
3. Seriously limited in performing normal activities; requires nursing care or hospitalization. Patient confined to bed or wheelchair or with significant intellectual impairment.
4. Unable to perform even minimal normal activities. Requires hospitalization and/or constant nursing care. Patient unable to communicate or in a coma.

11.3 APPENDIX 3: ON-STUDY EVALUATIONS

Cycle/ Visit	H&P	Ophtho Exam	Toxicity Assess	MMSE	Clin. Labs	Cr. Cl.	Screen	MRI	PET	CSF	HIV/ CD4	EBV	Research Blood	Neuro-psych	Adherence
Cycle 1	X		X	X	X	X		X[2]	X[2]	X[2]	X[2]	X	X		
Cycle 2	X		X	X	X	X			X						
Cycle 3	X		X		X	X		X			X	X	X		
Cycle 4	X		X		X	X									
Cycle 5	X		X		X	X		X			X	X	X		
Cycle 6	X		X		X	X									
Cycle 7	X		X		X	X		X			X	X	X		
Cycle 8	X		X		X	X									
CR by MRI		X[1]							X	X					
End Rx	X	X[1]	X	X	X			X	X	X	X	X	X	X	X
Follow-up															
Q2 mo x3															
Month 2	X				X			X			X	X	X		
Month 4	X				X			X			X	X	X		
Month 6	X				X			X			X	X	X	X	X
Q3 mo x6															
Month 9	X				X			X			X	X	X		
Month 12	X				X			X	X		X	X	X	X	X
Month 15	X				X			X			X	X	X		
Month 18	X				X			X			X	X	X	X	X
Month 21	X				X			X			X	X	X		
Month 24	X				X			X			X	X		X	X
Q6 mo x 2															
Month 30	X				X			X			X	X	X		
Month 36	X				X			X			X	X	X	X	
Q12 mo x2		(Optional Clinical Follow up)													
Month 48	X				X						X				
Month 60	X				X						X				

1. Repeat if abnormal at baseline.

H&P= History and Physical, including ECOG; Ophtho Exam = Ophthalmology exam for orbital involvement; Clin. Labs = CBC with differential and Retic, Chem. 20, PT/PTT, Urine: UA, β-hCG (women); Cr. Cl. = estimated creatinine clearance by Cockcroft-Gault formula (Appendix 4), Serology = Hepatitis and Toxoplasmosis serologies, RPR; CSF = CSF cell count, glucose, protein, EBV, molecular pathology, flow cytometry ; HIV/CD4 = Baseline HIV Serology, genotype if indicated, HIV viral load, and lymphocyte subset TBNK; EBV = EBV quantitative PCR, blood; Neuropsych, See Appendix 5; Adherence, see Appendix 6. BL = Baseline Studies; End Rx = End of therapy, Cycle 8, Day 28 (Month 1 follow-up).

For patients that relapse, re-staging will include: MRI-brain with contrast, CNS FDG-PET, CSF studies, quantitative EBV-PCR, and ophthalmologic examination. See Section 3.8.5.

On-Study Evaluations for Best Available Radiation Sparing Therapy (no Methotrexate)

Cycle/ Visit	H&P	Ophtho Exam	Toxicity Assess	MMSE	Clin. Labs	Cr. Cl.	Serology	MRI	PET	CSF	HIV/ CD4	EBV	Research Blood	Neuro-psych	Adherence
Screen/BL	X	X			X	X	X	X	X	X	X				
Day 1	X		X	X	X	X					X	X	X		
Day 3	X		X		X				X						
Day 5	X		X		X						X	X	X		
Day 8	X		X	X	X										
Day 15	X		X	X	X						X	X	X		
Day 21	X		X	X	X										
CR by MRI		X[1]							X	X					
End Rx	X	X[1]	X	X	X			X	X	X	X	X	X	X	X
Follow-up															
Week 2	X		X		X										
Week 4	X		X		X			X			X	X	X		
Q2 mo x3															
Month 2	X				X			X			X	X	X		
Month 4	X				X			X			X	X	X		
Month 6	X				X			X			X	X	X	X	X
Q3 mo x6															
Month 9	X				X			X			X	X	X		
Month 12	X				X			X	X		X	X	X	X	X
Month 15	X				X			X			X	X	X		
Month 18	X				X			X			X	X	X	X	X
Month 21	X				X			X			X	X	X		
Month 24	X				X			X			X	X		X	X
Q6 mo x 2															
Month 30	X				X			X			X	X	X		
Month 36	X				X			X			X	X	X	X	
Q12 mo x2	(Optional Clinical Follow up)														
Month 48	X				X						X				
Month 60	X				X						X				

1. Repeat if abnormal at baseline.

H&P= History and Physical, including ECOG; Ophtho Exam = Ophthalmology exam for orbital involvement; Clin. Labs = CBC with differential and Retic, Chem. 20, PT/PTT, Urine: UA, β-hCG (women); Cr. Cl. = estimated creatinine clearance by Cockcroft-Gault formula (Appendix 4), Serology = Hepatitis and Toxoplasmosis serologies, RPR; CSF = CSF cell count, glucose, protein, EBV, molecular pathology, flow cytometry ; HIV/CD4 = Baseline HIV Serology, genotype if indicated, HIV viral load, and lymphocyte subset TBNK; EBV = EBV quantitative PCR, blood; Neuropsych, See Appendix 5; Adherence, see Appendix 6. BL = Baseline Studies; End Rx = End of therapy, Day 28 (Week 1 follow-up.)

For patients that relapse, re-staging will include: MRI-brain with contrast, CNS FDG-PET, CSF studies, quantitative EBV-PCR, and ophthalmologic examination.

11.4 APPENDIX 4: CALCULATION OF CREATININE CLEARANCE

I. **Calculation: Cockcroft-Gault formula for Creatinine Clearance (CrCl) estimate**
   A. Male CrCl = (140 - age) x (weight) / (sCr x 72)
   B. Female CrCl = (140 - age) x (weight) x 0.85 / (sCr x 72)
   C. Annotation
      1. Where creatinine clearance is expressed in mL/min
      2. Where age is in years
      3. Where weight is Actual Body Weight in kilograms
      4. Where sCr is Serum Creatinine in mg/dL
   D. Efficacy
      1. As accurate as CrCl measured from a 24-hour urine collection in many cases
      2. Exceptions: See 24-hour CrCl indications below
      3. For estimated creatinine clearance of <100 mL/min, a 24-hour urine collection should be obtained *plus* a serum creatinine at the end of the collection period to determine a measured creatinine clearance (per section 3.4.2.1.1).
   E. Resources: http://www.kidney.org/professionals/

II. **Calculation: Measured Creatinine Clearance from 24-hour urine collection**
   A. CrCl = (uCr x uV) / (sCr x 1440)
   B. Annotation
      1. Where CrCl is Creatinine clearance in mL/min
      2. Where uCr is Urine Creatinine in mg/dL
      3. Where sCr is Serum Creatinine in mg/dL
      4. Where uV is 24-hour urine volume in mL
      5. Where 1440 represents number of minutes in 24 hours
   C. Indications (where calculation above is inaccurate)
      1. Altered protein intake
         a. Vegetarian diet
         b. Creatine Supplementation
      2. Altered muscle mass and muscle injury
         a. Malnutrition, muscle wasting, muscle trauma
         b. Amputation
      3. Females
      4. Unstable renal function (changing serum creatinine)
      5. Obesity

11.5 APPENDIX 5: NEUROPSYCHOLOGICAL TEST BATTERY

At entry into the study, patients will be administered a comprehensive neuropsychological battery of tests. The major domains of functioning to be evaluated for the comprehensive and monitoring assessment batteries are indicated below. The specific tests to be used for the protocol will be selected by the NCI/MICC neuropsychology group. Such tests have been shown to provide an effective assessment of functioning in similar chronically ill populations. The tests do not require complicated instrumentation and can be done with high reliability and validity by trained personnel. Repeated neuropsychological assessments will be administered to the patient according to the schedule specified in the protocol. The monitoring battery, which may be administered at shorter intervals, includes tests less subject to practice effects. The same domains should be assessed using the same test instruments throughout the study.

Comprehensive Assessment

General Cognitive
Language/Verbal
Visual-Perceptual
Processing Speed
Memory (working, immediate and delayed, visual and auditory)
Executive Function
Attention
Fine Motor
Mood/QOL

Monitoring assessment

General Cognitive
Language/Verbal
Visual-Perceptual
Processing Speed
Working memory
Executive Function
Attention
Fine motor
Mood/QOL

--Additional neuropsychological tests may be administered to more comprehensively assess the patients if needed to further explore specific areas of functioning

--The comprehensive assessment is administered at baseline and then annually, whereas the monitoring assessment is administered at the half-year assessments.

11.6 APPENDIX 6: ADHERENCE ASSESSMENT

Baseline, 6, and 12 months	**Estimated Time**
Knowledge Questionnaire	5 minutes
Pills Identification Test	5 minutes
Interview	10 minutes
	20 minutes total
Baseline, 3 and 9 months	
Interview	10 minutes
	10 minutes total

11.7 APPENDIX 7: KNOWLEDGE QUESTIONNAIRE

Hosp #:	Protocol:
Name:	Date:

Please circle the best response for the questions below. Circle *one and only one* response for each question.

1. HIV is a virus that:
   a) can be cured with a vaccine.
   b) cannot be controlled with medication.
   c) can often be controlled with antiretroviral medicine taken the way it is prescribed.
   d) can be controlled with antiretroviral medication in people who have received the vaccine.

2. It is important for people to take HIV medicine the way it is prescribed because this will help the body:
   a) get a higher viral load.
   b) build up resistance to the medicine.
   c) keep a strong immune system.
   d) all of the above.

3. Resistance means:
   a) HIV resists the process of changing into AIDS.
   b) immune cells fight with HIV cells.
   c) HIV learns to grow even with medicine in the body.
   d) HIV is killed by the medicine.

4. A measure of "viral load" tells us:
   a) How many different viruses are in someone's blood.
   b) How many T-cells a person has.
   c) How much HIV is in someone's blood.
   d) What kinds of HIV cells are in someone's saliva.

5. If you run out of HIV medicine, you should:
   a) schedule an appointment at NIH to get more.
   b) call the nurse or doctor right away to get more.
   c) borrow some pills from a friend until you can get more from the pharmacy.
   d) both B and C are correct.

6. The best thing to have is:
   a) high viral load and low T-cells.
   b) low viral load and low T-cells.
   c) high viral load and high T-cells.
   d) low viral load and high T-cells.

7. The <u>best</u> way to fight HIV is:
   a) take HIV medicines the way they are prescribed.
   b) eat healthy foods.
   c) get the HIV vaccine.
   d) exercise every day.

8. Which of these statements is NOT true?
   a) A person can only be resistant to one medicine at a time.
   b) Doctors can do a test to see which HIV medicines work best for a person.
   c) If you become resistant to a medicine, the medicine probably won't work for you.
   d) There are many different types or "strains" of HIV.

9. If a person doesn't take his medicine every day,
   a) the medicine will work better.
   b) his red blood cells will be destroyed.
   c) his T-cells will become resistant to the medicine.
   d) the HIV will become resistant to the medicine.

10. If a person skips doses of her HIV medicine,
    a) her viral load will probably go to zero.
    b) she will probably gain weight.
    c) her CD4 cells will probably double.
    d) her body could become resistant to the medicine.

11.8 APPENDIX 8: RECRUITMENT ANNOUNCEMENT

Clinical Trial for AIDS-related Primary Central Nervous System Lymphoma
HIV and AIDS Malignancy Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Individuals with AIDS-related primary central nervous system (brain) lymphoma are invited to participate in a clinical trial designed to study the feasibility of treating this cancer without using the radiation therapy approaches traditionally used in this setting. Patients are invited to contact the NCI if they are HIV-infected and have a brain mass, even if the diagnosis for the cause of the brain mass has not been made. In certain circumstances, the diagnostic procedures may also be done at the National Institutes of Health.

AIDS-related primary brain lymphoma is a serious and life threatening complication of HIV infection. Few individuals with this cancer survive for one year. Radiation therapy to the brain is the treatment commonly administered. Radiation therapy can make the lymphoma go away. However, many patients have recurrence of the lymphoma after radiation therapy, or develop other AIDS complications that limit survival. With the availability of highly active antiretroviral therapy, many individuals with AIDS-related primary brain lymphoma may be able to control the HIV infection long-term and go on to good health if the lymphoma can be cured. Many not HIV-infected people treated with radiation therapy for brain lymphoma survive several years, but have severe brain problems related to the radiation treatment. Therefore, in not HIV-infected people, chemotherapy has become the standard of care for treating this cancer. Survival with a healthy brain in such people is improved with this strategy. This clinical trial will assess whether chemotherapy will lead to several years or longer survival without brain problems in people with AIDS-related primary brain lymphoma.

The treatment being studied in this experimental trial is to give a combination of high-dose methotrexate, leucovorin, rituximab, and highly active antiretroviral therapy. This treatment is designed to treat the lymphoma without causing additional toxicity to the CD4+ lymphocytes. CD4+ lymphocyte recovery is essential for the long-term survival prospects of people with advanced AIDS. High-dose methotrexate is the core of most treatments for this tumor in HIV-unrelated primary brain lymphoma and it is used in that setting in conjunction with other medications. When given with leucovorin, high-dose methotrexate can usually be given without causing the expected toxicity to the bone marrow and to the immune system. Rituximab is being given because it is a monoclonal antibody that targets the lymphoma cells and helps the methotrexate kill the cancer cells. Since rituximab does not attach to the CD4+ lymphocytes, it may be possible to give methotrexate, leucovorin, and rituximab along with highly active antiretroviral therapy and simultaneously treat the cancer and the HIV. Some patients may not be able to receive high-dose methotrexate due to problems with their kidneys or heart. In such cases, treatment may still be possible, and will generally include rituximab and antiretroviral therapy. It may be possible to commence immune system recovery during

the lymphoma therapy. Immune system recovery may be essential not only to prevention of other AIDS complications, but also to prevention of recurrent brain lymphoma.

The study is designed to test the ability of this treatment to make the cancer go away, to see the extent of immune recovery that is feasible with the treatment, and to see whether the cancer comes back or not. Also, a series of tests will be done to measure the effects of this therapy on brain function, to see whether this therapy is less toxic to the brain than would be potentially expected with radiation therapy.

Frequent visits and prolonged stays at the NIH may be necessary since this disease is a very serious condition causing many complications. Each treatment will require a week or longer in the hospital. The treatments will be repeated up to 8 times depending on how well it is tolerated, and the effects on the tumor. All of the diagnostic tests and medical treatments administered at the National Institutes of Health are free of charge. If the cancer goes away, treatment for HIV may continue for up to three years while additional tests on your immune system and brain function are made.

Transportation costs to the NIH will be paid for those who do not live close to the NIH campus.

For further information about coming to the NIH for consultation and possible enrollment into this clinical trial, contact Dr. Robert Yarchoan at 240-760-6075, or 1.800.243.2732, and then hit 4.

11.9 APPENDIX 9: RITUXIMAB COMBINED WITH HIGH-DOSE METHOTREXATE WITH LEUCOVORIN RESCUE HAART AND CHEMOTHERAPY ADMINISTRATION, METHOTREXATE MONITORING AND SUPPORTIVE CARE GUIDELINES

Baseline/Admission:

1. Determine HAART regimen. Tenofovir sparing regimens preferred due to potential overlapping renal toxicity. Pharmacogenetic testing for abacavir; check HLA B*5701
2. PCP prophylaxis; monthly pentamidine or atovaquone preferred
3. MAC prophylaxis: azithromycin 1200 mg /m^2 weekly for CD4 < 100 cells/microL
4. HSV prophylaxis: Valacyclovir 1000 mg/daily
5. Evaluate for thrush daily
6. Check Cockcroft-Gault estimate of creatinine clearance. If < 100mL/min, order 24 hour urine collection for creatinine clearance
7. Daily weights

Day -1 Each Cycle:

1. Check Cockcroft-Gault estimate of Creatinine clearance. If < 100mL/min, order 24-hour creatinine clearance.
2. Confirm at least 7 days serum methotrexate ≤ 0.05 mcmol/L from previous cycle to next dose of methotrexate.
3. Evaluate for possible methotrexate dose adjustments based on toxicities from previous cycle.

Day 1 of each cycle:

1. Review all concomitantmedications prior to day 1 of each cycle. Discontinue any drug that would have interaction with methotrexate excretion. See list below. No filgrastim within 24 hours before methotrexate administration.
2. Rituximab 375 mg/m^2
3. Start IV fluid hydration by 18:00, using one of the protocol specified alkalinized IVF order sets in CRIS for 06-C-0051
4. Order q6 hour urine pH ≥ 7.0 (00:00, 6:00, 12:00, 18:00) and **STRICT URINE OUTPUT** monitoring record urine output every hour
5. Draw the following blood work until methotrexate level is < or equal to 0.05 mcmol/L:
    a. Hepatic panel every other day
    b. CBC/Diff, Chem 7 daily

Day 2:

1. 7 am: notify MD of urine pH and hourly UOP. If UOP < 100mL/hour or urine pH <7 or >8, adjust IVF.
2. Methotrexate 6000 mg/m^2. Order within the 06-C-0051 CRIS order set. Include Sodium Bicarbonate 45mEq as an additive. (May be adjusted between 25-75mEq depending on individual patient needs).
    a. For creatinine clearance 60-100 mL, adjust methotrexate dose using formula: (Creatinine Clearance)/100 x 6000 mg/m^2
    b. For creatinine clearance < 60ml/min, methotrexate **will <u>NOT</u> be administered**.
    c. If episodic AST/ALT elevation > 10X upper limit normal on previous cycle, decrease methotrexate by 25%
3. Goal Start time: administer methotrexate starting between 9:00-10:00 am

Day 3-End Leucovorin Rescue:

1. Start leucovorin 100 mg/m^2 IV q6 hours starting 24 hours **AFTER <u>INITIATION</u>** of methotrexate administration.
2. Serum methotrexate monitoring levels is now performed at Children's Hospital 7 days a week at 2 p.m. Specimens collected for methotrexate monitoring must be delivered to the NIH CC Department of Laboratory Medicine's Chemistry Service in the morning so they can be delivered to Washington's Children's Hospital and processed in a timely manner. This process is designed to obtain same day results for patients on 06-C-0051 so the clinical team can make protocol defined treatment decisions, such as stopping leucovorin or IV fluids, or implementing treatment of delayed methotrexate clearance with glucarpidase (Voraxaze®).
3. 24 hours **AFTER <u>INITIATION</u>** of methotrexate, draw red top tube for serum methotrexate level. Methotrexate Levels need to be **drawn at scheduled time.**
4. Repeat every 24 hours: 48 hr, 72 hr, 96 hr, until methotrexate monitoring no longer required.
5. Check methotrexate levels same day, evaluate for delayed methotrexate clearance. Notify Research team for:
    a. Methotrexate > 10 mcmol/L at 24 hours
    b. Methotrexate > 1 mcmol/L at 48 hours
    c. Methotrexate > 0.1 mcmol /L at 72/hours
    d. For **delayed methotrexate** elimination see pages 26 and 27 3.4.3), Table 1 (Leucovorin dose at 24, 48, 60 and 72 hours), and 3.4.4 for use of glucarpidase guidelines.

6. Stop **intravenous** leucovorin and switch to oral leucovorin 25 mg PO (Alternative 10 mg/m^2 PO) q6 hours
    a. If methotrexate level is <0.5 mcmol/L, see section 3.3.4.3.2
    b. Patient does <u>NOT</u> have delayed methotrexate clearance (Defined, Point 5 above.)
    c. Discuss discontinuing IVF research team

7. Stop **oral** leucovorin and urine monitoring once serum methotrexate level is ≤ 0.05 mcmol/L

Medications to avoid during Methotrexate administration:

1. Asparaginase
2. Aspirin
3. Acidic drugs
4. Amoxicillin
5. Cephalosporins
6. Chloramphenicol
7. Cholestyramine
8. Cotrimoxazole
9. Cyclosporine
10. Diclofenac
11. Ibuprofen
12. Indomethacin
13. Ketoprofen
14. Mercaptopurine
15. Mezlocillin
16. Naproxen
17. Non-steroidal anti-inflammatory drugs
18. Omeprazole
19. Penicillins
20. Piperacillin (±tazobactam)
21. Probenecid
22. Proton pump inhibitors (e.g., dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole)
23. Pyrimethamine
24. Salicylates
25. Sulfonamides
26. Sulindac
27. Tetracyclines
28. Tolmetin
29. Trimethoprim
30. Trimethoprim/sulfamethoxazole
31. Vaccines, Live (viral and bacterial)