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Phase II Study of Chemotherapy (Doxorubicin, Methotrexate and Leucovorin) in Combination with Antiviral-Based Therapy (Zidovudine + Hydroxyurea) for AIDS,Immunocompromised, or Immunocompetent Patients with Relapsed or CNS Positive Epstein Barr Virus Associated Lymphoma

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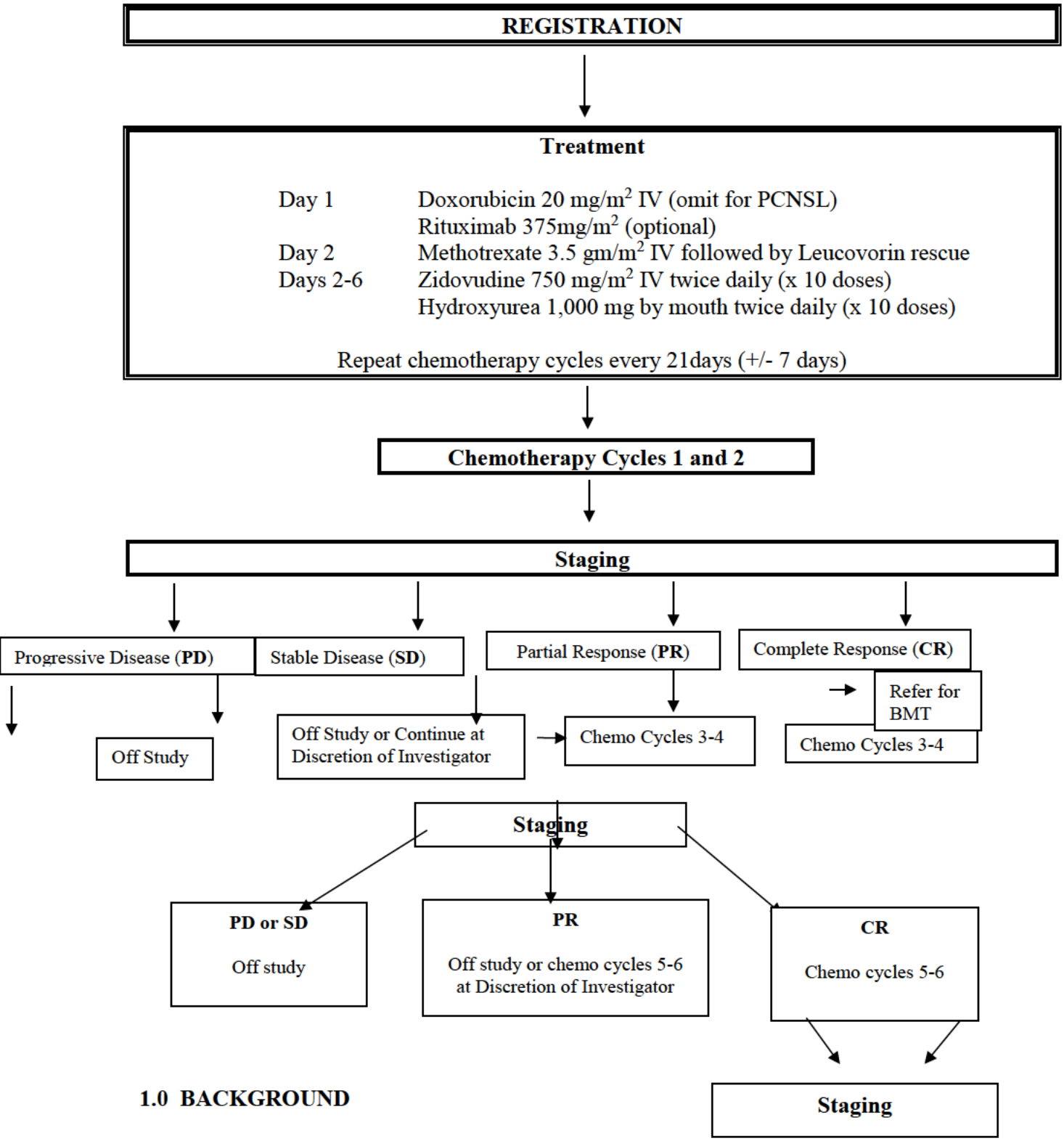
STUDY SCHEMA



1.0 BACKGROUND

1.1 Study Disease

The epidemiology and pathogenesis of Hodgkin and aggressive non-Hodgkin's lymphoma (NHL) is quite variable. In developing nations and immune compromised populations, the majority of these tumors are associated with Epstein Barr Virus (EBV). Although these NHLs share some histologic and molecular features with those that occur in the non-immune compromised, there are clear distinctions (1-3). In the U.S., a large percentage of aggressive lymphomas in immunocompromised patients are associated with EBV. This is true for both Acquired Immunodeficiency Syndrome (AIDS) associated and post-transplant lymphomas (4, 5). Recent studies have indicated that a substantial genetic heterogeneity in EBV gene expression exists among both Burkitts type and Large Cell (Immunoblastic) variants of NHL (8). Some interesting reports on the biology of EBV associated lymphomas suggest that in some cells in these tumors the herpes virus is not strictly latent (10). An important association was made between the presence of lytic transcripts or proteins and favorable response to chemotherapy in African patients with endemic BL (10). It is likely that treatment with chemotherapy or Zidovudine (ZDV) might enhance the expression of EBV genes and subsequent response especially when these approaches are used together (11).

A principal hypothesis that is central to our trial is that high-grade lymphomas associated with gamma herpesviruses appear to be dependent on constitutive expression of the promiscuous transcription factor nuclear factor kappa B (NF-κB) (16). Hodgkin and Reed-Sternberg (HRS) cells also highly express this transcription factor. Inhibition of NF-κB results in apoptosis of these tumors (17). In addition, NF-κB appears to play an important role in maintaining viral latency (18). Pharmacologic induction of EBV from latency can render virus-infected lymphomas susceptible to antiviral nucleoside analogs, such as ganciclovir (GCV), and ZDV, which can be readily phosphorylated by the viral-encoded kinases and serve as highly specific therapeutic agents (19, 20). We have found that ZDV potently induces apoptosis in primary EBV+ BL lines (12). There is precedent for ZDV as an agent with activity in viral lymphomas. ZDV and Interferon alpha have activity in a viral associated malignancy (Human T cell Lymphotropic Virus Type I [HTLV-I]) adult T cell leukemia (21). ZDV exhibits marked activity in certain EBV associated lymphomas. We have recently demonstrated that ZDV inhibits NF-κB and induces the EBV lytic cycle (12). ZDV is phosphorylated by the EBV thymidine kinase (EBV TK), a lytic protein. Therefore, ZDV is unique in its ability to both induce (EBV) and kill EBV positive BL cells (22). Other investigators have demonstrated that the conventional chemotherapeutic agents that we will use in this protocol (doxorubicin, methotrexate) also induce the EBV lytic program (11, 20). Our objective is to develop therapies for aggressive EBV+ lymphomas with agents that can be easily administered especially in immunocompromised patients. Hydroxyurea enhances phosphorylation of ZDV through inhibition of ribonucleotide reductase and methotrexate markedly enhances the phosphorylation of ZDV through the inhibition of thymidylate synthesis (23). Since we have demonstrated that ZDV inhibits NF-κB and induces the viral lytic cycle we hypothesize that this combination of doxorubicin/methotrexate/ZDV/hydroxyurea will be a unique targeted therapy for these poor prognosis EBV positive lymphomas. We recently reported a retrospective analysis of 19 patients with aggressive EBV+ non-Hodgkin's lymphoma, including 9 cases of AIDS-associated primary central nervous

system lymphoma (AIDS-PCNSL) treated with ZDV-based chemotherapy (24). Our results demonstrate that high-dose ZDV-methotrexate is efficacious in treating highly aggressive systemic EBV+ lymphomas in the upfront setting. In primary EBV+ lymphoma cell lines, the combination of ZDV with hydroxyurea resulted in synergistic EBV lytic induction and cell death. Further, ZDV-hydroxyurea treatment resulted in dramatic responses in patients with AIDS-PCNSL. The combination of ZDV with chemotherapy, especially lytic-inducing agents, should be explored further in clinical trials for the treatment of EBV-related lymphomas. Both ZDV and methotrexate have excellent CNS penetration thus making the combination of these drugs ideal for the treatment of lymphomas involving CNS and sanctuary areas.

1.2 Rationale

By combining a variety of agents that potentiate ZDV we hope to induce remission in aggressive EBV-associated lymphomas such as those occurring under immunosuppression (i.e. post-transplant) and in the setting of HIV/AIDS. Most therapies for aggressive B cell lymphomas are based upon intensive chemotherapeutic regimens, expensive modalities (bone marrow transplant, Rituximab), or experimental approaches (gene therapy, cytotoxic T cell infusion) that are difficult to implement in heavily pre-treated and immunocompromised patients. Therapy outcome for HIV-associated PCNSL and relapsed aggressive B cell lymphomas is very poor. Even curable lymphomas such as Burkitt Hodgkin lymphoma, and NHL, especially in the HIV setting, are extremely difficult to treat in relapse and/or after stem cell transplant failure. We propose a novel therapeutic approach that exploits the presence of EBV in lymphomas; antiviral mediated disruption of viral latency, resulting in EBV reactivation and drug phosphorylation by endogenous EBV kinases followed by cell death. This study was originally designed to show efficacy in relapsed aggressive EBV-associated lymphomas. However, cooperative groups generally exclude patients with central nervous system (CNS) involvement from clinical trials. We have expanded eligibility to include patients with untreated EBV+ CNS lymphomas in the upfront setting.

2.0 OBJECTIVES

2.1 Primary Objectives

The primary objective of this phase II study is to determine efficacy (complete response rate) of patients with EBV+ lymphomas presenting with CNS involvement or systemic relapse treated with high dose parenteral zidovudine (ZDV), oral hydroxyurea and combination chemotherapy with doxorubicin, methotrexate (MTX), and leucovorin.

2.2 Secondary Objectives

2.2.1 Evaluate 1-year overall and failure-free survival of this treatment.
2.2.2 Assess the toxicity of this treatment regimen.
2.2.3 Assess the effect of this treatment on HIV viral load and T-cell subsets
2.2.4 Measure EBV viral load in peripheral blood before, after treatment, and during

surveillance in order to correlate the presence of with tumor load and disease status.

2.2.5 Measure EBV reactivation in circulating peripheral blood memory B-cells before and after treatment with chemotherapy/ZDV in order to assess the drug effect on EBV latency.

2.2.6 Determine baseline tumor EBV gene expression profile to assess viral thymidine kinases. (BXLF1/vTK and BGLF4/PK), EBV latency pattern (I, II or III) and lytic phase.

2.2.7 Measure immune activation markers and inflammation in peripheral blood in response to treatment and EBV reactivation.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Any stage, histologically or cytologically documented intermediate to high grade relapsed or refractory EBV+ non-Hodgkin's or Hodgkin's lymphoma, or any treated or untreated patients with EBV+ lymphoma involving CNS. Patients with relapsed or refractory monomorphic (monoclonal) post-transplant lymphoproliferative disease (PTLD) are also eligible.

3.1.2 Patients who are HIV+ or negative. Documentation of HIV infection can be done at any time prior to study entry. Documentation may be serologic (positive ELISA and positive Western blot), molecular (positive HIV viral RNA), or other federally approved licensed HIV test. Prior documentation of HIV seropositivity is acceptable.

3.1.3 Tumors must be positive for EBV. This may be done either by EBER stain on the original tumor or the biopsy of relapsed disease (if performed). Biopsy of relapsed disease is desirable but not mandatory. If stains for LMP-1 done outside are positive, EBER does not need to be done.

3.1.4 All patients, except those who have CNS involvement, must have relapsed or progressed from at least one previous chemotherapy based regimen.

3.1.5 Measurable or non-measurable tumor parameter(s). Non-measurable tumor parameter(s) is defined as not having bi-dimensional measurements (e.g., gastric or marrow involvement), but can be followed for response by other diagnostic tests such as gallium scan, PET imaging and/or bone marrow biopsy.

3.1.6 Age ≥ 18 years.

3.1.7 Karnofsky performance status (KPS) ≥ 50%/Eastern Cooperative Oncology Group (ECOG) Performance Score 0, 1, 2 (See Appendix I).

3.1.7 Patients must have adequate end organ and bone marrow function as defined below:

3.1.7.1 Absolute neutrophil count ≥ 1,500 cells/mm^3 and platelets ≥ 75,000 cells/dL unless cytopenias are secondary to lymphomatous involvement of bone marrow or due to HIV-related thrombocytopenia. All patients must be off colony stimulating factor therapy at least 24 hours prior to institution of Cycle 1 chemotherapy.

3.1.7.2 Adequate hepatic function: Serum glutamic-oxaloacetic transaminase (SGOT) ≤ 5 times the upper limit of normal. Total bilirubin ≤ 2.0 mg/dL (unless elevated secondary to lymphomatous involvement of liver or

biliary system or due to other HIV medications [e.g., indinavir, tenofavir or atazanavir]). Patients who are negative for Hepatitis B, or if infected with Hepatitis B, receiving anti-Hepatitis B therapy are eligible. All subjects will be required to be screened for Hepatitis B and C. Per IDSA and AASD guidelines, those subjects that show no immunity, defined by the lack of Hepatitis B surface antibody, and show evidence of chronic infection (i.e. HBsAg+, HBcore+, HBsAB-) will be required to be on anti-Hepatitis B therapy, during the study, in order to be eligible. Patients will be permitted to enroll in the study provided liver function tests meet criteria listed under Section 3.1.7.2 above, and there is no evidence of cirrhosis. The exact Hepatitis B therapy will be at the discretion of the infection disease specialist or investigator. However all patients who present with acute hepatitis B or show normal transaminases and are HBsAg+ and IgM+ for Hepatitis core antigen will not be eligible for trial enrollment. Subjects who are Hepatitis C antibody positive, with or without a positive Hepatitis C RNA level, will be permitted to enroll in the study provided liver function tests meet criteria listed under 3.1.7.2 above, and have no evidence of cirrhosis. Patients diagnosed with Hepatitis C less than 6 months from trial enrollment, will be considered to have Acute Hepatitis C and will be excluded from study unless Hep C viral load is undetectable.

3.1.7.3 Creatinine $\leq$ 2.0 mg/dL or creatinine clearance $\geq$ 60 mL/min unless due to renal involvement by lymphoma.

3.1.8 Concurrent radiation, with or without steroids, for emergency conditions secondary to lymphoma (CNS tumor, cord compression, etc.) will be permitted.

3.1.9 Females with childbearing potential must have a negative serum pregnancy test within 7 days prior of entering into the study. Men and women must agree to use adequate birth control if conception is possible during the study. Women must avoid pregnancy and men avoid fathering children while in the study and for 6 months following the last study drug treatment.

3.1.10 Able to give consent.

3.1.11 Patients already receiving erythropoietin or G-CSF are eligible, although G-CSF therapy must be discontinued at least 24 hours prior to receiving chemotherapy.

3.1.12 The maximum cumulative dose of doxorubicin allowed is 450 mg/m^2. Patients who have previously received doxorubicin with a cumulative dose of 350 mg/m^2 or greater are eligible but MAY NOT receive doxorubicin under protocol.

3.1 Exclusion Criteria

3.2.1 Concurrent active malignancies, with the exception of *in situ* carcinoma of the cervix, non-metastatic, non-melanomatous skin cancer, or Kaposi sarcoma not requiring systemic chemotherapy.

3.2.2 Myocardial infarction (MI) within 6 months prior to study entry, New York Heart Association (NYHA) Class II or greater heart failure, uncontrolled angina, severe, uncontrolled ventricular arrhythmias, clinically significant pericardial disease, or

electrocardiograph evidence of acute ischemic or active conduction system abnormalities.

3.2.3 Left Ventricular Ejection Fraction (LVEF) that is less than the lower institutional limits of normal as assessed by Multiple Gated Acquisition (MUGA) scan or echocardiogram within 6 weeks prior to registration.

3.2.4 Subjects with viral hepatitis who do not meet the criteria listed on 3.1.7.2 will be not be eligible. All patients who present with acute hepatitis B including those with normal transaminases who are HBsAg+ and IgM + for hepatitis core antigen will not be eligible. Subjects who are Hepatitis B core antibody positive are eligible only if they start or are on prophylactic therapy. A hepatitis B viral load should be confirmed negative on all patients who are hepatitis B core antibody positive, but hepatitis B antigen negative. Patients refusing to take any anti-hepatitis B therapy during study will also be excluded. Patients diagnosed with Hepatitis C are eligible if they meet criteria listed on 3.1.7.2.

3.2.5 Psychological, familial, sociological or geographical conditions that do not permit treatment and/or medical follow-up required to comply with the study protocol.

3.2.6 Patients may not be receiving any other investigational agents.

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Patients with mycobacterium avium will not be excluded.

3.2.8 Pregnant or breast-feeding women.

3.2 Enrollment Procedures

To enroll a patient, the study team will follow the University of Miami Sylvester Comprehensive Cancer Center Subject Enrollment Requirements SOP.

4.0 TREATMENT PLAN

Treatment will be administered on an inpatient basis. Treatment cycles will be given every 21days (+/- 1 week), depending upon recovery from any hematologic or non-hematologic toxicity as specified in Section 6.0. A treatment cycle is defined below. Each day will be calculated as 24 hours day +/- 4 hours.

4.1 Agent Administration

4.1.1 Doxorubicin 20 mg/m^2 intravenously will be administered on Day 1 in patients with systemic (non-primary CNS) lymphoma as per institutional guidelines at a standard rate of 2mg per minutes/maximum infusion time 1mg per minute. For bilirubin ≥3.0 mg/dL due to hepatic involvement, the initial dose will be decreased by 50%. The maximum cumulative dose of doxorubicin allowed is 450 mg/m^2. Patients who have previously received doxorubicin with a cumulative dose of 350 mg/m^2 or greater are eligible but MAY NOT receive doxorubicin under protocol.

4.1.2 Rituximab 375 mg/m^2 will be administered on Day 1 in subjects who have CD20+ lymphoma provided baseline CD4 count is at least 50 cells/mm^3 unless investigator

feels adding this drug will not be beneficial after prior use and failure.

4.1.3 Urine alkalinization will begin on Day 1 with normal ½ saline or 5% dextrose, plus sodium bicarbonate.

4.1.4 On Day 2 when urine output is ≥ 150 mL/hr for approximately four consecutive hours, urine pH is ≥ 7.0 and after at least 1 liter of fluids, give Methotrexate 5 gm/m^2 IV in 1000 mL 0.9% sodium chloride over approximately 4 hours x 1 dose only. NOTE: Methotrexate may be omitted during the first cycle of treatment in patients felt to be at risk for severe toxicity due to debilitated condition, renal function, or in post-transplant setting, and can be added in subsequent cycles at the discretion of the investigator.

4.1.5 Leucovorin 10 mg/m^2 IV over 15 minutes-2 hours every 6 hours for at least 10 doses (see Section 4.1.8) – start approximately 24 hours after the BEGINNING of the methotrexate infusion.

4.1.6 Methotrexate level will be obtained approximately 48 hours after the END of the methotrexate infusion and the Leucovorin dose adjusted accordingly.

4.1.7 Zidovudine 750 mg/m^2 IV over approximately 1 hour twice daily will begin on Day 2 (after methotrexate) and continue for a total of 10 doses. (see Section 4.1.8)

4.1.8 Hydroxyurea 1000 mg by mouth twice daily will begin on Day 2 and continue for a total of 10 doses. (see Section 4.1.8)

4.1.9 Once methotrexate level is normal AND the subject has received 5 doses of intravenous zidovudine, the remaining doses of leucovorin and zidovudine may be given orally and the subject discharged from the hospital at the discretion of the investigator. Subject will be provided with a drug diary to record the remaining doses to be taken at home (APPENDIX VII).

4.1.8.1 Leucovorin 25 mg every 6 hours for at least a total of 10 doses. The number of doses beyond 10 will be prescribed at the discretion of the investigator.

4.1.8.2 Zidovudine 1200 mg orally twice daily for a total of 10 doses.

4.1.8.3 Hydroxyurea 1,000 mg orally twice daily will be given for a total of 10 doses.

4.2 Concurrent Medication

4.2.1 Prevention of herpetic reactivation and EBV viremia: Oral Valganciclovir 450 mg twice daily x 10 doses along zidovudine.

4.2.2 Central Nervous System Treatment and Prophylaxis: Patients without CNS involvement may receive intrathecal chemotherapy as prophylaxis at the discretion of the investigator or treating physician. If lymphomatous CNS involvement is confirmed in cereprospinal fluid (CSF), these subjects must receive aggressive IT chemotherapy per institutional standards. Although the specific regimen may vary at each center, a suggested regimen is listed below:

- Cytarabine 50 mg
- Methotrexate 12 mg
- Hydrocortisone 50 mg

Intrathecally administer twice a week until CSF is negative, then weekly x 1 month and then monthly x 6 months.

4.2.3 Prophylaxis against *Pneumocystis carinii* is required in HIV+ subjects, with the specific regimen at the discretion of the Investigator. Recommended options include trimethoprim-sulfamethoxazole (160-800 mg Mondays, Wednesdays, Fridays), dapsone 100 mg daily, or atovoquone 1500 mg with food once daily).

4.2.4 Quinolone antibacterial prophylaxis in JHIV+ subjects: Patients with a CD4 count of $< 100/mm^3$ at baseline or whose CD4 count decreases below $100/mm^3$ during therapy are required to receive quinolone prophylaxis. Begin quinolone prophylaxis during each cycle no later than Day 8 of chemotherapy and continue until documented recovery from neutropenia (ANC $\geq 1000/mm^3$). If subjects are allergic or intolerant of quinolones an alternative antibiotic will be used.

4.2.5 Antiretroviral therapy (HAART): HIV+ subjects on an antiretroviral regimen should be receiving treatment that is in accordance with the current International AIDS Society guidelines. The specific agents are at the discretion of the Investigator and use of agents currently available on an expanded access basis is allowed, but the use of experimental antiretroviral agents or those containing zidovudine (including Combivir® and Trizivir®) are prohibited. Changes to HAART therapy may be made if medically necessary (toxicity, failure of regimen, etc.). **Antiretroviral naïve subjects:** Subjects who are not on HAART at study entry must begin therapy **AFTER** Cycle 1 of chemotherapy has been completed. Changes to HAART therapy may be made if medically necessary (toxicity, failure of regimen, etc.).

4.2.6 Prophylaxis against other common opportunistic infection (OI) is encouraged in HIV+ subjects, dependent upon the subject's CD4 cell count at study entry, and at the discretion of the Investigator. Topical and/or antifungal agents are permitted. Prophylaxis against mycobacterium avium is recommended if CD4 cells are $< 100/mm^3$.

4.2.7 Prevention of tumor lysis syndrome (Cycle 1 only): For patients with evidence of high tumor burden (bone marrow involvement, LDH > twice the upper limit of normal) it is recommended subjects receive allopurinol 300 mg 24 hours prior to the initiation of chemotherapy followed by a minimum of 300 mg daily for at least the first seven treatment days. It is also recommended that appropriate IV hydration be maintained and serum electrolytes, BUN, phosphorous, creatinine, calcium and uric acid be monitored closely. Allopurinol dosing may be adjusted and electrolytes replaced or corrected at the discretion of the treating physician.

4.2.8 All antibiotics may be administered as clinically indicated.

4.2.9 Topical and/or antifungal agents are permitted.

4.2.10 Growth factors may be used as clinically indicated.

4.2.11 Growth Factor (GF) therapy with G-CSF, GM-CSF, or pegfilgrastim will be used in all subjects after each chemotherapy cycle, beginning at least 48 hours after completion of methotrexate.

4.2.12 Nausea and Vomiting: Nausea and vomiting should be managed according to standard practice as outlined in the published American Society of Clinical Oncology (ASCO) document "Preventing and Treating Nausea and Vomiting

Caused by Cancer Treatment." Briefly, antiemetic agents including, but not limited to, 5HT-3 antagonists, aprepitant, lorazepam, diphenhydramine, or phenothiazines may be considered.

4.3 Drug Treatment Schedule

4.3.1 Chemotherapy cycles will be repeated every 21days (+/- 1 week) depending upon blood counts and recovery from other toxicity. Chemotherapy cycles will be given every 21days (+/- 1 week) as long as the following criteria are met:

- ANC $\geq 1000/mm^3$
- Platelet count $\geq 75{,}000/mm^3$

4.4 Duration of Therapy

4.4.1 Patients will be evaluated for response after the second, fourth and sixth cycles (if given) of therapy. Patients will receive two cycles of therapy beyond initially documenting a CR. A minimum of 4 and a maximum of 6 cycles of therapy will be given. Patients with PR after cycles 2 and 4, who remain on study at the investigator's discretion, will receive a maximum of 6 cycles of therapy.

4.4.2 Patients with stable disease (SD) after 2 cycles may remain on study or be removed from study at the discretion of the investigator. Patients with SD after 4 cycles will be removed from the study.

4.4.3 If CR is achieved after cycle 2, the patient will be referred for stem cell transplant evaluation to begin search for stem cell donor at the discretion of the investigator also receive up to 2 additional cycles of chemotherapy. Patients who are not candidate will be followed as per Section 5.3 once they have completed cycles 3 and 4 of chemotherapy.

5.0 Clinical and Laboratory Evaluations

5.1 Baseline/Pretreatment Evaluation (Appendix II)

5.1.1 Complete medical history within 4 weeks prior to enrollment unless otherwise indicated.

5.1.1.1 Date of initial and/or relapsed diagnosis of lymphoma. A copy of the pathology report must be available in the medical record. Unstained slides must be submitted for pathology correlative studies (see Appendix V)

5.1.1.2 Documentation of the presence or absence of "B" symptoms (unexplained fevers, night sweats, involuntary weight loss > 10% of normal body weight).

5.1.1.3 History of other symptoms related to non-Hodgkin's lymphoma.

5.1.1.4 History of drug allergies.

5.1.1.5 Current medication list.

5.1.1.6 Previous therapy received for this lymphoma, including total cumulative dose of previously received Doxorubicin (if applicable).

5.1.2 Complete physical examination includes: performance status score (Appendix I), vital signs, weight, height, body surface area, neurologic examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical examination.

5.1.3 **Laboratory and other tests within 7 days prior to enrollment unless otherwise indicated** :

5.1.3.1 Hematology: CBC, differential, and platelet count.
5.1.3.2 Serum electrolytes, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), LDH, total protein, albumin, calcium, phosphorous, AST, ALT, uric acid.
5.1.3.3 Assessment for Hepatitis C antibody, Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis B surface antigen (HBsAg). A baseline Hepatitis B or C viral load should be obtained on all patients who are Hepatitis B core antibody positive, Hepatitis B antigen positive, and/or Hepatitis C positive respectively.
5.1.3.4 T-cell subsets (in HIV + patients only)
5.1.3.5 HIV viral load (in HIV + patients only)
5.1.3.6 Serum pregnancy test where indicated within **7 days of treatment initiation** and at any time during protocol therapy if pregnancy is suspected.
5.1.3.7 EKG (within 14 days) prior to enrollment.
5.1.3.8 Determination of LVEF by MUGA scan or echocardiogram (within 6 weeks of enrollment). Patients will not be enrolled if LVEF is below institutional limits.
5.1.3.9 EBV viral load (Two 7 ml green top tubes) (See Appendix III).
5.1.3.10 Measurement of EBV reactivation in PBMCs (Two 10 ml yellow top tubes) (See Appendix IV). This will be done at baseline (Day 0 or -1) prior to starting treatment under protocol.
5.1.3.11 Measurement of immune activation markers and inflammation associated cytokines in peripheral blood (one 10 ml purple top tubes with EDTA or equivalent). This will be done at baseline (Day 0 or -1) prior to starting treatment under protocol. (See Appendix VIII).

5.1.4 Staging Evaluation: The following studies will be done for baseline evaluation of the extent of disease within 28 days of treatment initiation.

5.1.4.1 CT scans of the brain, neck (if involved by adenopathy), chest, abdomen, and pelvis with contrast unless contraindicated, in which case nuclear imaging methods for lymphoma staging can be used or non-contrast CTs. CT/PET may substitute for CT scans.
5.1.4.2 Bone marrow biopsy and aspirate are optional, but should still be done whenever possible and/or at the discretion of the principal investigator within 6 weeks prior to patient registration. Bone marrow aspirate will

be sent for lymphoma markers and flow cytometry. If performance of bone marrow biopsy will delay treatment initiation (e.g., falling on a weekend or holiday), it may be delayed but must be performed to confirm CR whenever that occurs.

5.1.4.3 Lumbar puncture (LP) with routine studies and cytology, unless L3 leukemia is present, will be done at the discretion of the physician if lymphomatous CNS involvement is suspected.

5.2 Evaluations During Treatment (Appendix II)

5.2.1 Physical examination including performance status score (Appendix I) will be repeated prior to each cycle of chemotherapy. Disease measurable on physical examination should be measured in two dimensions.

5.2.2 The following must be performed within 48 hours prior to beginning of each cycle of chemotherapy:

5.2.2.1.1 CBC, differential and platelet count

5.2.2.1.2 Serum electrolytes, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), LDH, total protein, albumin, calcium, phosphorous, AST and ALT, uric acid

5.2.2.1.3 Baseline evaluation laboratory tests may be used toward cycle 1 requirements, if done within 48 hours of treatment initiation.

5.2.3 EBV viral load will be obtained at the beginning of cycle 1 (prior to the administration of Doxorubicin) (as in 5.1.3.10), on cycle 1 prior to the administration of ZDV 20-24 hours after the administration of doxorubicin, after the 5th dose of intravenous ZDV cycle 1 (to determine if ZDV induces the EBV lytic cycle) and within the last 7 days of the end of cycle 1. Thereafter, EBV viral load will be done within the last 7 days of the end of cycle 2, 4, (minus 1-week window) and after cycle 6 (2-6 weeks after last treatment) (See Appendix III).

5.2.4 Measurement of EBV reactivation in PBMCs will be obtained at the beginning of cycle 1, prior to the administration of Doxorubicin (as in 5.1.3.11), on Day 2 of Cycle 1 prior to the administration of ZDV 20-24 hours after the administration of Doxorubicin, and after the 5th dose of ZDV (See Appendix IV).

5.2.5 Measurement of immune activation markers and inflammation associated cytokines in peripheral blood (one 10 ml purple top tubes with EDTA or equivalent). This will be done at completion of cycles 2, 4 (minus 1-week window for each) and after cycle 6 (2-6 weeks after last treatment).

5.2.6 HIV studies (in positive patients only): HIV-1 viral load and T-cell subsets following completion of cycle 2 (minus 1-week window) and cycle 6 (2-6 weeks after last treatment)

5.2.7 Restaging evaluation: CT scans (and other studies as indicated) will be performed following completion of cycles 2, 4 and 6 (minus 1-week window for each), and 2 to 6 weeks after completion of cycle 6 treatment.

5.2.8 If the patient had initial bone marrow involvement, then a repeat bone marrow biopsy should be performed when the patient has no other evidence of disease in order to determine CR status.

5.3 Post-Treatment Evaluation for Patients Not Receiving Bone Marrow Transplant (Appendix II)

5.3.1 EBV viral load will be obtained after cycle 6 (2-6 weeks after last treatment) as per section 5.2.3, and at least every three months for one year (+/- 2 weeks) (See Appendix III). EBV viral load will also be obtained upon disease progression or relapse.

5.3.2 Measurement of immune activation markers and inflammation associated cytokines in peripheral blood (one 10 ml purple top tubes with EDTA or equivalent) will be obtained upon disease progression or relapse

5.3.3 The following procedures and studies should be repeated post-therapy at least every 3 months for 2 years (+/- 4 weeks) unless indicated otherwise below, every 6 months for the third-fifth years, or as clinically indicated. These procedures and studies are required at these intervals until disease progression or death. No additional studies are required after disease progression. (Whichever occurs first, see section 12.0).

- 5.3.2.1 History
- 5.3.2.2 Physical exam/tumor measurement
- 5.3.2.3 Performance status
- 5.3.2.4 The following labs: CBC, differential and platelet count, serum electrolytes, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), LDH, total protein, albumin, calcium, AST, ALT
- 5.3.2.5 Adverse events/toxicity notation
- 5.3.2.6 CT scans of the brain, neck (if initially involved), chest, abdomen, and pelvis with contrast unless contraindicated, in which case nuclear imaging methods for lymphoma staging can be used or non-contrast CTs will be performed every 6 months for two years, or more often if clinically indicated (+/- 4 weeks).
- 5.3.2.7 HIV studies (in positive patients only): HIV-1 viral load and T-cell subsets will be performed 2-6 weeks after last treatment (as in 5.2.6), and at least every 3 months (per standard practice) for up to one year after the completion of chemotherapy (+/- 4 weeks).

5.4 Final Evaluations for Patients Accepted For Bone Marrow Transplant

Patients referred and accepted for bone marrow transplant will have a complete physical examination, blood drawn for the following studies: CBC, differential and platelet count Serum electrolytes, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), LDH, total protein, albumin, calcium, AST, ALT and EBV viral load, and a toxicity evaluation. No additional studies are required. Patients will be followed at least yearly for 5 years for survival only.

6.0 DOSING DELAYS/DOSE MODIFICATIONS

6.1 Hematologic Toxicity

6.1.1 No dose modifications will be made for anemia. This will be managed by transfusion of packed cells and/or administration of growth factors at the discretion of the investigator. We do expect severe pancytopenia and thrombocytopenia.

6.1.2 If ANC <1,000 cells/mm^3 start G-CSF 3-5 mcg/kg (daily) and repeat CBC weekly until ANC > 1000 cells/mm^3.

6.1.3 If platelets < 75,000 cells/mm^3 repeat weekly until platelets are > 30,000 cells/mm^3 unless thrombocytopenia is due to HIV-related immune thrombocytopenia. Platelet transfusions may be given at the discretion of the physician.

6.1.4 If on day one of any cycle, ANC < 1000 cells/mm^3 and/or platelets < 30,000 cells/mm^3, delay up to 2 weeks until ANC and platelets have increased above these levels. Treatment with G-CSF or pegfilgrastim is encouraged.

6.2 Non-Hematologic Toxicity

6.2.1 Hepatic toxicity: Dose adjustment for hyperbilirubinemia

6.2.1.1 Bilirubin 1.2 to 3.0 mg/dl, reduce doxorubicin dose by 50%.

6.2.1.2 Bilirubin 3.1 to 5.0 mg/dl, reduce doxorubicin dose to 25% of full dose.

6.2.1.3 Bilirubin >5.0 mg/dl doxorubicin should not be administered.

6.2.1.4 If bilirubin> 3.0 mg/dl for more than 30 days and is not associated with the presence of hepatic involvement by NHL, the patient will be removed from study.

6.2.1.5 No dosage adjustment is required for isolated hyperbilirubinemia associated with the use of indinavir, tenofovir, or atazanavir.

Note: Persons at high risk of viral hepatitis infection should be screened before initiation of rituximab. Subjects with chronic viral hepatitis will be eligible provided the meet criteria listed under Section 3.1.7.2. Subjects with chronic Hepatitis B, or Hepatitis B core antibody should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis during and for up to several months following rituximab therapy. Any subject who develops HBV reactivation or acute Hepatitis B as defined by the presence or reappearance of hepatitis B surface antigen, a positive IgM hepatitis B core antibody, and a positive viral load will be permanently removed from the study. Any patient with Hepatitis B or C who have worsening liver function necessitating drug adjustment as defined in Section 6.2.1 will also be removed from study. Rituximab and any concomitant chemotherapy should be discontinued and appropriate treatment including antiviral therapy initiated. There are insufficient data regarding the safety of resuming rituximab therapy in patients who develop hepatitis subsequent to HBV reactivation.

6.2.2 Cardiac toxicity: If clinical findings suggesting congestive heart failure are present, doxorubicin will be discontinued and evaluation by echocardiogram or MUGA will be performed. Some patients will have received doxorubicin prior to this protocol, although at total doses far below the threshold for toxicity.

6.2.3 Renal toxicity: Methotrexate dose will be decreased by 20% in the case of grade 3 or 4 renal toxicity on the prior cycle.

6.2.4 Other viral infections: The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or postmarketing reports. The majority of patients received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus (progressive multifocal leukoencephalopathy (PML), cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of rituximab and have resulted in death.

7.0 AGENT FORMULATION AND PROCUREMENT

7.1 *Zidovudine (Retrovir, AZT)*

7.1.1 Classification: Zidovudine, a thymidine analogue, is an inhibitor of the replication of some retroviruses including HIV.

7.1.2 Mechanism of Action: Zidovudine triphosphate interferes with the HIV viral RNA dependent DNA polymerase (reverse transcriptase) and thus, inhibits viral replication. *In vitro*, zidovudine triphosphate has been shown to be incorporated into growing chains of DNA by viral reverse transcriptase.

7.1.3 Metabolism: The mean half-life of peak serum concentration is approximately 1 hour. Most of the drug is excreted through the kidneys.

7.1.4 Human toxicity: The dose-limiting toxicity is anemia. Other toxicities include:
More common: fever, chills or sore throat; pale skin; headache, myalgia, back pain, rigors; paresthesia; constipation; malaise and weakness; nausea and vomiting; difficulty in sleeping; weight loss. Neutropenia and thrombocytopenia may also occur.

Less common: bluish-brown colored bands on nails; changes in skin color.

Rare: Abdominal discomfort; confusion; convulsions; diarrhea; general feeling of discomfort; loss of appetite; mood or mental changes.

Please refer to the approved package insert for complete prescribing and toxicity information.

7.1.5 Pharmaceutical Data/Formulation: Zidovudine is supplied in 20ml vials as a sterile solution in water for injection with 10 mg of Zidovudine per ml. Zidovudine is also available in 100 mg capsules and as syrup (50 mg/5ml).

7.1.6 Storage and stability: The capsules are stored at 15-30°C, and should be protected from the sun. The I.V. solution, after dilution, is physically and chemically stable for 24 hours at room temperature and 48 hours if refrigerated.

7.1.7 Administration: Zidovudine I.V. infusion is administered intravenously at a constant rate over one hour. Zidovudine should be diluted in D5W to achieve a concentration of less than or equal to 4 mg/ml prior to administration.

7.1.8 Supplier: Commercially available.

7.2. Doxorubicin (Adriamycin)

7.2.1 Classification: anthracycline antibiotic.

7.2.2 Mechanism of action: Doxorubicin binds tightly with DNA, inhibits nucleic acid synthesis and causes DNA strand breaks. Although active throughout the cell cycle, cells in S phase are most sensitive.

7.2.3 Metabolism: Doxorubicin is primarily excreted by the liver, and any liver impairment will increase toxicity. Some of the drug is excreted in the urine. The drug has a very short plasma half-life of < 20 minutes. Animal studies indicate cytotoxic levels persist in tissue for as long as 24 hours.

7.2.4 Human Toxicity: Moderate to severe myelosuppression with the nadir for leukopenia occurring at 10-14 days. Other common side effects include alopecia, and stomatitis, which is dose-related and may be severe. Drug- induced cardiomyopathy, which may result in congestive heart failure is a cumulative dose-dependent effect and risk becomes considerable at total doses exceeding 500 mg/m^2. Doxorubicin is a vesicant causing severe local necrosis at the site of injection if extravasation occurs. Nausea and vomiting are frequent. Other side effects are listed in the table below.

Common (> 30%)	Uncommon (< 30%)	Rare (< 5%)
Esophagitis **Hair loss** **Infection** **Leukopenia** **Nausea** **Red colored urine** **Stomatitis** **Vomiting**	**Congestive heart failure** **Darkening of the soles and palms** **Diarrhea** **Hyperpigmentation of the fingernails** **Uric acid nephropathy**	**Allergic dermatitis** **Allergic reaction** **Anaphylaxis** **Bronchospasm** **Drug fever** **Hives** **Shortness of breath** **Skin rash** **Wheezing**

Please refer to the approved package insert for complete prescribing and toxicity information.

7.2.5 Pharmaceutical Data/Formulation: Doxorubicin is available in 10, 20, 50, 100, and 150 mg vials for intravenous use. It should be diluted with 0.9% sodium chloride to yield a final concentration of 2 mg/mL.

7.2.6 Storage and Stability: Store under refrigeration 2°C to 8°C (36°F to 46°F). Protect from light. Retain in carton until time of use. Multi-dose vial contains no preservatives. Doxorubicin Hydrochloride for Injection, USP, is supplied in single dose vials as a sterile red orange lyophilized powder. The vials are packed in individual cartons. Store under refrigeration 2°C to 8°C (36°F to 46°F). After reconstitution the solution is stable for 7 days at room temperature and 15 days under refrigeration 2°C to 8°C (36°F to 46°F). Discard unused portion.

7.2.7 Administration: Administer slowly over 3 to 5 minutes with a rapid flowing IV. Care in the administration of doxorubicin hydrochloride will reduce the chance of peri-venous infiltration. It may also decrease the chance of local reactions such as urticarial and erythematous streaking. On intravenous administration of

doxorubicin, extravasation may occur with or without an accompanying stinging or burning sensation and even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the injection or infusion should be immediately terminated and restarted in another vein. If it is known or suspected that subcutaneous extravasation has occurred, local infiltration with an injectable corticosteroid and flooding the site with normal saline has been reported to lessen the local reaction. Because of the progressive nature of extravasation reactions, the area of injection should be frequently examined and plastic surgery consultation obtained. If ulceration begins, early wide excision of the involved area should be considered.

7.2.8 Supplier: Commercially available

7.3 Methotrexate (Methotrexate sodium, MTX, Mexate®, Mexate-AQ®, Folex®, Folex PFS®, Abitrexate®, Rheumatrex®, Amethopterin).

7.3.1 Classification: Antimetabolite.

7.3.2 Mechanism of action: Methotrexate inhibits the enzyme dihydrofolate reductase, thereby blocking the conversion of folic acid to its active form, tetrahydrofolic acid. Inhibition of this enzyme reduces purine synthesis and the conversion of deoxyuridylate to thymidylate that inhibits the synthesis of DNA, RNA and proteins.

7.3.3 Metabolism: Methotrexate given IV reaches an immediate peak with an unknown duration. Methotrexate is excreted unchanged in the urine, except at high doses when it is partially metabolized to hydroxy-MTX and excreted.

7.3.4 Human Toxicity: Myelopsuppression, with the nadir for anemia at 6-13 days, for leukopenia at 4-7 days, and for thrombocytopenia at 5-12 days. *Common:* anorexia; azotemia; bacterial infection; cutaneous vasculitis; gastroenteritis; gastric ulcers; stomatitis; hyperuricemia; nausea and vomiting; nephropathy.
Uncommon: renal failure; acne; cirrhosis; demyelination; hair loss; itching; pneumonitis; pulmonary fibrosis; skin rash.
Other side effects: Dermatologic: photosensitivity; furunculosis; depigmentation or hyperpigmentation; telangiectasia; skin desquamation (exfoliative dermatitis) and bullae formation; folliculitis. Gastrointestinal: diarrhea; anorexia; hematemesis; melena. Genitourinary: Renal dysfunction: dose-related, more likely to occur in patients with already compromised renal function, dehydration, or on other nephrotoxic drugs, manifested by increased creatinine, hematuria. Hepatic: Increased SGOT, mild and transient; hepatic fibrosis and cirrhosis, more likely to occur in patients receiving long-term continuous or daily methotrexate treatment. Neurologic: tiredness, weakness, confusion, ataxia, tremors, irritability, seizures, coma. Allergic: Fever and chills; rash; urticaria; anaphylaxis. Ocular: Conjunctivitis; excessive lacrimation; cortical blindness has occurred with high doses. Pulmonary: Cough dyspnea. Other: Malaise; osteoporosis (aseptic necrosis of the femoral head); hyperuricemia; reversible oligospermia.

Please refer to the approved package insert for complete prescribing and toxicity information.

7.3.5 Pharmaceutical Data/Formulation: Lyophilized 20, 50, 100, and 250 mg vials are reconstituted with sterile water, normal saline or 5% dextrose to a concentration no greater than 25 mg/ml. The 1000 mg vials are reconstituted with 19.4 ml to provide a concentration of 50 mg/ml. Higher doses (> 100 mg) are often further diluted with 100 ml or more of 0.45%-0.9% sodium chloride or 5% dextrose.

7.3.6 Storage and stability: Store at room temperature protected from light. Reconstituted solutions are stable at room temperature for at least 1 week. Solutions (50 mg/100 ml) in PVC bags of 5% dextrose may be frozen at -20 C for at least 30 days when thawed in 2 minutes by microwave radiation. There is no loss of potency after 5 freeze-thaw cycles. Doses of > 80 mg/week should be accompanied by leucovorin rescue.

7.3.7 Administration: Usually administered by IV bolus (< 100 mg) or slow IV infusion over 30 minutes or longer (> 100 mg). Has also been given intrathecally, intra-muscularly, orally, intra-arterially, intraperitoneally and intravesicularly. For patients who are to begin methotrexate therapy at a dose of 1 gm/m^2 or greater: Proper functioning of kidneys must be documented. Proper hydration and alkalinization of urine must be maintained.

7.3.8 Supplier: Commercially available

7.4 Hydroxyurea (Other Names: Hydrea, hydroxycarbamide (rINN), NSC-32065, SQ-1089,WR 83799 ®)

7.4.1 Classification**:** Miscellaneous agent (substituted urea).

7.4.2 Mechanism of Action: Inhibits ribonucleotide reductase enzyme system thus inhibiting DNA synthesis. It is S-phase specific.

7.4.3 Metabolism: Hydroxyurea reaches its peak 2 hours after oral administration and its duration is 24 hours.

7.4.4 Human Toxicity: Hematologic: Thrombocytopenia, leukopenia, anemia, megaloblastic erythropoiesis. Gastrointestinal: Nausea, vomiting, diarrhea, constipation, mucositis, anorexia. Dermatologic: Maculopapular rash, facial erythema, pruritus, alopecia, radiation recall phenomenon. Neurologic: Headache, drowsiness, dizziness, disorientation, hallucinations, convulsions. Renal: Hyperuricemia, dysuria, increased BUN and serum creatinine, proteinuria. Liver: Transient elevation of hepatocellular enzymes, jaundice. Other: Pulmonary edema; self-limited hepatitis and a flu-like syndrome (case report); acral erythema (case report).

Please refer to the approved package insert for complete prescribing and toxicity information.

7.4.5 Pharmaceutical Data/Formulation: Available in 200, 300, 400, and 500 mg capsules.

7.4.6 Storage and Stability: Stored in tight containers at room temperature. Avoid excessive heat.

7.4.7 Administration: Oral.

7.4.8 Supplier: Hydroxyurea is commercially available.

7.5 Leucovorin: (Other Names: Leucovorin Calcium, Wellcovorin, citrovorum factor, folinic acid, 5-formyl tetrahydrofolate, LV, LCV)

7.5.1 Classification: Tetrahydrofolic acid derivative.

7.5.2 Mechanism of Action: Leucovorin acts as a biochemical cofactor for 1-carbon transfer reactions in the synthesis of purines and pyrimidines. Leucovorin does not require the enzyme dihydrofolate reductase (DHFR) for conversion to tetrahydrofolic acid. Leucovorin can potentiate the cytotoxic effects of fluorinated pyrimidines (i.e., fluorouracil and floxuridine). After 5-FU is activated within the cell, it is accompanied by a folate cofactor, and inhibits the enzyme thymidylate synthetase, thus inhibiting pyrimidine synthesis. Leucovorin increases the folate pool, thereby increasing the binding of folate cofactor and active 5-FU with thymidylate synthetase.

7.5.3 Metabolism: Oral leucovorin has its onset of action in 20-30 minutes, reaches its peak in 2-3 hours and has duration of 3-6 hours. Intravenous leucovorin has its onset of action in 5 minutes, reaches its peak at 10 minutes and has duration of 3-6 hours.

7.5.4 Human Toxicity: Hematologic: Thrombocytosis. Dermatologic: Skin rash. Gastrointestinal: Nausea, upset stomach, and diarrhea. Allergic: Skin rash, hives, pruritus. Pulmonary: Wheezing (possibly allergic in origin). Other: Headache; may potentiate the toxic effects of fluoropyrimidine therapy, resulting in increased hematologic and gastrointestinal (diarrhea, stomatitis) adverse effects.
Please refer to the package insert for complete prescribing and toxicity information.

7.5.5 Pharmaceutical Data/Formulation: The 50 and 100 mg vials for injection are reconstituted with 5 and 10 ml of sterile water, respectively, resulting in a 10 mg/ml solution. The 350 mg vial is reconstituted with 17 ml of sterile water resulting in a 20 mg/ml solution. Due to the increased preservative content with the higher doses of bacteriostatic water, only sterile water should be used for reconstitution of leucovorin doses greater than 10 mg/m^2. Also available in 5, 10, 15 and 25 mg tablets.

7.5.6 Storage and Stability: All dosage forms are stored at room temperature. At concentrations of 0.5-0.9 mg/ml the drug is chemically stable for at least 24 hours at room temperature under normal laboratory light.

7.5.7 Administration: Intravenous infusion over 15minutes- 2 hours. Compatibilities: Leucovorin (0.5-0.9 mg/ml) is chemically stable for at least 24 hours in normal saline, 5% dextrose, 10% dextrose, Ringer's injection or lactated Ringer's injection. Leucovorin is also compatible with fluorouracil.

7.5.7 Availability: Commercially available.

7.6 Rituximab

7.6.1 Rituximab is a chimeric mouse/human anti-CD 20 monoclonal antibody that has been shown to bind human C1q, mediate complement-dependent cell lysis and lyse human target cells through antibody dependent cellular cytotoxicity. It has

documented anti-tumor activity in low grade or follicular B-cell non-Hodgkin's lymphoma and was recently FDA-approved for that indication. Rituximab will not be supplied by PMB for this protocol. The agent must be commercially obtained.

7.6.2 Adverse Events and Potential Risks List for Rituximab

The Adverse Event and Potential Risks list provides a single list of reported and/or potential adverse events (AE) associated with Rituximab.

Common adverse events (frequency >20%), occurring among patients receiving Rituximab (MoAb C2B8 anti CD20, chimeric):
• Nausea • Chills, fever • Infusion related reaction • Decrease in lymphocyte count

Occasional and serious adverse events (frequency ≤ 20%), occurring among patients receiving Rituximab (MoAb C2B8 anti CD20, chimeric):	
• Anemia which may require blood transfusions • Anemia • Blood and lymphatic system disorders - Other (Hyperviscosity: Waldenstrom's) • Febrile neutropenia • Myocardial infarction • Sinus tachycardia • Supraventricular tachycardia • Abdominal pain • Diarrhea • Nausea • Edema limbs • Fatigue • Pain • Allergic reaction, serum sickness • Skin and subcutaneous tissue disorders - Other (angioedema) • Rash maculo-papular • Urticaria • Flushing • Hypertension • Hypotension	• Infections and infestations - Other (Activation of Hepatitis B, C, CMV, parvovirus B19, JC virus, varicella zoster, herpes simplex, West Nile virus) • Infections and infestations - Other (Infection in HIV Positive Patients) • Neutrophil count decreased • Platelet count decreased • White blood cell decreased • Hyperglycemia • Hypocalcemia • Hypokalemia • Arthralgia • Back pain • Myalgia • Tumor pain • Dizziness • Headache • Lethargy • Seizure • Acute kidney injury • Allergic rhinitis • Bronchospasm • Cough • Dyspnea • Hypoxia • Pneumonitis • Sore throat • Hyperhidrosis • Pruritus

Rare but serious adverse events (frequency ≤3), among patients receiving Rituximab (MoAb C2B8 anti CD20, chimeric):
• Anaphylaxis • Tumor Lysis Syndrome • Nervous system disorders - Other (progressive multifocal leukoencephalopathy) • Adult respiratory distress syndrome • Erythema multiforme • Stevens-Johnson syndrome • Toxic epidermal necrolysis

Also reported on rituximab trials but with the relationship to rituximab still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Bone marrow hypocellular; Hemolysis

CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Cardiac disorders - Other (cyanosis); Left ventricular systolic dysfunction; Sinus bradycardia; Ventricular fibrillation

EYE DISORDERS - Conjunctivitis; Eye disorders - Other (ocular edema); Uveitis; Watering eyes

GASTROINTESTINAL DISORDERS - Constipation; Dyspepsia; Dysphagia; Gastrointestinal obstruction[3]; Gastrointestinal perforation[4]; Mucositis oral

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain

INFECTIONS AND INFESTATIONS - Infections and infestations - Other (Opportunistic infection associated with >=Grade 2 Lymphopenia)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture

INVESTIGATIONS - Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Cardiac troponin I increased; Cardiac troponin T increased; Creatinine increased; Investigations - Other (hyperphosphatemia); Investigations - Other (LDH increased); Weight loss

METABOLISM AND NUTRITION DISORDERS - Anorexia; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hyperuricemia; Hypoglycemia; Hypomagnesemia; Hyponatremia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis

NERVOUS SYSTEM DISORDERS - Nervous system disorders - Other (Cranial Neuropathy NOS); Peripheral motor neuropathy; Peripheral sensory neuropathy; Pyramidal tract syndrome; Reversible posterior leukoencephalopathy syndrome; Syncope

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Depression; Insomnia

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Epistaxis; Pharyngolaryngeal pain; Pleural effusion; Pulmonary edema; Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans)

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Skin and subcutaneous tissue disorders - Other (paraneoplastic pemphigus)

VASCULAR DISORDERS - Phlebitis; Thromboembolic event; Vasculitis

Note: Rituximab in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.6.3 Method of Administration

The administration of rituximab will be accomplished by slow IV infusion.

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

Rituximab infusions may be administered to patients in an outpatient clinic setting. Oral pre- medication (650 mg of acetaminophen and 50 mg diphenhydramine hydrochloride) will be administered 30 to 60 minutes prior to starting each infusion of rituximab**.** A central intravenous line will be established. During the rituximab infusion, the patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored every 15 minutes times 4 or until stable and then hourly until the infusion is discontinued. Available at the bedside prior to rituximab administration will be epinephrine for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment for the emergency management of anaphylactic reactions. IV pumps such as the IMED 960 may be used with the rituximab infusion. DO NOT INFUSE CONCOMITANTLY with another IV solution or IV medications. Prime the line with the rituximab solution such that approximately 30 mL are delivered. This will saturate the tubing. If a delay in administration of the infusion occurs after the product is prepared, the properly identified container may be kept refrigerated at 2-8°C (36-46 degrees F) for 24 hours and at room temperature for an additional 12 hours. The initial dose rate at the time of the first rituximab infusion should be 50 mg/hr for the first hour. If no toxicity is seen, the dose rate may be escalated gradually (50 mg/hr increments at 30 minute intervals) to a maximum of 400 mg/hr. If the first dose of rituximab is well tolerated, the starting flow-rate for the administration of subsequent doses will be 100 mg/hr then increased gradually (100 mg/hr increments at 30 minute intervals) not to exceed 400 mg/hr. Rituximab may be given at a slower infusion rate if it is consistent with institutional policy.

8.0 CORRELATIVE/SPECIAL STUDIES

Parallel translational studies will be performed, see Appendix II, as part of this protocol. An important question is the effect of our regimen on EBV viral load and modulation of gene expression profile. This is particularly relevant in view of the recent finding that quantification of EBV DNA is useful for monitoring patients with nasopharyngeal carcinoma and lymphoma (25, 26). It will also be particularly important to determine the effect of chemotherapy on viral reactivation (lytic cycle) from tumor and latently infected peripheral blood memory B cells, since we hypothesize a higher proportion of these harbor latent virus, and the viral load in the presence of high dose ZDV. See Appendices III and IV for collection instructions.

We will also perform studies on available primary tumors to determine: (1) pattern of EBV latency and (2) expression of EBV kinases (BGLF/vPK and BXLF1/vTK). The antivirals used in this protocol (ZDV and ganciclovir) are excellent substrates for the viral kinases. These studies are highly relevant and will provide important information as to whether the expression patterns of EBV change in tumors from patients with relapsed lymphomas.

Finally, we will measure the effects of drug therapy used in this protocol on HIV infection (in positive subjects), immune activation markers in peripheral blood T-cells, and plasma cytokines in order to investigate whether treatment results in immune re-constitution or exhaustion, and the effect of treatment and disease status with inflammation. These studies have not been done previously within the context of a HIV or EBV lymphoma trials.

9.0 MEASUREMENT OF EFFECT

All patients will be evaluated for clinical response by physical examination following each chemotherapy cycle and by imaging studies at the conclusion of cycles 2, 4 and at the conclusion of cycle 6.

9.1 Response Assessment

Response is assessed on the basis of clinical, radiologic, and pathologic (i.e. bone marrow) criteria.

1. CT scans remain the standard for evaluation of nodal disease. Brain, thoracic, abdominal, and pelvic CT scans will be performed for staging even if those areas were not initially involved because of the unpredictable pattern of recurrence in NHL. Neck CT scans will be required if disease involving this area was present at baseline.
2. A bone marrow aspirate and biopsy should only be performed to confirm a CR if they were initially positive or if it is clinically indicated by new abnormalities in the peripheral blood counts or blood smear.

9.2 Definition of Response

9.2.1 Complete Response (CR)

9.2.1.1 Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities [e.g. lactate dehydrogenase (LDH) or calcium] definitely assignable to NHL.

9.2.1.2 All lymph nodes and tumor masses must have disappeared or regressed to normal size (less than or equal to 1.5 cm in their greatest transverse diameters for nodes >1.5 cm before therapy).

9.2.1.3 Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to less than or equal to 1 cm in their greatest transverse diameter after treatment, or by more than 75% bin the sum of the products of the greatest diameters (SPD).

9.2.1.4 The spleen, if considered enlarged before therapy on the basis of a CT scan, must have decreased in size and must not be palpable on physical examination. Similarly, other organs considered to be enlarged before therapy due to involvement by NHL, such as liver and kidneys, must have

decreased in size.

9.2.1.5 If the bone marrow was involved by NHL before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site.

9.2.1.6 No new sites of disease.

9.2.1.7 Typically FDG-avid lymphoma: in subjects with no pre-treatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size in permitted as long as it is PET negative.

9.2.1.8 Variably FDG-avid lymphomas/FDG activity unknown: in subjects without a pre-treatment PET scan, or if a pre-treatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to 1.0 cm in their short axis after treatment.

9.2.2 Clinical Complete Response (CCR):

Clinical complete responders will be grouped with complete responders if they qualify for inclusion by meeting all of the following criteria:

9.2.2.1 No abnormal palpable lymph nodes. In the event there is a suspicious or equivocal palpable lymph node, a negative biopsy is required.

9.2.2.2 No "B" symptoms (unexplained fevers, night sweats, involuntary weight loss greater than 10% normal body weight).
Chest radiograph and/or CT chest scan must be normal or, if mediastinal widening persists, the abnormal extra width must be diminished by at least 50% compared with pretreatment radiographs and there must have been no progression for eight weeks or more after the completion of therapy.

9.2.2.3 Residual opacified lymph nodes must return to normal as demonstrated by appearance on plain film of the abdomen or repeat lymphangiogram. If abnormal, there must have been at least a 50% reduction in the abnormal enlargement of the lymph nodes and there must have been no change in size for at least eight weeks after the completion of therapy. Architecture should be ignored for this type of evaluation.

9.2.2.4 CT abdomen/pelvic scan should be normal, or if abnormal, at least an 80% reduction from the initial abnormal enlargement of the measurable lesions should be seen and maintained for at least eight weeks after the completion of therapy.

9.2.3 Partial Response (PR) requires the following:

9.2.3.1 At least ≥ 50% decrease in sum of the product diameter (SPD) of the six largest dominant nodes or masses. These nodes or masses should be selected according to the following features: (a) they should be clearly measurable in at least two perpendicular dimensions, (b) they should be

from disparate regions of the body as possible, and (c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

9.2.3.2 No increase in the size of the other nodes, liver, or spleen.

9.2.3.3 Splenic and hepatic nodules must regress by at least 50% in the SPD or, for single nodules, in the greatest transverse diameter.

9.2.3.4 With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and not measurable disease should be present.

9.2.3.5 Bone marrow assessment is irrelevant for determination of a PR if positive before treatment. However, if positive, the cell type should be specified in the report, (e.g., large cell lymphoma or small neoplastic B cells). Subjects who achieve CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders. When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, subjects should be considered partial responders.

9.2.3.6 No new sites of disease.

9.2.3.7 Typically FDG-avid lymphoma: in subjects with no pre-treatment PET scan or when the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.

9.2.3.8 Variably FDG-avid lymphomas/FDG activity unknown: in subjects without a pre-treatment PET scan, or if a pre-treatment PET scan was negative CT criteria should be used.

9.2.4 Stable disease is defined as less than a PR (see above) but is not progressive disease (see below).

9.2.4.1 Typically FDG-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.

9.2.4.2 Variably FDG-avid lymphomas/FDG –avidity unknown: subjects without a pre-treatment PET scan or if the pre-treatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

9.2.5 Relapsed Disease (after CR)/Progressive Disease (after PR, SD)

9.2.5.1 Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0 cm. Lymph nodes 1.0 x 1.0 cm will not be considered as abnormal for relapse or progressive disease.

9.2.5.2 Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In subjects with no prior history of pulmonary lymphoma, new lung

nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

9.2.5.3 At least a 50% increase from nadir in the SPD of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.

9.2.5.4 At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.

9.2.5.5 Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (< 1.5 cm in its long axis by CT.

9.2.5.6 Measurable extra nodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable (e.g., pleural effusions, bone lesions) will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical examination, it is found to be histologically negative.

9.2.6 Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.

9.2.7 Time to response is defined as time from the first dose of chemotherapy until documentation of first response.

9.2.8 Time to progression is defined as time from initiation of chemotherapy to documentation of first progression.

9.2.9 Response duration is defined as the time from first documentation of response to documentation of first progression.

9.2.10 Failure–free survival (FFS) will be measured from the date of treatment initiation until date of documented disease progression, relapse after response, or death from any cause. For patients alive and free of relapse or progression, follow-up time will be censored at the last documented date of failure-free status.

9.2.11 Overall survival (OS) will be measured from the date of initiation of study treatment until date of death from any cause. In the absence of death, the follow-up will be censored at date of last contact (censored observation).

10.0 ADVERSE EVENT REPORTING

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

10.1 Definitions

10.1.1 **Adverse Event (AEs) –** Any unfavorable and unintended sign (including any clinical significant abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment of procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). Each AE is a unique representation of a specific event used for medical documentation and scientific analysis.

10.1.2 **Serious Adverse Event (SAE)** – Any adverse event (except those listed in section 10.2.5) occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

SAEs are defined by the FDA and therefore seriousness (not severity) serves as a guide for defining regulatory reporting obligations for patient/subject safety. **Serious** is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

10.1.3 **Expected events** are those that have been previously identified as resulting from administration of the agent(s).

10.1.4 An adverse event is considered **unexpected** when either the type of event or the severity of the event is *not* listed in the: current NCI Agent-Specific Adverse Event List; investigator's brochure; drug package insert; or the drug information section of this protocol.

10.1.5 The definition of *related* is that there is a reasonable possibility that the drug caused the adverse experience.
Attribution categories:

Definite – The adverse event is *clearly related* to the investigational agent(s).
Probable – The adverse event is *likely related* to the investigational agent(s).
Possible – The adverse event *may be related* to the investigational agent(s).
Unlikely – The adverse event *is doubtfully related* to the investigational agent(s).
Unrelated – The adverse event *is clearly NOT related* to the investigational agent(s).

10.2 Adverse Events with Commercial Agents

The following adverse reactions must be reported to the Principal Investigator and the Institutional Review Board in the manner described below.

Commercial Agents: Zidovudine, Hydroxyurea, Doxorubicin, Methotrexate, Leucovorin

10.2.1 Investigators are required to report adverse events which fit the following criteria *within 10 working days* of the time the investigator becomes aware of them:

10.2.1.1 Event is **UNANTICIPATED** (An unanticipated event is any adverse experience where the nature, severity or frequency is not identified in the drug package insert or described in the protocol. Events which are already cited in the drug package insert or protocol are not unanticipated and do not have to be reported),

AND

Event is **RELATED** to the study design, procedures, or drug/device (possibly, probably, definitely related, or unknown). If the adverse event is clearly not related to the study drug, device, procedures, or washout process, it would not represent a risk to other subjects in the research and, therefore, does not have to be reported.

10.2.2 Do not report adverse events that are clearly not related to the study drug, device, procedures, or washout process.

10.2.3 Any death, regardless of causality or relationship to the protocol, must be reported within 24 hours of the knowledge of the event. Attribution to treatment or other cause must be provided.

10.2.4 Clinical laboratory results that are outside of the normal ranges are adverse events if they meet criteria as per 10.2.1.1

10.2.5 In this protocol the following clinical laboratory abnormalities are expected and will not be reported as SAEs except those ≥ Grade 3

- Anemia
- Leucopenia (including neutropenia and lymphopenia)
- Thrombocytopenia
- HIV viral load (if applicable)
- T Cell subsets (if applicable)

11.0 DATA REPORTING

11.1 Records to be kept

Paper Case Report forms (CRFs) will be provided for each subject. Subjects will be identified by a Patient Identification Number assigned by coordinating site study team upon registration. Any requested information that is not obtained as specified in the protocol should have an explanation noted on the paper CRF as to why the required

information was not obtained. SAEs and AEs will be entered in Velos eResearch program for each subject.

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the project coordinator.
11.2.2 The timeline for forms submission can be found in Appendix V.
11.2.3 Record Retention
11.2.3.1 Federal regulations require that records of drug disposition, source documentation, CRFs, and all reports of this investigation shall be retained by the principal investigator for a minimum of 2 years following notification by the PI that the IRB has been notified of the study's discontinuation.

12.0 CRITERIA FOR DISCONTINUATION OF THERAPY

12.1 Permanent Withdrawal

After enrollment, the patient will be permanently withdrawn from study treatment for any of the following reasons:

12.1.1 Development of an intercurrent illness that prevents further administration of treatment.
12.1.2 Unacceptable adverse events. Progressive disease at any time while on study.
12.1.3 Voluntary withdrawal.
12.1.4 The investigator has the right to remove subjects from study for clinical reasons, which he or she believes to be life threatening or resulting in significant morbidity to the subject.

12.2 Permanent Withdrawal Evaluations

Subjects going off treatment prior to completion of therapy or for progressive disease will have a complete physical examination, a toxicity evaluation and blood drawn for the following studies: CBC, differential and platelet count Serum electrolytes, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), LDH, total protein, albumin, calcium, , AST, ALT and EBV viral load. No additional studies are required. Subjects that achieve CR will be followed at least yearly for survival only, as well as those who voluntarily withdraw consent, are removed by the principal investigator from the study for clinical reasons, received or not bone marrow transplant.

13.0 STATISTICAL CONSIDERATIONS

13.1 Overview

The primary objective of this phase II study is to determine the clinical response of patients with relapsed EBV+ associated non-Hodgkin's or Hodgkin's lymphoma or post-transplant lymphoproliferative disease treated with high dose parenteral zidovudine and oral hydroxyurea and combination chemotherapy with Doxorubin, Methotrexate, and Leucovorin. Secondary objectives include evaluate 1-year overall survival and failure-free survival, toxicity, and correlative studies endpoints, such as, EBV reactivation, circulating EBV viral load, and immune response parameters.

13.2 Definitions

13.2.1 At the time signed informed consent is given, a patient is considered to be on study.

13.2.2 Evaluable patients will be *study-eligible patients* who initiate the study treatment, regardless of the number of treatment cycles completed. All evaluable patients will be assessed for treatment response, toxicity, disease progression, and survival.

13.2.3 Patients are considered to be *on treatment* as long as they continue to receive study treatment. Patients who are *off treatment* will remain on study and will be followed for toxicity, response, progression, and survival.

13.2.4 Exclusions from study (*not evaluable patients*):

13.2.4.1 Patients who are enrolled on study but not treated (do not receive any dose of study treatment) will be excluded from all analyses, including analyses of failure-free and overall survival, and safety. Reasons for such withdrawals, such as eligibility not confirmed or consent withdrawn, will be characterized.

13.2.4.2 Any patient who is treated but later found to be ineligible for study (a "protocol violation") will be withdrawn from study but followed for survival and toxicity. Such patient experience will be characterized separately from that of evaluable patients, whose experience will represent the main basis for evaluating the therapeutic regimen of this phase II trial.

13.2.5 *Failure–free survival (FFS)* will be measured from the date of treatment initiation until date of documented disease progression, relapse after response, or death from any cause. For patients alive and free of relapse or progression, follow-up time will be censored at the last documented date of failure-free status.

13.2.6 *Overall survival (OS)* will be measured from the date of initiation of study treatment until date of death from any cause. In the absence of death, the follow-up will be censored at date of last contact (censored observation).

13.3 Statistical Plan:

Study Size: We plan to enroll a total of 26 evaluable patients over a period of 4 years (5-6 patients per year) and follow them for a minimum of 1 year. The resulting study falls within our capacity for enrollment and provides sufficient precision for our main objective.

Efficacy: Assessment of treatment efficacy will be made with regard to the rate of overall response (complete, complete/unconfirmed, or partial responses), failure-free survival, and overall survival. The overall response rate (CR+PR) and rates of the individual categories of best clinical response (CR, PR, SD, and PD) will be estimated by the percentage of patients achieving each specific response type. The precision of these estimates will be characterized by the corresponding 90% confidence intervals using the exact binomial method (28). The time to onset and duration of response will be summarized (median and range) for each response type.

Analysis of primary and secondary endpoints will be based on all evaluable patients, i.e., patients who are study eligible and initiate the study treatment, regardless of whether they receive the complete first cycle of treatment. Secondary analyses will consider evaluable patients receiving 3 or more cycles of treatment.

Demographic (age at diagnosis, gender, and race/ethnicity), and other baseline characteristics (performance status, previous therapies, molecular and viral characteristics of EBV lymphoma: EBV viral load, protein expression) will be summarized using plots and descriptive statistics (28) counts and percentages, range, median, mean, and standard deviation, as appropriate.

Toxicity: Adverse events and toxicities will be tabulated by severity, attribution to treatment, and treatment cycle according to the most recent NCI CTCAE criteria. A patient-level summary by worse grade adverse event will be included.

Treatment feasibility: The feasibility of administering the per-protocol treatment will be characterized by the percentage of evaluable patients who initiate and complete each treatment cycle. Number of cycles of therapy required to achieve best response, total number of cycles completed, reasons for study withdrawal, laboratory data, and concomitant medications, will be summarized descriptively.

Efficacy: Assessment of treatment efficacy will be made with regard to the rate of overall response (complete, complete/unconfirmed, or partial responses), failure-free survival, and overall survival for all subjects and separate disease entities.

The overall response rate (CR+PR) and rates of the individual categories of best clinical response (CR, PR, SD, and PD) will be estimated by the percentage of patients achieving each specific response type. The precision of these estimates will be characterized by the corresponding 90% confidence intervals using the exact binomial method (28). The time to onset and duration of response will be summarized (median and range) for each response type. Failure-free survival (FFS) and overall survival (OS) will be estimated by the Kaplan-Meier method. Estimates of FFS and OS rates at 3, 6, 9, and 12 months will be reported along with corresponding 90% confidence intervals (29). Median failure-free time and survival, if achieved, will be estimated along with corresponding 90% confidence intervals. The amount of patient follow-up will be characterized descriptively by the range and median for patients who experience the event (progression or death) and for those who do not (censored observations). To the extent possible with 26 patients, Cox proportional hazards regression (29) will be used to assess whether FFS and OS are affected by demographic and baseline disease characteristics, or any correlative laboratory or clinical parameters. Given the small number of patients, all analyses are essential exploratory in nature and no adjustments for multiple comparisons will be made.

Survival. We plan to enroll a total of 26 evaluable patients over a period of 4 years (5-6 patients per year) and follow them for a minimum of 1 year. The resulting study falls within our capacity for enrollment at multiple clinical sites and provides sufficient precision for our main objective. It is expected that the study treatment will result in a median overall survival of 6 months or better, that is a 6-month survival rate of 50% or higher.

Study precision can be expressed in terms of the 90% confidence interval (90% CI) for the 6-month survival rate. Assuming 50% of the 26 evaluable patients remain alive for 6 months, and no losses to follow-up, Peto's approximation (27) for the standard error of the 6-month survival rate is 9.8%, and the corresponding 90% CI is 33.9% to 66.1%. Such a finding would establish with high confidence (95%) that the true 6-month survival rate for this treatment regimen is not less than 33.9%. We note further that the estimated study precision based on 26 patients is only slightly less than that for a much larger investigation attaining a similar result. Specifically, a study of 36 patients (38% more patients) in which 50% are alive at 6 months would yield a 90% CI of 36.3% to 63.7%, which is only slightly narrower than the interval based on 26 patients (width 27.4% v. 32.2%).

Table 13.1 below illustrates possible study findings on survival based on 26 patients, assuming no patient is lost to follow up within 6 months of treatment initiation. The first row shows the expected 6-month survival rate of 50%, which “rules out" 6-month survival rates smaller than 33.9%. The second row shows that a less favorable study outcome of 38.5% alive at 6 months would only "rule out" 6-month survival rates smaller than 22.8%. A more favorable finding shown in the third row, namely 6-month survival rate of 61.5%, would indicate with high confidence (95%) that the true 6-month survival rate is at least 45.8%. The far right column of this table presents the median time to progression, derived under the assumption of exponential survival, corresponding to the 6-month rates in the second column.

Table 13.1 Possible outcomes for survival with 26 patients

# of pts alive/26 with min 6-month follow-up	6-month survival rate	90% confidence interval*	Median survival time**
Expected study finding			
13/26	50%	33.9% - 66.1%	6 months
Less favorable finding			
10/26	38.5%	22.8% - 54.2%	4.4 months
More favorable finding			
16/26	61.5%	45.8% - 77.2%	8.6 months

* Based on Peto's formula for the standard error and assuming no censoring within the first 6 months of follow-up.

** Based on the 6-month OS rates and under the assumption of exponential survival.

13.4 Correlative Studies

13.5.1 Determining EBV gene or viral protein (kinase) and latency/lytic gene expression patterns on available tissue in patients with relapsed Epstein Barr Virus associated lymphomas and any association with treatment response and survival. Tissue will be stored for future studies, which may include immunohistochemistry or in situ hybridization for BGLF4/vPK, BXLF1/vTK, LMP-1, BZLF-1, and EBV gene expression array studies, genome wide gene expression arrays, next generation sequencing, and the use of new genomic or proteomic technologies that become available in the future

13.5.2 Measurements of plasma circulating EBV viral loads (by quantitative EBV PCR) obtained at baseline, at various times during treatment, and post treatment will be summarized using plots and descriptive statistics, in order to study the effects of chemotherapy/AZT on blood circulating memory B cells which harbor latent virus, peripheral blood mononuclear cells will be evaluated for EBV reactivation using our specialized EBV gene expression array (Dr. Dittmer laboratory). Measurements of the effects of drug therapy on HIV infection (in positive subjects) and immune activation markers in peripheral blood T-cells will investigate immune re-constitution or exhaustion.

14.0 DATA AND SAFETY MONITORING

14.1 Role of Research Team and Data and Safety Monitoring Committee (DSMC)

The Research Team will continuously monitor study accruals, toxicities, and response to treatment, as measured by overall survival. The UM/Sylvester Comprehensive Cancer Center's Data and Safety Monitoring Committee (DSMC) will monitor this protocol according to the Cancer Center's DSM plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study.

DSMC oversight of the conduct of this trial includes ongoing review of adverse event data and periodic review of response to treatment, as measured by overall survival. The DSMC also reviews reports from internal audits of protocol compliance and data integrity conducted by the University of Miami, Office of Research Compliance Assessment. The guidelines in this section are offered for DSMC consideration in assessing adverse events and response to study treatment.

14.2 Early stopping guidelines

We propose the following guidelines for the DSMC in its review of accumulating data on toxicity and response to treatment, as measured by overall survival. These guidelines were developed using Bayesian methods, which can be applied at any stage of enrollment without pre-specification of the number of interim analyses to be performed, or the number of patients evaluable for toxicity or survival at the time such assessments are made (30,31). Under the Bayesian method, we assign a prior probability (level of belief at the start of the trial) to a range of possible values for the true rate. As data on treated patients become available, this probability distribution is revised and the resulting posterior probability becomes the basis for recommending either early termination or continuation of the study. In the sections that follow, we provide specific stopping guidelines based on posterior probabilities for interim monitoring of toxicity and efficacy over the course of this trial. Underlying assumptions for the prior distribution are also presented.

Safety: Early stopping due to toxicity

If a treatment-related (possible, probable, or definite) death occurs, enrollment will be suspended and continuation of the study will be reassessed by the DSMC. We do not plan to stop this study early due to toxicity because we expect 80% of study patients will experience grade 3-4 toxicity related to treatment such as neutropenia, thrombocytopenia, anemia, neutropenia fever, mucositis, etc. Such toxicities are manageable by medications and or delays in treatment. Due to the poor prognosis of these patients criteria for stopping the study will be based on survival and not toxicity.

Early stopping due to lack of efficacy: monitoring the survival

Although we expect that study treatment will lead to increased median survival to about 6 months (that is, 6-months survival rate of 50%), to ensure patient safety the DSMB will monitor the accumulating study data for the possibility of a reduced survival time on study. Such interim monitoring will be done by computing the Bayesian posterior probability (**PPr)** that the median survival is less than or equal to 3 months based on interim data and a prior distribution. As a guideline for early stopping, if the posterior probability is 80% or greater, that is,

PPr (median survival ≤ 3 months | current data and prior) ≥ 0.80,

then the DSMB will consider stopping the study early. If the study is stopped early

under this circumstance, then enrollment of new patients would be terminated and study treatment would be stopped for the currently enrolled patients.

The posterior probability will be computed based on interim study data and a prior distribution representing a proponent's degree of belief in the target survival improvement, since such an individual needs to be convinced of potential harm. Under assumption of exponential distribution for overall survival time, the proponent's belief can be represented by a normal distribution for the log hazard rate centered around a mean value corresponding to the expected 6 months median survival (mean = log hazard rate= log [(-log 0.5)/ 6] = $\log_e$ (0.1155)= -2.1583). The variance (σ^2= 0.6783) is derived by assigning a 20% probability (weak prior belief) to the possibility that the median overall survival is ≤3 months (log hazard rate= log [(-log 0.5)/ 3] = $\log_e$ (0.2310) =-1.4651).

Table 14.2 provides hypothetical illustration of the posterior probability calculation described above based on patient enrollment, observed number of deaths, and total follow-up (in months) considering all patients enrolled in the study by the time of interim analysis. (Total follow-up, which is the person-time at risk, and observed number of deaths, are used for calculating the data-driven estimates of hazard rate and median survival).

Table 14.2. Hypothetical Example of Interim Results Regarding Survival

Patients	Total follow-up[1] (months)	Observed number of deaths	Expected[2] number of deaths if median is		Posterior probability that median survival is ≤ 3 months
			3 mos.	6 mos.	
5	5	2	1.16	0.59	0.516
5	7	4	1.62	0.81	0.867
8	15	4	3.47	2.60	0.424

[1] Sum of follow-up time from start of treatment to date of death, or to last date of documented alive at the time of interim analysis.

[2] Expected = Total follow-up x [(-log 0.5)/Md], Md = median = 3, 6.

Taking the second row of table 14.2 as an example, if 4 out of 5 patients have died and the total follow-up time is 7 months, the posterior probability that the median survival time is smaller than or equal to 3 months is 0.867. Thus, an interim analysis finding such as this would suggest that early termination of the study should be considered since there is a high chance (86.7%) that the median time to progression is ≤ 3 months. An example of a more favorable potential outcome is given in row 3. Specific calculations will be provided to the DSMC for interim review of actual trial data.

14.3 Interim Review of Safety Data

The protocol management team, consisting of two of the investigators for this study AND the statistician, will continuously monitor study accruals, toxicities, and response to treatment.

The protocol management team may convene at their discretion to review the accumulated safety data. Accrual will continue as long as the protocol management team has no clinical concerns regarding the incidence of Grade 3 or 4 adverse events and the relationship of the events to treatment with the study drugs.
The protocol management team will have access to all available safety data and will carefully review the data for safety issues. The protocol management team may close the trial if there are major safety problems, especially Grade 4 adverse events.

Suspension of Study:
If safety concerns arise during the study, the DSMC may suspend, amend or terminate the study. The study may be suspended by the DSMC until the situation or safety concern has been resolved.

14.4 Study Termination

The DSMC retains the right to terminate the study for any other cause, suspending patient enrollment and related study materials from the study site at any time. Specific instances, which may precipitate such termination at a site, are as follows:

- Deviation from protocol requirements
- Inaccurate and/or incomplete data recording on a recurrent basis
- Unauthorized use of investigational products or administration to any subject not enrolled as part of the protocol
- Delinquent fulfillment of obligation on the part of the Investigator with regard to adverse reaction reporting, unacceptable patient enrollment or other responsibilities as outlined in this protocol.

The DSMC will Review the Following:

1. DSMC will review:
2. Number of patients enrolled to date
3. Summary of all adverse events regardless of grade and attribution.
4. Dose modifications
5. Deviations
6. Data collection/entry

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APPENDIX I

Performance Status Scale

Karnofsky Performance Scale		ECOG Performance Status Scale	
Percent	**Description**	**Grade**	**Description**
100	Normal, no complaints, no evidence of disease.	0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
90	Able to carry on normal activity; minor signs or symptoms of disease.		
80	Normal activity with effort; some signs or symptoms of disease.	1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
70	Cares for self, unable to carry on normal activity or to do active work.		
60	Requires occasional assistance, but is able to care for most of his/her needs.	2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
50	Requires considerable assistance and frequent medical care.		
40	Disabled, requires special care and assistance.	3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
30	Severely disabled, hospitalization indicated. Death not imminent.		
20	Very sick, hospitalization indicated. Death not imminent.	4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
10	Moribund, fatal processes progressing rapidly.		
0	Dead.	5	Dead.

APPENDIX II – STUDY CALENDAR

	Baseline/ Pre-treatment See Sec 5.1 for timeframes	**Chemo Therapy Day 1 each cycle**	**Cycle 1 Only[1]**	**After Cycles 2, 4 and 6**	**Post Therapy Each Visit**	**Early Discontinuation/BM Transplant**
Complete History	X					
Physical Exam/	X	X			X[3]	X
Tumor Measurement	X	X			X[3]	
Performance Status	X	X			X[3]	
CBC, Diff, Platelets	X[8]	X			X[3]	X
Blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, calcium, phosphorous, AST, ALT, uric acid	X[8]	X	X		X[3]	X
Serum Pregnancy Test within 7 days (Females)	X					
HIV viral load	X			X[4]	X[4]	
T-cell subsets	X			X[4]	X[4]	
Hepatitis B Core Ab, Surface Ab & Surface Ag,,and hepatitis C Ab. A baseline Hep B and Hep C viral load should be obtained in Hep B Core Ab (+) and Surface antigen (+) cases, and Hepatitis C Ab (+) cases respectively.	X			X[9]		
EBV Viral Load	X[1]		X[1]	X[4]	X[4]	X[4]
EBV reactivation	X[1]		X[1]			
Immune activation and inflammation markers	X			X[5]		X[5]
EKG	X					
MUGA or Echo	X					
CT scans	X			X	X[6]	
Bone Marrow	X			X[2]		
Lumbar Puncture	X					
Concurrent Medications	X					
Toxicity Notation	X	X				X

X[1] EBV viral load will be obtained at the beginning of cycle 1 prior to the administration of Doxorubicin. On cycle 1 also prior to ZDV administration, after the 5th dose of intravenous ZDV, and within the last 7 days of the end of

cycle 1 (Appendix III). Blood for measurement of EBV reactivation will be obtained prior to the initiation of treatment, on Day 2 prior to ZDV administration, and after the 5th dose of intravenous ZDV of cycle 1 (Appendix IV).

X[2] If the patient had initial bone marrow involvement, then a repeat bone marrow biopsy should be performed when the patient has no other evidence of disease in order to determine CR status.

X[3] Post therapy visits will occur (unless otherwise noted) every 3 months for 2 years, every 6 months for the third-fifth years and yearly thereafter. See Section 5.3 for more details. (+/- 4 weeks)

X[4] EBV viral load, HIV-1 viral load, and T-cell subsets will be obtained after completion of cycle 2 (minus 1-week window), post cycle 6 (2-6 weeks after last treatment), and every 3 months (+/- 4 weeks) for one year.

X[5] Immune activation and inflammation markers will be obtained after completion of cycle 2 and 4 (minus 1-week window), post cycle 6 (2-6 weeks after last treatment), and/or anytime upon disease progression, or disease relapse during follow-up period

X[6] CT or CT/PET will be performed every 6 months (+/- 4 weeks) for two years or more often if clinically indicated.

X[7] Uric acid and Phosphorus are only required at baseline (+/- 2 weeks)

X[8] Baseline evaluation laboratory tests may be used toward cycle 1 requirements, if done within 48 hours of treatment initiation.

X[9] HBV and/or HCV viral load in subjects who were HBV antigen or core antibody positive, or HCV antibody positive with undetectable viral particles or viral load at baseline, at the end of cycles 2, 4, and 2-6 weeks post Day 1 of cycle 6.

APPENDIX III: EBV VIRAL LOAD SPECIMEN COLLECTION AND PREPARATION

Collect two 7 ml green top (heparin) tubes and keep at room temperature until processing. EBV viral load will be obtained at the following times: the beginning of cycle 1,prior to the administration of doxorubicin, on cycle 1 prior to the administration of ZDV 20-24 hours after the administration of doxorubicin (to determine whether Doxorubicin induces EBV proliferation), after the 5th dose of intravenous ZDV cycle 1 (to determine if ZDV induces the EBV lytic cycle), and within the last 7 days of the end of cycle 1. Thereafter, EBV viral load will be done within the last 7 days of the end of cycles 3 and 6. During follow-up EBV viral load will be obtained upon disease progression or relapse, or after completion of treatment during progression-free time while on study at least every three months for one year.

Sample Processing: Peripheral blood will be collected and centrifuged at 2 x 15 minutes (at 1,500 x g). Plasma samples will be separated and transferred into sterile polypropylene tubes. The plasma samples will be stored in aliquots at -80°C until further processing. DNA from plasma will be extracted using QIAamp MinElute Virus Spin Kit (QIAGEN, Valencia, CA). DNA samples will then be subjected to real time PCR analysis in a Roche LightCycler 2.0 system (Roche, Indianapolis, IN) using Taqman probes for the EBV EBNA-1 gene [redacted]

[redacted]

APPENDIX IV
MEASUREMENT OF EBV REACTIVATION IN PERIPHERAL BLOOD MEMORY B CELLS BEFORE AND AFTER TREATMENT

Collect two yellow cap top tubes and keep at room temperature until processing.

Venous blood will be collected in 2 yellow top tubes (10 ml capacity) prior to the initiation of treatment and on Day 2 of cycle 1 prior to the administration of ZDV 20-24 hours after the administration of doxorubicin, and after 5th dose of ZDV on cycle 1 only. Peripheral blood mononuclear cell (PMBC) will be freshly isolated by centrifugation using Lymphoprep (ficol). The extracted lymphocytes will be centrifuged, and cryopreserved at 80°C (+/- 10°C) in the form of cell pellets for later use. This material will serve as the source for RNA, to be extracted using standard methods, for EBV gene expression array studies to be performed by [redacted]

APPENDIX V: DETERMINING EBV GENE EXPRESSION PATTERN AND VIRAL ENCODED KINASES

If tissue is available, 10-15 unstained (blank) paraffin tissue sections will be prepared for immunohistochemistry or *in situ* hybridization for BGLF/vPK, BXLF1/vTK, LMP-1, and BZLF1 (to be performed at Ohio State University), and EBV gene expression array studies [redacted]. If excess tissue is available if subject consents, tissue will be stored for future studies that may include genome wide gene expression arrays, next generation sequencing, methylation studies, and the use of new genomic or proteomic technologies that may become available in the future.

[redacted]

APPENDIX VI: FORMS SUBMISSION TABLE

Form	Baseline	Cycle 1	Cycle 2, 4, 6	Post-therapy	PRN
Registration	X				
Eligibility	X				
History	X				
Prior Medications	X				
Vital Signs & Physical Exam	X	X	X	X	
Hematology	X	X	X	X	
Chemistries and LFTs	X	X	X	X	
Study Drug Treatment		X	X		
Concomitant Medications		X	X	X	
Adverse Events		X	X	X	
Lymphoma Evaluation	X		X	X	
Comments					X
Missed Visits					X
Off Study					X
Death					X

APPENDIX VII: DRUG DIARY

YOU MUST KEEP THIS DIARY AND BRING IT TO EVERY APPOINTMENT.

Study ID #: _____
Cycle ____

Please record in the chart below the date and time that your dose was taken. Be sure to record the doses when you take them, and avoid writing entries for several days at once. When you are finished, bring this diary with you when you next see the Doctor or Study Staff. In the "Comments" section write any problems you are having with the medicines or if you missed a dose and why or if you only took part of the medicine.

	Leucovorin 25 mg Every 6 hours (Times)	Zidovudine 1200 mg twice daily (Times)	Hydroxyurea 500 mg twice daily (Times)	**Comments**
Date:				
Date:				
Date:				
Date:				
Date:				
Date:				

APPENDIX VIII
MEASUREMENT OF IMMUNE ACTIVATION MARKERS AND INFLAMMATION IN PERIPHERAL BLOOD T CELLS

Collect 8-10 ml of venous blood in one purple cap top tube (10 ml capacity) and keep at room temperature until processing and overnight shipping to the University of Miami Miller School of Medicine, Center for AIDS Research (CFAR) laboratory core [redacted]. If unable to ship from Monday through Wednesday, or during holidays, plasma PMBC will be processed per processing instructions below. Blood will be collected prior to the initiation of treatment, at the end of cycles 2, 4, and 6, and/or upon disease progression or disease relapse during follow-up period. Extracted plasma will be subjected to measurement of inflammatory cytokines using Multiplex Luminex assays. The extracted lymphocytes will be subjected to 12-color cytometry studies for detection of immune cell activation marker profile (i.e. CD38, HLA-DR, Ki-67, PD-1).

Sample Processing:

Plasma separation:
Approximately 4 ml of peripheral blood will be centrifuged at 2 x 15 minutes (at 1,500 x g). Plasma samples will be separated and transferred into sterile polypropylene tubes. The plasma samples may be stored in aliquots at -80°C until further processing.

PBMC isolation:
Approximately 4 ml of freshly isolated blood will be centrifuged using Lymphoprep (ficol) method. The extracted lymphocytes will be cryopreserved in freezing media containing DMSO in liquid Nitrogen tank.

Specimen Shipment: Samples must be labeled with date and time of collection, specimen type (i.e. blood, plasma, or PBMCs) and protocol subject ID #. Samples may ONLY be shipped on Monday through Wednesday (except during holiday weeks) to the following address:

[redacted]