Abbreviated Title: EPOCH-RC in recurrent lymphoma
CC Protocol #: 10-C-0011 J
Version Date: 08/03/2018
NCT Number: NCT01030900

PHASE II TRIAL OF ALEMTUZUMAB (CAMPATH) AND DOSE-ADJUSTED EPOCH-RITUXIMAB (DA-EPOCH-R) IN RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL AND HODGKIN LYMPHOMAS

NCI Principal Investigator: Wyndham H. Wilson, M.D., Ph.D. [A-F]
Lymphoid Malignancies Branch (LYMB)
Center for Cancer Research (CCR)
National Cancer Institute (NCI)
National Institutes of Health (NIH)
Building 10, Room 4N115
9000 Rockville Pike
Bethesda, MD 20892
Phone: 240-760-6092
Email: wilsonw@mail.nih.gov

Commercial Agents:
EPOCH-R = etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab, alemtuzumab

Investigational Agents:

Not applicable.

PRÉCIS

Background:

- Two signatures of the microenvironment were recently identified that are predictive of outcome in patients with newly diagnosed DLBCL treated with R-CHOP. These signatures, called 'stromal 1' and 'stromal 2', are associated with genes expressed by infiltrating mononuclear cells. The stromal 2 signature, which includes genes associated with angiogenesis, is predictive of an inferior outcome. Based on these observations, we are interested in targeting the reactive cells in the microenvironment as a therapeutic strategy in patients with relapsed and refractory DLBCL. Along the same principles, we are also including patients with relapsed Hodgkin lymphoma (HL). The surrounding reactive cells around Hodgkin Reed Sternberg (HRS) cells are now not thought to be bystander cells and they appear to provide important survival signals to HRS cells.
- CD52 is one such promising target that is highly expressed in most of these infiltrating cells and on most DLBCL although not on HRS cells specifically. Anti-CD52 antibodies may have therapeutic value by depleting reactive B and T cells, and monocytes from the microenvironment.
- The dose of alemtuzumab in combination with DA-EPOCH is 30 mg IV, as determined by a prior study done in patients with untreated peripheral T-cell lymphoma. The main toxicities of this combination are myelosuppression and opportunistic infections.
- An important component of this study will be to obtain tumor tissue for gene expression profiling and to assess microenvironment signatures and look at other molecular signatures and targets before treatment and in patients who progress and ultimately correlate response and outcome with these various end-points.

Objective:

- Assess response, progression free survival (PFS) and overall survival (OS) in relapsed/refractory DLBCL and Hodgkin Lymphoma.

Eligibility:

- Previously treated or refractory classical large B-cell lymphomas, Grey-zone lymphoma and Hodgkin lymphoma, including Lymphocyte predominant Hodgkin Lymphoma (LPHL).
- Age $\geq$ 18 years with adequate organ functions.
- HIV negative and no active CNS lymphoma.

Study Design:

- Patients will receive 30mg of Alemtuzumab on day 1 of therapy, followed by Rituximab on day 1 and dose-adjusted EPOCH chemotherapy days 1-5, up to six cycles of therapy.
- Tumor biopsies will be done before treatment, after 1 cycle of therapy and at relapse.
- It is anticipated that up to 10-15 patients per year may be enrolled onto this trial. Thus, accrual of up to 52 patients is expected to require approximately 4-5 years.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

Assess response, progression free survival (PFS) and overall survival (OS) in relapsed/refractory DLBCL and Hodgkin lymphoma.

1.1.2 Secondary Objective

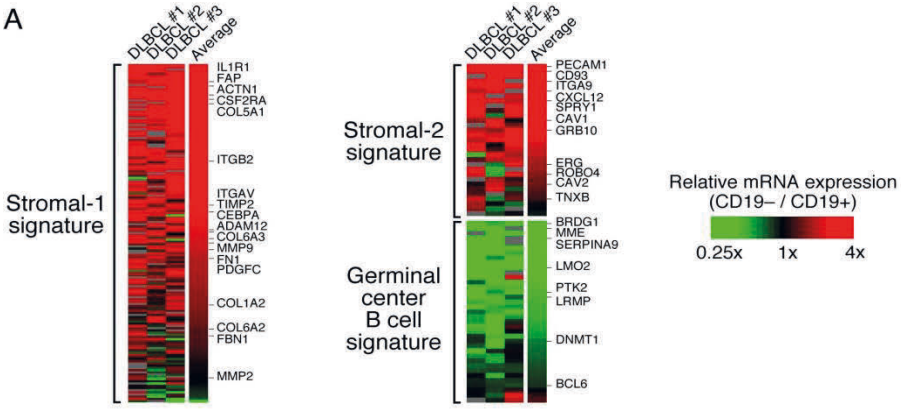
Correlate clinical outcomes with microenvironment/stromal molecular signatures by gene expression profiling and immunohistochemistry on study and at relapse after DA-EPOCH-RC.

1.2 BACKGROUND AND RATIONALE

1.2.1 Hypothesis

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) – although the addition of rituximab to anthracycline-based chemotherapy has significantly improved the outcome of patients with the disease, it remains incurable in up to 50% of cases. We are interested in building upon insights from the molecular characterization of DLBCL, which includes the identification of three distinct subtypes (ABC, GCB and PMBL) by gene expression profiling – these subtypes are associated with different prognoses [1]. This has paved the way for the development of therapeutic strategies that target specific pathways. Indeed, we recently demonstrated that the ABC type may be preferentially targeted (when compared to the GCB type) by the addition of bortezomib to anthracycline-based chemotherapy [2].

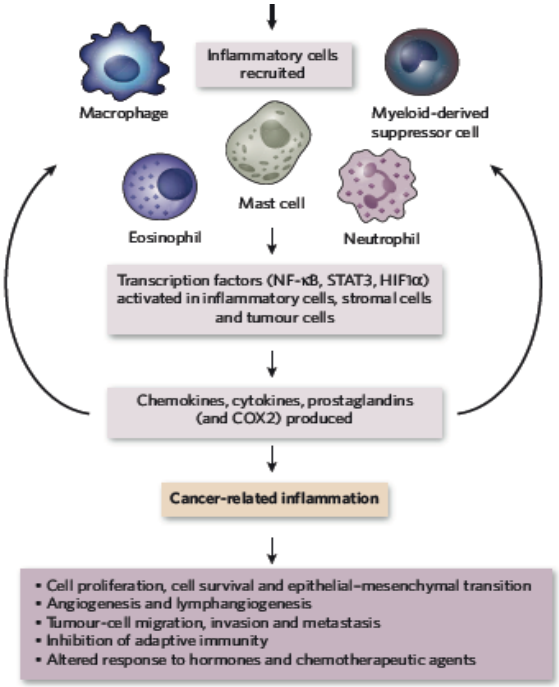
Recent work led by Dr. Staudt's group has identified two signatures of the microenvironment that are predictive of R-CHOP outcome in DLBCL[3]. These signatures, called stromal 1 and stromal 2, are associated with genes expressed by infiltrating mononuclear cells including macrophages, natural killer cells, T-cells and myeloid cells. The stromal 2 signature, which includes genes associated with angiogenesis, is predictive of an inferior outcome. Based on these observations, we are interested in targeting the microenvironment as a therapeutic strategy.



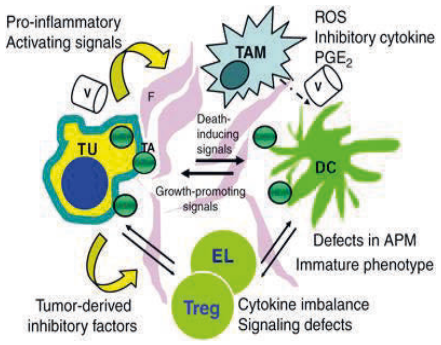
The microenvironment is usually tumor dependent and may involve tumor-induced interactions. It is comprised of tumor cells, stroma, blood vessels, infiltrating inflammatory cells and a variety of other associated tissue cells. Potential strategies include targeting the infiltrating "normal" cells and/or tumor angiogenesis. We hypothesize that targeting the infiltrating cells more

specifically is a potentially important strategy because the microenvironment may promote tumor survival through multiple mechanisms, such as angiogenesis and pro-survival signals.

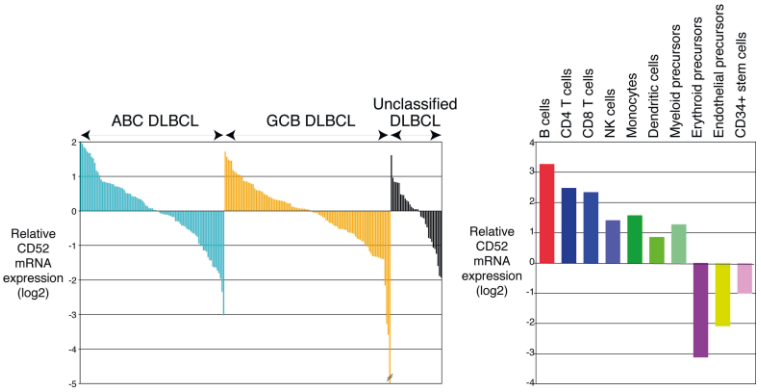
Immune cells present in the tumor microenvironment include those mediating adaptive immunity, T lymphocytes, dendritic cells and occasional B cells, as well as effectors of innate immunity, macrophages, polymorphonuclear leukocytes and natural killer cells[4]. Although various immune effector cells (e.g. cytotoxic T lymphocytes) are recruited to the tumor site, their anti-tumor functions may be downregulated in response to tumor-derived signals. This is because the tumor microenvironment is enriched in regulatory T cells (T_{reg}), tumor-associated macrophages (TAM) as well as myeloid suppressor cells (MSC)[5-7]. All three immune cell types act in concert to promote tumor growth and suppress immune cell functions. It has been proposed that NF-κB pathway plays a key role in activation of signaling in cancer cells as well as tumor-infiltrating leukocytes. NF-κB activation in these cells leads to secretion of TNF-α or other pro-inflammatory cytokines (e.g. IL-6 and IL-8) which are responsible for cell proliferation, angiogenesis and tumor-cell migration (see figure below) [8, 9].



Regulatory T cells (T_{reg}) are capable of suppressing proliferation of other T cells in the microenvironment through contact-dependent mechanisms or IL-10 and TGF-β secretion[10-12]. Likewise, TAMs inhibit lymphocyte functions through release of inhibitory cytokines such as IL-10, prostaglandins or reactive oxygen species (ROS) [13, 14]. Myeloid suppressor cells (MSC) are bone marrow-derived immature dendritic cells[15]. They, on the other hand, promote tumor growth and suppress immune cell functions through copious production of an enzyme involved in L-arginine metabolism, arginase 1, which synergizes with inducible nitric oxide synthase (iNOS) to increase superoxide and nitric oxide production, thereby blunting lymphocyte responses[16]

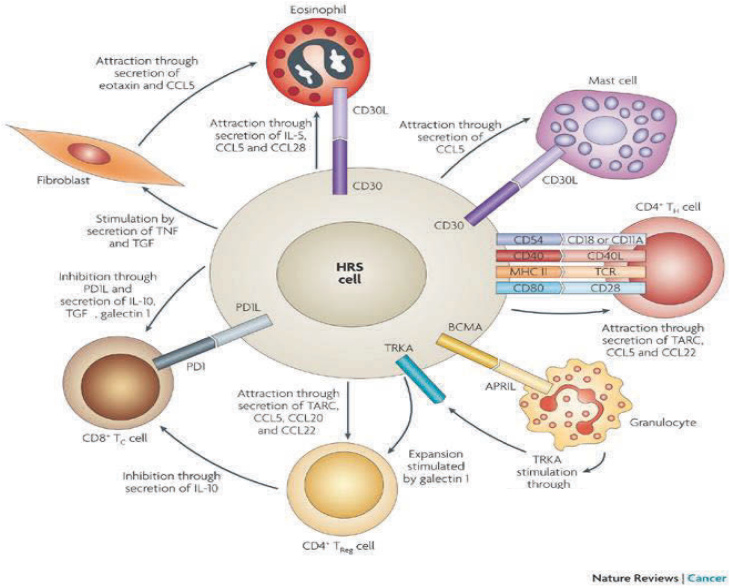


A promising therapeutic target is CD52 which is highly expressed on most DLBCL, including both ABC and GCB types, and on many of the infiltrating cells (monocytes, macrophages, dendritic cells and myeloid precursors) in the microenvironment (Gene Expression Profiling of CD52 shown below). Furthermore, Rodig et al. reported CD52 expression by immunohistochemical techniques in 75% of DLBCL[17].



We are also interested in investigating the efficacy of this combination in patients with relapsed or refractory Hodgkin lymphoma (HL). Hodgkin's lymphoma is characterized by an infiltration of many different types of cells of the immune system into the lymphoma tissue, including T cells, B cells, plasma cells, neutrophils, eosinophils and mast cells, such that the Hodgkin reed Sternberg (HRS) cells themselves usually represent only about 1% of cells in the tumor. There is evidence that many of the cells are actively attracted by HRS cells. For example, HRS cells secrete CCL5 (RANTES), CCL17 (TARC) and CCL22, which attract T_H2 cells and T_{reg} cells[18-20]. The secretion of IL-5, CCL5, CCL28 and granulocyte–macrophage colony-stimulating factor by HRS cells presumably causes the recruitment of eosinophils into the Hodgkin's lymphoma microenvironment. CCL5 additionally attracts mast cells. HRS cells also secrete IL-8, which attracts neutrophils. These reactive cells are not bystander cells, as previously thought. By contrast, they frequently provide survival signals to HRS cells[21]. The role of the microenvironment in supporting the survival and growth of HRS cells is best illustrated by the difficulty of establishing HRS-derived cell lines. Several observations indicate that HRS cells are dependent on survival signals received from other cells: it is difficult to grow HRS cells in culture; HRS cells do not survive in immunodeficient mice; HRS cells are rarely found in peripheral blood; and, even when they metastasize into non-lymphoid organs, they are embedded

in their typical microenvironment[22]. Some examples of these survival signals include triggering of CD40 signalling[23], activation of transmembrane activator and calcium-modulator and cyclophilin-ligand interactor (TACI) and B-cell maturation antigen (BCMA) through production of their ligand APRIL by neutrophils [24], and perhaps activation of CD30 through CD30L-expressing mast cells and eosinophils [25 26].



HRS cells also orchestrate their cellular microenvironment to evade an attack by cytotoxic T cells or natural killer cells. A considerable fraction of infiltrating $CD4^+$ T cells are T_{reg} cells, which have been shown to have immunosuppressive activity on Hodgkin's lymphoma-infiltrating cytotoxic T cells [27]. HRS cells may further modulate their cellular microenvironment by shifting the T_H response from an anti-cellular T_H1 response to a humoral T_H2 response, which often has tumor-promoting activities. HRS cells also produce the immunosuppressive cytokines IL-10 and TGFβ, and galectin 1 and prostaglandin E_2, which inhibit T-cell effector functions [28, 29 30]. Therefore, it is ironic that the host's own immune cells are providing survival factors to the malignant cells, creating an 'immune-betrayal' phenomenon. Eliminating these reactive cells from the microenvironment may deprive HRS cells from critical survival factors and may lead to their growth arrest and death. CD52 is not expressed by HRS cells; however, anti-CD52 antibodies may have therapeutic values in HL by depleting reactive B and T cells and monocytes from the microenvironment. Therefore, alemtuzumab, a monoclonal antibody to CD52, is a very interesting drug to investigate in both these diseases, given the high CD52 expression in DLBCL tumor cells and in the surrounding microenvironment of both these tumors.

Over the past few years, we have been conducting a study of alemtuzumab in combination with DA-EPOCH in untreated peripheral T-cell lymphoma (PTCL) and have determined the safe dose of alemtuzumab in combination with DA-EPOCH to be 30 mg IV[31]. The main toxicities were myelosuppression and opportunistic infections.

Based on our hypothesis, we wish to investigate this combination (DA-EPOCH-RC) in patients with relapsed/refractory aggressive DLBCL and Hodgkin lymphoma. An important component of this study will be to obtain tumor tissue for gene expression profiling and to assess microenvironment signatures and look at other molecular signatures and targets before treatment, after 1 cycle of therapy and in patients who progress. Ultimately, we would like to correlate the

responses and outcomes with these various end-points. Of the DLBCL subtypes, we are particularly interested in investigating the efficacy of this combination in T-cell rich DLBCL and in elucidating the molecular signature of these tumors and correlating that with response. These tumors have not been well molecularly characterized and may significantly benefit from the addition of alemtuzumab.

1.2.2 EPOCH in Relapsed lymphomas

The dose-adjusted EPOCH (DA-EPOCH) regimen was initially studied in 131 patients with relapsed or resistant lymphomas[32]. 46% of the group generally had a poor prognosis as determined by a high-intermediate or high International Prognostic Index (IPI) scores. All patients were extensively pretreated having received a median of 8 (1-17) different drugs. 57% of patients had received all 5 drugs of the DA-EPOCH regimen and 88% had received at least 4 of the agents. All but 4 (6%) patients had previously received doxorubicin. Among 125 assessable patients, 93 (74%) achieved objective responses, including 30 (24%) complete and 63 (50%) partial responses. To determine if the infusional schedule increased efficacy, we analyzed the response to EPOCH in patients who showed no response to their last combination regimen (i.e. resistant disease). In this group of 42 patients, 57% responded to EPOCH. Among patients with chemotherapy-sensitive disease, 83% responded with 33% complete responses as shown in the table below.

Category	No	CR	(%)	PR	(%)	RR	(%)
Total	125	30	(24%:)	62	(50%:)	92	(74%)
Sensitive Disease	81	27	(33%:)	40	(49%:)	67	(83%)
Resistant Disease	42	2	(5%:)	22	(52%:)	24	(57%)
Dox-Resistant	20	0	(0%)	10	(50%)	10	(50%)
No of Regimens							
1	67	21	(31%:)	33	(51%:)	54	(81%)
2	26	5	(19%)	14	(54%:)	19	(73%)
≥ 3	30	3	(10%)	14	(47%:)	17	(57%)

High-dose therapy with stem-cell transplant (SCT) is generally believed to provide the best outcome for patients with chemosensitive relapsed aggressive lymphomas. Hence a clinically important role for salvage chemotherapy is to cyto-reduce patients who are candidates for SCT. In this study, 28 patients with aggressive lymphomas who had relapsed after CR, 89% responded to EPOCH, including 54% who had complete responses, and became potential candidates for SCT. Notably, among the 33 patients with sensitive aggressive lymphomas who did not receive a stem cell transplant, 18% were event-free and 37% were alive at 5 years, suggesting that EPOCH alone may provide effective salvage for a proportion of patients. Furthermore, 19 patients who had chemotherapy-resistant disease, 7 (37%) responded to EPOCH, including 1 CR. Overall, patients had a median OS of 17.5 months with 41% and 26%, respectively, alive at 3 and 6 years, and a median EFS of 7 months, with 15% and 10%, respectively, event-free at 3 and 6 years. The main toxicities included brief neutropenia, usually less than 4 days and occurring around day 10 to 14 of treatment, and minimal gastrointestinal toxicity. Thrombocytopenia below 50,000/μL occurred on 24% of cycles, and neutropenia occurred on 48% of cycles with hospitalization for neutropenia with fever on 18% of cycles. There were three infection-related deaths. Neurological toxicity necessitating vincristine reduction occurred in 10% of cycles. Cardiac toxicity, a major limitation of doxorubicin, was rarely seen (3% of patients) despite no maximum allowable doxorubicin exposure. We observed a modest and clinically insignificant decline in the median EF over multiple cycles of EPOCH; a paired t test (p_2) analysis comparing cycle 0-1 vs. cycle 2-3, cycle 4-5 and cycle ≥ 6 revealed a difference of -2.58%, (0.13); -5.5%, (0.0038) and -

6.32%, (0.020), respectively. Although the patients were heavily pretreated, the toxicity profile of EPOCH allows delivery of 71%, 92%, 92% and 93% of planned cyclophosphamide, doxorubicin, vincristine and etoposide, respectively.

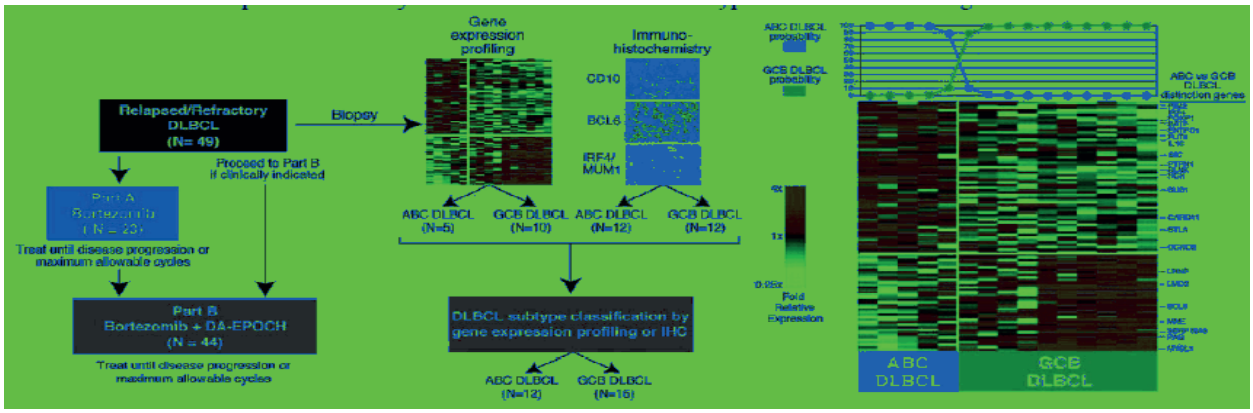
1.2.3 EPOCH-Rituximab in Relapsed lymphomas

With the advent of Rituximab and its addition to CHOP, that significantly improved the outcome of DLBCL and R-CHOP soon became the new standard in untreated DLBCL[33-35]. At our institution we have also shown that DA-EPOCH-R to be an effective therapy in untreated DLBCL. Jermann et al. reported the use of EPOCH-Rituximab combination as salvage therapy in a phase II study[36]. Patients with relapsed or refractory CD20-positive large B-cell and mantle-cell lymphoma were offered treatment with rituximab 375 mg/m2 intravenously (i.v.) on day 1, doxorubicin 15 mg/m2 as a continuous i.v. infusion on days 2-4, etoposide 65 mg/m2 as a continuous i.v. infusion on days 2-4, vincristine 0.5 mg as a continuous i.v. infusion on days 2-4, cyclophosphamide 750 mg/m2 i.v. on day 5 and prednisone 60 mg/m2 orally on days 1-14. Fifty patients, with a median age of 56 years (range 23-72), entered the study. Twenty-five had primary diffuse large B-cell lymphoma, 18 transformed large B-cell lymphoma and seven mantle-cell lymphoma. The median number of prior chemotherapy regimens was 1.7 (range one to four). The median number of treatment cycles was four (range one to six). Objective responses were obtained in 68% of patients (28% complete responses, 40% partial responses). Nineteen patients received consolidating high-dose chemotherapy with autologous stem-cell transplantation. The median follow-up was 33 months. Three patients developed a secondary myelodysplastic syndrome. The median overall survival was 17.9 months; the projected overall survival at 1, 2 and 3 years was 66, 42 and 35%, respectively. The median event-free survival was 11.8 months; the projected event-free survival at 1, 2 and 3 years was 50, 30 and 26%, respectively.

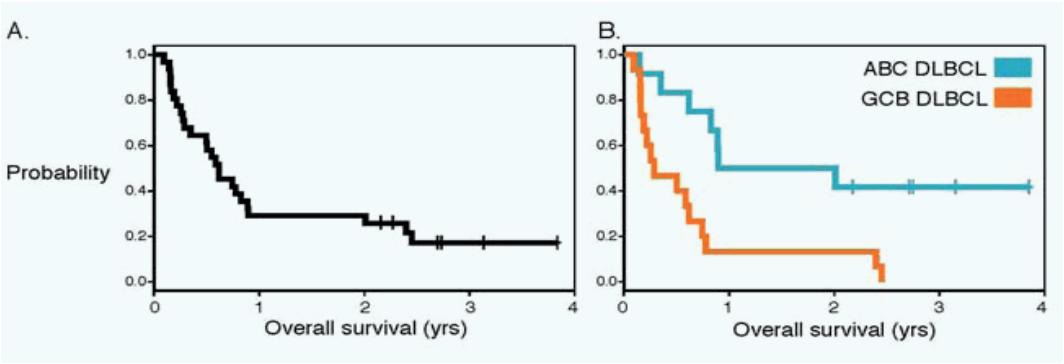
1.2.4 EPOCH-Bortezomib in Relapsed DLBCL

We have published our results correlating the outcomes of the different molecular subtypes of DLBCL with DA-EPOCH-R[37]. We analyzed 72 untreated patients with GCB and ABC DLBCL. Similar to recent findings with R-CHOP, the ABC subtype tended to be associated with a worse PFS compared with GCB DLBCL. These findings suggest that DA-EPOCH-R outcome is impacted by the biology associated with ABC subtype, possibly driven by NFκB activation[1, 38, 39].

To further study the hypothesis that inhibition of NFκB might sensitize ABC but not GCB DLBCL to chemotherapy. We did a study combining bortezomib, a proteasome inhibitor, with DA-EPOCH (DA-EPOCH-B) in 49 patients with relapsed or refractory DLBCL to investigate if ABC compared to GCB DLBCL was more responsive to DA-EPOCH-B[2]. In vitro, bortezomib blocks degradation of phosphorylated IκBα and consequently inhibited NFκB activity in ABC DLBCL cell lines. The study was divided into 2 parts (see figure below), in Part A patients received bortezomib alone if they were clinically able; patients who progressed or was too ill to justify bortezomib alone, they received DA-EPOCH-B in part B.



Among 23 patients in part A, there was only 1 partial response, indicating bortezomib alone has no activity. Overall, all 44 patients in Part B, 15 (34%) responded including 8 complete responses. Similar response rates were also seen in the 27 patients with de novo DLBCL. Among all patients with de novo DLBCL, the median survival was 8 months. However, when we analyzed the responses and overall survival in the de novo GCB and ABC DLBCL patients who received DA-EPOCH-B, there were significant differences between the 2 groups. The overall response rate was 13% in GCB DLBCL compared to 83% in ABC DLBCL (P = 0.0004). It was particularly striking that 41.5% of patients with ABC DLBCL achieved complete remission compared to only 6.5% for those with GCB DLBCL. The median overall survival was 3.4 and 10.8 months, respectively (P = 0.0026) for the ABC and GCB subtypes (see figure below). This study highlights the fact that because of genetic differences between the DLBCL subtypes, preferential activity to a targeted agent may be seen in one particular subtype over another. This further underscores the importance of pairing molecular characterization and clinical outcome in DLBCL for the rational development of targeted agents in this disease.



1.2.5 EPOCH-Alemtuzumab in untreated T-cell lymphomas

Alemtuzumab is a humanized antilymphocyte monoclonal antibody engineered by grafting the rodent hypervariable complementarily determining regions into a human immunoglobulin molecule. It is directed at CD52, a 12 amino acid protein that is highly glycosylated and linked to the cell membrane by phosphytidylinositolglycan linkage. CD52 is highly expressed on membrane lipid shafts of all B and T lymphocytes at most stages of differentiation (except plasma cells), as well as on monocytes, macrophages, eosinophils, natural killer cells and dendritic cells[40-42]. However, monocytes appear to be resistant to Alemtuzumab-mediated lysis. Monocytes disappear from the peripheral circulation but reappear shortly after treatment

suggesting a sequestration without lysis or regeneration from a precursor pool that is CD52-negative. It is estimated that there are 5 x 10^5 antibody sites per lymphocyte and the antigen does not modulate from the cell surface. Alemtuzumab is thought to mediate cell lysis through complement[43, 44] or antibody-dependent cell-mediated cytotoxicity[45-47]. Recent studies have also shown Alemtuzumab may induce rapid cell death in vitro through both a non-classic caspase-independent[48, 49] and caspase dependent pathways[50, 51]. The function of CD52 is not known but a recent paper suggested that CD52 may contribute to the activation of T-regulatory cells[52].

The combination of Alemtuzumab and DA-EPOCH chemotherapy has been studied in 29 patients with untreated CD52-positive T-cell lymphomas[6]. A single infusion of Alemtuzumab (30, 60, or 90 mg) was given over 12 hours before each cycle of chemotherapy; patients were premedicated with prednisone 12 hours before the Alemtuzumab infusion was initiated. DA-EPOCH was initiated immediately following completion of the Alemtuzumab infusion. Toxicity during the first cycle of treatment was used to determine Alemtuzumab dose escalation. Patients were treated with escalated doses of Alemtuzumab in cohorts of three patients; 4 patients were treated with 30 mg, and subsequent cohorts were treated with 60 and 90 mg. All but 3 were stage III or IV at presentation.

Among the 28 patients evaluated for response there were 15 complete remissions, 8 partial remissions, 2 progressive diseases and 4 who were not evaluable for response. The median duration of complete response is 9 months with nine patients in continuous complete remission for 3-58 months. Most patients experienced grade 1–2 allergic and infusional reactions manifested as fever, chills, and urticaria. However, 4 patients developed bone marrow aplasia that prevented further treatment, 1 patient at the 30mg dose level, 1 patient at the 60 mg dose level (cycle 3 and 5) and 2 patients at the 90 mg dose level (cycle 4 and 5) of Alemtuzumab. Three of these four patients were CMV antigen positive and were treated with oral or intravenous ganciclovir. There was more myelosuppression in patients treated with campath and EPOCH compared with those treated with EPOCH alone, 29% vs. 8% had platelet counts of less than 25K, 100% vs. 60% for grade 4 neutropenia, 35% vs. 15% incidence of febrile neutropenia. But with the 30 mg dose of Alemtuzumab myelosuppression was equivalent to that observed with EPOCH alone or in combination with rituximab.

We evaluated the ability to dose escalate EPOCH chemotherapy in patients treated with Alemtuzumab and found that there was no significant difference in dose escalation or reduction with the regimen. All but one patient developed grade 4 lymphopenia. Documented infections were common and the most common pathogens detected were viral with 20 patients experiencing viral infection or reactivation. The most common virus detected was cytomegalovirus that was generally asymptomatic and detected only by a positive CMV PCR reaction. Another viral pathogen not commonly seen with EPOCH alone or with rituximab was hemorrhagic cystitis associated with BK virus infection in the urine, occurring in 8 patients, which resolved despite continued treatment. Four patients had rising EBV DNA titers in the blood but there were no cases of EBV lymphoproliferative disease. Two patients had reactivation of HSV despite prophylaxis. Other viral infections included influenza, rotavirus and respiratory syncytial virus. Bacterial infections, both gram positive and gram negative, occurred in 11 patients with staphylococcal infections as the most common organism isolated. Four patients had fungus isolated but only one was thought to have tissue invasion. Four patients died during treatment with Alemtuzumab and EPOCH. Two patients died of neutropenic sepsis, one died of disseminated toxoplasmosis, and one had a witnessed cardiac arrest during EPOCH

chemotherapy infusion that was thought to be related to cardiac arrhythmia. The 30 mg dose level of Alemtuzumab appears safe to administer in combination with DA-EPOCH and there has been no bone marrow suppression that prevented completion of therapy in any patient treated at the 30 mg dose of Alemtuzumab. Based on the results of this study, the 30mg dose was chosen as the treatment dose for Alemtuzumab in our current study and we have also incorporated CMV and toxoplasmosis prophylaxis routinely for all patients.

1.2.6 EPOCH in Relapsed Hodgkin Lymphoma

The EPOCH infusional regimen has also shown to be effective and well tolerated and should be considered as salvage therapy prior to stem cell transplant or for palliation in patient with incurable Hodgkin lymphoma. We performed a study in 54 patients with relapsed/refractory Hodgkin lymphoma[53]. Patients received fixed dose EPOCH chemotherapy (etoposide 200 mg/m^2, vincristine 1.6 mg/m^2 (no cap) and doxorubicin 40 mg/m^2 CIVI x 96-hrs D1-4; cyclophosphamide 750 mg/m^2 IV D5 and prednisone 60 mg/m^2 daily D1-6) with G-CSF q21 days. Histology included nodular sclerosis 34 (64%), mixed cellularity 3 (25%), and lymphocyte depleted 5 (9%) classical HL, and nodular lymphocyte predominant HL 1 (2%). Most of the patient had advanced disease, with stage III in 11 (21%) and IV in 30 (56%). In addition, 24 patients (45%) had more than 2 prior regimens. Overall, 44% of patients achieved complete remissions, and a progression-free survival (PFS) and overall survival (OS) of 41% and 21% respectively, with a median follow-up of 68 months. The median progression-free (PFS) and overall survivals (OS) are 10 and 40 months at 68 months median follow-up. Among 33 pts who underwent stem-cell transplant, median PFS was 15 months.

Combination of Rituximab and chemotherapy in Relapsed Hodgkin Lymphoma: CD20 antigen is infrequently expressed by HRS cells. The approach of using rituximab to deplete the CD20+ B cells from the HL microenvironment was studied by investigators from MD Anderson. A total of 22 heavily pretreated patients with classical HL were treated with 6-weekly doses of rituximab, of whom six demonstrated CD20 expression by HRS cells. In all, five patients (22%) achieved partial or complete remissions and eight additional patients had stable disease[54]. In a follow-up study, the same investigators combined rituximab with ABVD chemotherapy to treat patients with newly diagnosed classical HL, and with gemcitabine to treat patients with relapsed classical HL In the first study, 52 patients with classical HL were treated with rituximab plus ABVD[55]. With a median follow-up of 32 months, the estimated event-free survival (EFS) was 82% and overall survival 100%. Importantly, the EFS was improved for all risk categories: for patients with a prognostic score of 0–1 the EFS was 92%, for scores 0–2, EFS was 86% and for scores 3–5, it was 73%. These data are encouraging, but has not been confirmed in a randomized trial. In the second study, rituximab was combined with gemcitabine for the treatment of patients with relapsed/refractory classical HL[56]. The median number of prior treatment regimens was four (range one to six) and 17 (65%) were refractory to their last regimen. Of the patients studied, 14 patients (54%) had received prior SCT, and 21 patients (81%) were previously treated with platinum-based therapy. Treatment was reasonably well-tolerated, with the most frequent toxicity being thrombocytopenia and neutropenia. A total of 13 patients (50%) responded to rituximab plus gemcitabine therapy, regardless of CD20 expression by HRS cells. These results compared favorably with the reported single agent activity of gemcitabine in less heavily pretreated patients.

Targeting the microenvironment with DA-EPOCH-R-C is a novel approach. We hypothesize that eliminating the reactive cells from the microenvironment may deprive malignant cells from

critical survival factors leading to cell death. Therefore, an important aspect of this study is to further understand these interactions which may lead to the identification of new molecular signatures and targets. Ultimately, the goal of this approach is to improve outcomes in patients with relapsed/refractory aggressive DLBCL and Hodgkin lymphoma.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Previously treated or refractory large B-cell lymphomas, Grey-zone lymphoma, Hodgkin Lymphoma, including Lymphocyte predominant Hodgkin Lymphoma (LPHL)

2.1.1.2 Confirmed pathological diagnosis by the Laboratory of Pathology, NCI.

2.1.1.3 Age ≥18 years.

2.1.1.4 ECOG performance 0-2

2.1.1.5 Laboratory tests: ANC ≥ 1000/mm3, platelet ≥ 75,000/mm3. Creatinine ≤1.5 mg/dL or creatinine clearance ≥ 60 ml/min; AST and ALT ≤ 5x ULN. Total bilirubin < 2.0 mg/dl except < 5mg/dL in patients with Gilbert's (as defined as > 80% unconjugated hyperbilirubinemia without other known cause); unless impairment due to organ involvement by lymphoma.

2.1.2 Exclusion Criteria

2.1.2.1 Active symptomatic ischemic heart disease, myocardial infarction or congestive heart failure within the past year. If ECHO is obtained, the LVEF should exceed 40%.

2.1.2.2 HIV positive, because of the unknown effects of combined therapy with chemotherapy and an immunosuppressive agent on HIV progression

2.1.2.3 Female subject of child-bearing potential not willing to use an acceptable method of birth control (i.e. a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study and two years beyond treatment completion.

2.1.2.4 Female subject pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative serum β-human chorionic gonadotrophin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for women without child-bearing potential.

2.1.2.5 Male subject unwilling to use an acceptable method for contraception for the duration of the study and one year beyond treatment completion.

2.1.2.6 Invasive or active malignancy in past 2 years.

2.1.2.7 Serious concomitant medical illnesses that would jeopardize the patient's ability to receive the regimen with reasonable safety.

2.1.2.8 Active CNS lymphoma. These patient have a poor prognosis and because they frequently develop progressive neurological dysfunction that would confound the

evaluation of neurological and other adverse events.

2.1.2.9 Systemic cytotoxic therapy within 3 weeks of treatment.

2.2 SCREENING EVALUATION

Evaluation pre-treatment (Tests to be done within 4 weeks of treatment except laboratory tests in 2.2.2 and 2.2.8 must be done within 72 hours of treatment):

2.2.1 Complete history and physical examination with assessment of performance status

2.2.2 Laboratory tests: CBC/differential; prothrombin time, partial thromboplastin time; total and direct bilirubin, AST, ALT, LDH, alkaline phosphatase; albumin, calcium, phosphate, uric acid, creatinine (creatinine clearance if serum creatinine > 1.5 mg/dl), and electrolytes.

2.2.3 Tumor biopsies will be obtained for flow cytometry for assessment of CD52 staining if accessible tissue is available. Where possible, tumor biopsies will be obtained by core needle biopsy or surgery pre-treatment. Laparotomy, thoracotomy, or biopsy of relatively inaccessible lymph nodes (i.e. high axillary nodes) will only be performed if needed for definitive diagnosis and not for research purposes alone.

2.2.4 HIV antibody; hepatitis B surface and core antigen; hepatitis C; and anti-HCV antibody

2.2.5 CMV, EBV and toxoplasmosis serologies

2.2.6 CMV antigen by PCR, serum BK virus by PCR, EBV viral load

2.2.7 Urinalysis and urine for BK virus by PCR

2.2.8 Urine HCG in women of childbearing potential.

2.2.9 Imaging Studies: CT chest, abdomen, and pelvis; CT or MRI of head if neurological signs or symptoms suggestive of lymphomatous involvement are present.

2.2.10 Electrocardiogram

2.2.11 Clinical PET (fluorine 18-FDG) scan within two weeks of starting treatment, but may be waived if this will delay treatment.

2.2.12 Unilateral bone marrow aspiration and biopsy.

2.2.13 Lumbar puncture for cell count, chemistry, cytology, flow cytometry if indicated.

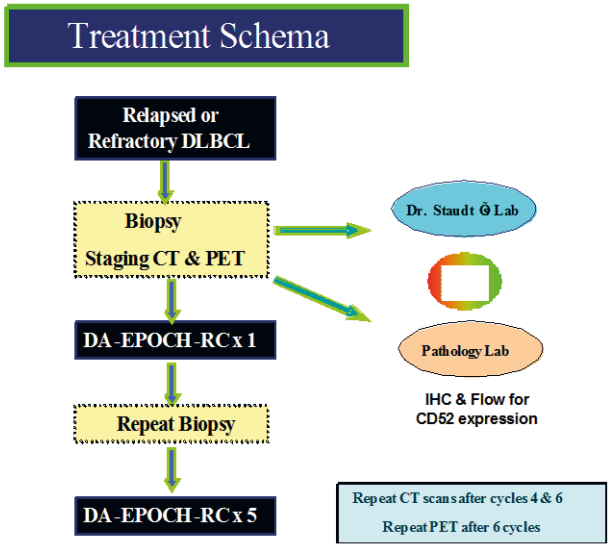
2.3 PATIENT REGISTRATION

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

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase II trial of Alemtuzumab in combination with DA-EPOCH-R given every 21 days for a total of 6 cycles of treatment. Six cycles of therapy will be administered based on clinical response evaluation. If a patient has progressive disease at any point during therapy, he/she will be removed from protocol treatment. Patients who are eligible for transplant will receive 2 cycles beyond stable disease or stop when they achieve CR. Patients not eligible for transplant will receive 2 cycles beyond stable disease, or 6 cycles if they achieve CR.



3.2 STUDY DRUG ADMINISTRATION (VIA CENTRAL CATHETER)

3.2.1 DA-EPOCH-R-Alemtuzumab Chemotherapy

See also **Appendix 1**.

Alemtuzumab will be given on day 1 of each treatment cycle. Patients will receive each treatment cycle until unacceptable toxicity, documentation of disease progression, completion of six cycles of therapy or other reasons for patient withdrawal, whichever comes first.

Patients should be pre-medicated as described in section **3.2.4**. Alemtuzumab will be administered as an IV infusion over approximately 12 hours. Alemtuzumab must not be administered via IV push or bolus. If infusional toxicities are observed, the infusion may be stopped for up to 60 minutes and resumed when toxicity resolved to baseline, or to grade 1 or less. If the patient has not had resolution of infusional toxicities within 60 minutes, contact the investigator for guidelines on when to resume infusion.

If anaphylaxis (Grade 4) hypersensitivity reactions occur, further treatment with Alemtuzumab will be discontinued. Hypersensitivity reactions should be managed according to procedures outlined in Table 1. Meperidine 25 mg IV should be administered for treatment of patients with severe rigors.

Table 1 Treatment Guidelines for Management of Hypersensitivity Reactions

	Treatment Guidelines
Severity of Symptoms	**Alemtuzumab**
Mild Localized cutaneous reactions such as mild pruritus, flushing, rash and chills	• Stop Alemtuzumab infusion until the patient is evaluated. • Following evaluation, the infusion may be restarted under observation and monitor subject; complete Alemtuzumab infusion at the initial planned rate. • Additional diphenhydramine 25-50 mg oral may be administered at the discretion of the treating physician. Chills without rigors can be managed with warm blankets.
Moderate Generalized pruritus, flushing, rash, dyspnea, fever, chills, rigors, and hypotension with systolic blood pressure >80 mmHg	• Stop Alemtuzumab infusion • Administer diphenhydramine 50 mg IV and monitor patient until symptoms resolve. Resume Alemtuzumab at one-half the initial infusion rate after recovery of symptoms; if symptoms develop after restarting the infusion, the infusion should be stopped and no additional Alemtuzumab should be administered at that time. • Additional oral or IV antihistamine may be administered. A maximum dose of diphenhydramine of 100 mg in a 6 hour period should not be exceeded. Rigors should be managed with IV meperidine 25 mg every 5-10 minutes to a maximum of 100 mg. • Give oral diphenhydramine, 50 mg and acetaminophen 650 mg 30 minutes prior to the next scheduled dose of Alemtuzumab.
Severe or Any reaction such as bronchospasm, generalized urticaria, angioedema, or systolic BP ≤80 mmHg)	• Immediately discontinue Alemtuzumab infusion • Bronchospasm, hypotension unresponsive to intravenous fluids, or angioedema may require administration of epinephrine 1 mg subcutaneously and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV or dexamethasone 20 mg IV, as needed, as well as supplemental oxygen. • Hypotension should be managed with intravenous fluids but if unresponsive may require more aggressive intervention such as phenylephrine infusion. • Monitor patient until resolution of symptoms.
Anaphylaxis Defined as a reaction that is life threatening and requires pressor and/or ventilator support or shock associated with acidemia and impairing vital organ function due to tissue hypoperfusion	• **Discontinue further treatment with Alemtuzumab**

3.2.2 Dose-Adjusted EPOCH-RC Regimen

All patients initiate therapy at Dose Level 1 of DA-EPOCH-RC shown below. Subsequent treatment doses are determined by hematological toxicity experienced on the previous cycle according to the dose-adjustment paradigm in Section **3.3.2**. Dose adjustment is based on

measurements of twice weekly CBC only (e.g., Monday and Thursday, or Tuesday and Friday), even if additional CBCs are obtained.

Cycle 1 Doses:

Drug	Dose	Route	Treatment Days
Infused Agents[1]			
Alemtuzumab	30 mg	IV	day 1
Rituximab	375 mg/m^2	IV	day 1
Etoposide	50 mg/m^2/day	CIV	1,2,3,4 (96 hours)
Doxorubicin	10 mg/ m^2/day	CIV	1,2,3,4 (96 hours)
Vincristine	0.4 mg/ m^2/day	CIV	1,2,3,4 (96 hours)
Bolus Agents			
Cyclophosphamide[2]	750 mg/m^2/day	IV	day 5
Prednisone[3]	60 mg/m^2 twice daily	PO	day 0-5[4]
Filgrastim	480 mcg	SC	days 6 to ANC recovery ≥ 5000/mm^3

[1]Begin the Rituximab immediately after Alemtuzumab infusion has completed. For the first cycle of treatment, Rituximab and the EPOCH infusion may be infused on days 2-6 if the Alemtuzumab infusion is prolonged due to infusion reaction.

[1]Begin the infusional agents immediately after Rituximab infusion has completed.

[1]Infusional agents should be administered through a central venous access device.

[2]Begin cyclophosphamide immediately after infusions are completed and administer over 30 minutes.

[3]Begin Prednisone 8-12 hours before start of Alemtuzumab with second dose given just prior to Alemtuzumab.

[4]A total of 10 doses of Prednisone 60 mg/m2 will be administered over 5 days.

3.2.3 Table of doses per level for adjusted agents:

Drugs	Drug Doses per Dose Levels							
	-2	**-1**	**1**	**2**	**3**	**4**	**5**	**6**
Doxorubicin (mg/m^2/day)	10	10	10	12	14.4	17.3	20.7	24.8
Etoposide (mg/m^2/day)	50	50	50	60	72	86.4	103.7	124.4
Cyclophosphamide (mg/m^2/day)	480	600	750	900	1080	1296	1555	1866

3.2.4 Premedication for Monoclonal Antibody Infusions

Premedicate 30-90 minutes before Alemtuzumab infusion with

- 650 mg acetaminophen PO
- 50 mg diphenhydramine PO
- Prednisone 60 mg/ m^2 PO. This would be the second dose of the 60 mg/ m^2 bid prednisone regimen that is part of the EPOCH therapy. The first dose is given 8-12 hours before Alemtuzumab.

Prophylaxis for tumor lysis syndrome will be administered at the discretion of the Principal Investigator according to institutional practice. Hydration and urine alkalinization will be administered according to the discretion of the Principal Investigator.

Premedicate 30-60 minutes before rituximab infusion with

- 650 mg acetaminophen PO
- 50 mg diphenhydramine PO

Rituximab infusion should begin immediately after the Alemtuzumab infusion has completed. See Section 9.2.4 for specific Rituximab administration instructions. There will be no dose adjustments for Rituximab.

3.3 DOSE MODIFICATIONS FOR TOXICITIES

3.3.1 Discontinue Alemtuzumab for:

- Grade 4 non-hematological toxicity other than easily correctable metabolic toxicities or infection.
- Grade 3 infusional or allergic toxicity

3.3.2 EPOCH Dose Adjustments

3.3.2.1 Hematological Toxicity

No dose adjustments at cycle 1 will be made for patients that have low neutrophil count or platelet count due to bone marrow involvement of disease. Drug doses may be modified from the following algorithm at the discretion of the investigator for severe life-threatening toxicity such as ICU admissions for sepsis. When two different rules give different answers for a particular dose decision, use the lower of the two dose options.

3.3.2.2 Dose-Adjustment Paradigm

- Dose adjustments above starting dose level (level 1) apply to etoposide, doxorubicin and cyclophosphamide
- Dose adjustments below starting dose level (level 1) apply to cyclophosphamide only.
- Drug Doses based on previous cycle ANC nadir:

  - If Nadir ANC ≥ 500/µl on all measurements : ↑ 1 dose level above last cycle
  - If Nadir ANC < 500/µl on 1 or 2 measurements : Same dose level as last cycle
  - If Nadir ANC < 500/µl ≥ 3 measurements(see**): ↓ 1 dose level below last cycle

  Or

  - If nadir platelet < 25,000/µl on 1 measurement : ↓ 1 dose level below last cycle.

- Proceed with the next cycle of therapy if ANC ≥ 1000/µl and platelets ≥ 75,000/µl on day 21, and filgrastim was stopped 24 hours before beginning treatment.

- If ANC < 1000/µl or platelets < 75,000/µl on day 21, delay up to 1 week. Filgrastim 480 mcg every day may be started for ANC < 1000/µl and stopped 24 hours before treatment. If counts still low after 1 week delay, ↓ 1 dose level below last cycle.

****Important: Measurement of ANC nadir based on twice weekly CBC only and must be 3 days apart. Only use twice weekly CBC for dose-adjustment, even if additional CBC's are obtained.**

3.3.2.3 NON-HEMATOLOGICAL TOXICITY

a. Sensory neuropathy

Grade	Vincristine Dose (%)
2	100
3	50
4	0

b. Motor neuropathy

Grade	Vincristine Dose (%)
1	100
2	75
3	25
4	0

If neuropathy resolves to a lower grade, doses for that lower grade may be reinstituted at investigator discretion. If the grade of neuropathy increases after being re-escalated, doses must be reduced for the appropriate toxicity grade and may not be re-escalated, even if neuropathy resolves again to a lower grade.

c. Hepatic Dysfunction

Vincristine dose will be decreased for elevated bilirubin as follows:

Bilirubin (mg/dL)	Vincristine Dose
>1.5 - <3.0	decrease by 25%*
≥ 3.0	decrease by 50%*

Vincristine dose will be re-escalated as hyperbilirubinemia improves.

d. Ileus

Constipation commonly occurs in patients receiving vincristine so patients should receive stool softeners as indicated. Occasionally, symptomatic ileus may occur and this should be treated with a vincristine dose reduction. Because the severity of ileus is dose related, it is usually unnecessary to stop the vincristine altogether. Furthermore, because the therapy administered in this study is potentially curative, every effort should be made to not unnecessarily reduce vincristine doses. The following guidelines for symptomatic ileus on a previous cycle should be followed.

1. Clinical ileus < 8 days with abdominal pain requiring narcotics and/or persistent nausea/vomiting >2 days: Reduce vincristine dose 25%.

2. Clinical ileus 8-12 days with abdominal pain requiring narcotics and/or persistent nausea/vomiting > 2 days: Reduce vincristine dose 50%.
3. Clinical ileus > 12 days with abdominal pain requiring narcotics and/or persistent nausea/vomiting > 2 days: Hold vincristine on next cycle. May restart at 50% reduction on subsequent cycle.

Recommended Bowel Regimen – goal of at least one soft bowel motion every 24 hours while on study

Adults: Sodium Docusate 100mg capsule; take one to two capsules once a day Day 1-7 of each cycle. If needed can double the frequency to two capsules every 12 hours. If needed add oral lactulose 15-30 ml prn/ q 6 hourly

e. Renal dysfunction secondary to lymphoma

Etoposide should be reduced 25% on cycle one for creatinine clearance < 50 cc/min. Etoposide should be returned to full dose (or escalated if indicated) once creatinine clearance > 50 cc/min. No other dose modifications for abnormal renal indices will be made for enrolled patients.

f. Other Non-Hematological Toxicity

Symptom grade	**Management**
≤ grade 2	Treat symptomatically
≥ grade 3	Hold next dose until ≤ grade 1 and, if further therapy is medically indicated • Reduce by 1 dose level If further toxicity at the reduced dose level • reduce by another dose level After 2 dose reductions • patient will be removed from study

3.3.3 Dose Modification for Obese Patients

All dosing is based on the patient's BSA as calculated from actual weight. There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.

3.3.4 Rituximab Infusional-related Adverse Events

Pretreatment for rituximab with diphenhydramine and acetaminophen using standard medical practice will be used in all patients. Side effects of rituximab may be infusion rate related and may be reduced by slower administration or premedication. Thus, dose reductions of rituximab will not be made. Rituximab will be discontinued for the duration of the cycles in patients with grade 4 allergic reactions. At the discretion of the PI, rituximab may be administered on the following cycles using slower infusion rates.

3.4 STUDY CALENDAR

Studies	Pre-therapy[A]	Day -1 or day 1 of each cycle	Bi-weekly during therapy	Post cycles 4 & 6	Follow-up[B]
Hx; PE; VS; PS	x	x			x
Tumor Measurement	x			x	
CBC/diff	x[F]	x	x		x
Electrolytes, Creatinine/BUN, ALT, AST, Bilirubin, LDH, Ca^{++}, Phos, Mg	x[F]	x			x
CMV PCR	x	x			x
HIV and Hepatitis B and C serology, Pregnancy Test	x				
Lymphocyte TBNK panel	x			x	x
Toxoplasmosis serologies, BK antigenemia, EBV Viral load, urine BK	x	x			
20 mL red top for serum storage	x	x			x
PET scan [C]	x			x (after 6 only)[C]	
CT chest/abdomen/pelvis[D]	x			x	x
Bone marrow biopsy & aspirate [E] and peripheral blood for flow cytometry where indicated	x			x[E]	
Tumor biopsy [G]	x				

A: Initial assessment may be performed within 4 weeks prior to starting treatment.

B: Follow-up to occur as per section 3.7.1.

C: Clinical PET scans may be performed at other time points throughout this protocol if the investigator deems it medically necessary. If obtaining PET scan will delay initiation of treatment, pre-treatment PET scan may be waived. PET may be obtained at the first post-treatment restaging in patients in CRu.

D: Extent of CT scans may be limited to assess sites of prior disease.

E: If flow cytometry or bone marrow positive, repeat test as indicated until negative

F: These laboratory tests must be obtained within one week of initial treatment.

G: Where possible, tumor biopsies will be obtained by core needle biopsy or surgery pre-treatment and after 1 cycle of treatment. Snap freeze and store tissue biopsies at -80°C.

3.4.1 Day 1 all cycles and day 21 last cycle: CBC/differential; electrolytes; mineral panel; AST, ALT, Bilirubin, LDH, Toxoplasmosis serologies, BK antigenemia and CMV PCR.

3.4.2 During cycles: CBC/differential BIW.

3.4.3 Restaging: Day 21, cycles 4 and 6. Repeat all initially positive staging tests, including peripheral blood flow cytometry.

3.4.4 Biopsies may be obtained for cytogenetics, immunophenotype, molecular analyses including Fluorescent In Situ Hybridization and polymerase chain reaction. Tissue will be frozen for microarray analysis. A fresh tissue biopsy of accessible lymph node or a core needle biopsy using a 16-18 gauge needle will be obtained if possible without major surgery or requirement for general anesthesia. Standard techniques will be used for biopsies which may include CT and/or ultrasound guided biopsy. The pretreatment lymph node biopsy will be used to perform all standard diagnostic tests. In addition, we will extract RNA and protein for research studies such as microarray. If patient consent is obtained, tumor tissue may be stored for future research assays which are related to this study and do not pose an increase in patient risk. General anesthesia will not be performed to obtain biopsies for research purposes but may be necessary when biopsies are medically indicated. However, conscious sedation may be used to obtain research biopsies. If a biopsy needs to be performed for medical reasons under general anesthesia, the obtained sample might be used for research purposes.

3.5 CONCURRENT THERAPIES (CONTINUED UNTIL CD4 COUNT IS >100 POST-TREATMENT)

3.5.1 **Pneumocystis jiroveci and Toxoplasmosis prophylaxis**: Recommendations: Trimethoprim/sulfamethoxazole 1 DS P.O. **twice daily** for three days each week. Monday, Wednesday, Friday is the preferred schedule.

- For patients who are allergic to trimethoprim/sulfamethoxazole: Alternatives include, in this order: aerosolized pentamidine administered every 3-4 weeks, atovaquone 1500 mg orally once a day and lastly Dapsone 50 - 100 mg orally once a day or 100 mg orally 2x/week. Atovaquone should be taken with food for improved absorption.
- Patients with positive toxoplasma antibodies should receive atovaquone 1500 mg orally once a day (instead of trimethoprim/sulfamethoxazole) and have their blood monitored on a weekly basis for Toxoplasma gondii by PCR. Atovaquone should be taken with food for improved absorption.

3.5.2 **Herpes Simplex prophylaxis:** Recommendation: acyclovir 400 mg twice daily or famciclovir 500 mg twice daily.

3.5.3 **Fungal prophylaxis:** Fluconazole will be administered as prophylaxis for fungal infections (200 mg daily). Fluconazole should be held during EPOCH infusions because of pharmacokinetic interactions.

3.6 POST-TREATMENT EVALUATION

3.6.1 For patients with response: Restage sites of disease q3 months for the first year, then q4 months for the second year, and then q6 months for 1 years, and yearly thereafter until disease progression. Laboratory tests: CBC/differential, mineral panel, electrolytes, AST, ALT, Bilirubin, and LDH. The timing for these visits may be adjusted $\pm$ 2 months.

3.6.2 Obtain lymphocyte TBNK panel and CMV PCR levels at all restaging visits until the CD4 count is greater than 100.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.7.1 Criteria for removal from protocol therapy

- Patient completes therapy as outlined in section **3.1**
- Progressive disease
- Intercurrent illness that prevents further administration of treatment
- General or specific changes in a patient's condition that render the patient unacceptable for further treatment as determined by that principal investigator
- Patient requests to be withdrawn from active therapy

3.7.2 Off Study Criteria

- Death
- Patient non-compliance which affects safety or endpoints of the study
- Patient voluntary withdrawal
- Physician's determination that withdrawal is in the patient's best interest
- Study is stopped or cancelled

3.7.3 Off Protocol Therapy and Off-Study Procedure

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

4 SUPPORTIVE CARE

4.1 PROPHYLAXIS FOR HEPATITIS B REACTIVATION

Patients who are positive for Hepatitis B core antibody (anti-HBc) and not acutely infected are at varying risk for reactivation of Hepatitis B. These patients will have quantitative PCR testing performed for Hepatitis B virus. Additionally, patients at high and moderate risk will receive appropriate prophylaxis for hepatitis B reactivation, e.g., lamivudine 100mg PO daily to continue

until 8 weeks after last chemotherapy with repeat quantitative PCR performed 4-8 weeks after stopping prophylaxis.

anti-HBc	HBsAg	Anti-HBs	Risk	HBV PCR	Prophylaxis
+ or −	+	+ or −	High	pre-tx, post 2, 4, 6 cycles	Yes
+	−	−	Moderate	pre-tx, post 2, 4, 6 cycles	Yes
+	−	+	Low	pre-tx, post 3, 6 cycles	No if PCR −

4.2 CMV ANTIGENEMIA

Patient management will be handled on a case-by-case basis in conjunction with the infectious diseases service.

4.3 FEBRILE NEUTROPENIA

Febrile neutropenia is a life-threatening complication requiring urgent broad-spectrum antibiotics. Management may be as an inpatient or outpatient depending on the clinical situation.

4.4 SYMPTOMATIC ANEMIA

Symptomatic anemia should be treated with appropriate red blood cell support, and is recommended if the hemoglobin falls below 8 mg/dl. Only irradiated leukodepleted blood products should be used.

4.5 THROMBOCYTOPENIA

Thrombocytopenia should be treated conservatively. In the absence of bleeding or planned invasive procedures, platelet transfusions should be given for platelets < 10,000/mm^3. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50,000/mm^3.

4.6 CENTRAL VENOUS ACCESS (REQUIRED FOR EPOCH ADMINISTRATION)

Possible lines include:

- temporary internal jugular line (preferred);
- PICC lines via the brachial vein;
- semi-permanent HICKMAN,
- GROSHONG catheters or medi-port implanted devices.

All devices will have nursing supervision to include patient self-care and cleaning/flushing of the devices.

4.7 TUMOR LYSIS SYNDROME (DOSE 1 ONLY)

At the discretion of the principal investigator, patients with high tumor burden may be treated with allopurinol 600 mg p.o. x 1 dose followed by 300 mg p.o. daily for up to 7 days. Hospitalization with aggressive IV hydration and urinary alkalization may be used.

4.8 GI PROPHYLAXIS

Omeprazole 20mg p.o. daily is given to patients for the entire duration while patient is on chemotherapy.

5 BIOSPECIMEN COLLECTION

5.1 PROCEDURES FOR SAMPLE COLLECTION

Sample collection is outlined in Section **3**.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

All specimens obtained in the protocol are used as defined in the protocol. Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.2.1 Procedures for stored serum specimens

The Clinical Support Laboratory, Leidos Biomedical Research, Inc., processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All laboratory personnel with access to patient information annually complete the NIH online course in Protection of Human Subjects. The laboratory is CLIA certified for CD4 immunophenotyping and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:

- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
- The database resides on a dedicated program server that is kept in a central, locked computer facility.
- The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
- Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
- The database sample entry program itself is accessed through a password protected entry screen.

- The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage. These facilities are operated under subcontract to Leidos Biomedical Research, Inc.
- Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection. Specific permissions will be required to view, input or withdraw samples from a collection. Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. The repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (generally the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.
- The Clinical Support Laboratory does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

5.2.2 Procedures for Collecting, Processing and Storing of Tumor Biopsies and/or Peripheral Blood Cells

- Orders for tumor biopsies and research blood samples collections should be placed in CRIS (Clinical Research Information System, Clinical Research Center, NIH, Bethesda, MD)
- Tumor biopsies will be submitted in native condition to the Department of Pathology, NCI, NIH and handled according to routine procedures. Material released for research studies will be documented on form NIH 2803-1. Initial processing of samples for research will depend on the size of the tumor biopsy. For core biopsies the research

sample will typically consist of 2 cores in a microcentrifuge vial snap frozen on dry ice. Surgical lymph node biopsies may in addition be processed for single cell suspension, additional vials of snap frozen tissue and OCT embedded tissue.

- Tumor and normal blood cells may be viably frozen, typically at concentrations of 20-100x10^6/mL in FCS with 10% DMSO using a temperature controlled freezing process to optimize sample viability. Samples will be transferred to Nitrogen tanks for long term storage.
- Tumor and normal blood cells can be further processed. Additional purification may be carried out by selection with magnetic beads binding to appropriate surface molecules, typically CD19. For analysis cells may be lysed to obtain RNA (using Qiagen manufactured kits are similar) or proteins (salt and/or triton containing buffers with addition of protease and phosphatase inhibitors). Integrity of RNA is monitored by gel electrophoresis and concentration of RNA or protein is measured spectrophotometrically.
- Research sample inventory and storage: All research samples are assigned a unique number and cataloged. Viably frozen cells are stored in a temperature controlled, alarm secured Nitrogen tank. Tumor biopsies and processed biologic material (RNA, protein) is stored at -80C in a temperature controlled, alarm secured -80C freezer.
- Tumor tissue may be stored by the NCI Department of Hematopathology for future research assays which are related to this study and do not pose an increase in patient risk. Tissue that is given to the technician will be assigned an accession number (HP#) in the HP Case Log book. A Patient background sheet will be filled out and filed with any accompanying paperwork in the black notebook. Final reports and any supplemental reports that follow will be added to these notebooks which are located in Room 2N110.
- Frozen Specimens: Tissue snap frozen or embedded in OCT is wrapped in aluminum foil labeled with the patient's name and accession number (HP#), put into a zip-lock bag, and stored in liquid nitrogen freezer. The liquid nitrogen freezers are monitored daily for temperature variations. A FileMaker Pro data base called HP Patient Information and Specimen Inventory is used for tracking the samples.

5.2.3 Protocol Completion/Sample Destruction

Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below for an indefinite amount of time.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist

with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All patients must have signed an Informed Consent and an on-study confirmation of eligibility form will be filled out before entering on the study.

Complete records must be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; specialty forms for pathology, radiation, or surgery; and off-study summary sheet, including a final assessment by the treating physician.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for a minimum of 30 days after removal from study treatment or until off-study, whichever comes first. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2**.

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

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

6.1.1 Data Collection/Recording Exceptions

As the toxicity profile of EPOCH-R is well defined and published, grade 1 clinical adverse events will not be recorded in the database. All hospitalizations, regardless of reason, will be recorded in the database with the reason of hospitalization and the duration of hospitalization noted.

6.1.2 Other Information

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

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

6.2 RESPONSE CRITERIA

From the International Workshop to Standardize Response Criteria for non-Hodgkin's Lymphomas. Responses must last for at least 4 weeks off treatment.

Complete Remission (CR): 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 (for example LDH) definitely assignable to the lymphoma. All lymph nodes must have regressed to normal size (less than or equal to 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in greatest diameter must have decreased to less than or equal to 1 cm or by more than 75 percent in the sum of the products of the greatest diameters. The spleen, if considered to be enlarged before therapy, must have regressed in size and not be palpable on physical examination. The bone marrow must show no evidence of disease by histology. Flow cytometry, molecular or cytogenetic studies will not be used to determine response. Response must persist for 1 month.

Complete response unconfirmed (CRu): As per complete remission criterion except that if a residual node is greater than 1.5 cm, it must have decreased by greater than 75 percent in the sum of the products of the perpendicular diameters.

Partial Response (PR): ≥ 50% decreased in SPD of 6 largest dominant nodes or nodal masses. No increase in size of nodes, liver or spleen and no new sites of disease. Splenic and hepatic nodules must regress by ≥ 50% in the SPD. Bone marrow is irrelevant for determination of a PR.

Relapsed disease (CR, CRu) requires the following: Appearance of any new lesion or increase by ≥ 50% in the size of the previously involved sites. Greater than or equal to 50% increase in greatest diameter of any previously identified node > 1 cm in its shortest axis or in the SPD of more than one node.

Progressive disease (PR, non-responders) requires the following: ≥ 50% increase from nadir in the SPD of any previously identified abnormal node for PRs or non-responders. Appearance of any new lesion during or at the end of therapy.

Stable Disease (SD): is defined as less than a PR but not progressive disease.

ALL assessment of clinical response will be made according to the NCI guidelines. The major criteria for judging response will include physical examination and examination of blood and bone marrow. All laboratory studies that are abnormal prior to study will be repeated to document the degree of maximal response.

6.3 TOXICITY CRITERIA

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. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section **8** (Pharmaceutical Information). A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html). Effective with Amendment G version date 05/08/2014, the CTCAE version to be used for adverse event reporting was changed from version 3.0 to version 4.0.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

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

7.1.2 Suspected adverse reaction

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

7.1.3 Unexpected adverse reaction

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

7.1.4 Serious

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

7.1.5 Serious Adverse Event

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

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

7.1.6 Disability

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

7.1.7 Life-threatening adverse drug experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 Non-compliance (NIH Definition)

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

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

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

The Protocol PI will report to the NCI-IRB and NCI CD:

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

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 DATA AND SAFETY MONITORING PLAN

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

The monitoring of this study will be done by the PI/Lead associate investigator on an ongoing manner so a DSMB or Safety Monitoring Committee will not be used.

8 STATISTICAL CONSIDERATIONS

The primary objectives of this study will be to determine if there is a moderately large fraction of patients who exhibit a clinical response to EPOCH-RC in relapsed or refractory DLBCL and to evaluate the safety and toxicity of this regimen.

The study will be conducted as a two-stage Simon optimal design (Simon R, *Controlled Clinical Trials*, 10:1-10, 1989) in order to rule out an unacceptably low 25% clinical response rate (PR+CR: p0=0.25) in favor of a targeted rate consistent with 45% (p1=0.45). With alpha=0.10 (probability of accepting a poor regimen=0.10) and beta = 0.10 (probability of rejecting a good regimen=0.10), the study will initially enroll 14 evaluable patients, and if 0 to 3 of the 14 are able to have a response then no further patients will be accrued. Response will be evaluated after 6 cycles or 18 weeks. If 4 or more of the first 14 patients have a response, then accrual would continue until a total of 44 patients have been treated. A temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 4 to 14 patients with a response in the total of 44 patients, then this would be an uninterestingly low rate, while if there were 15 or more patients of the 44 who have a response, this would be sufficiently interesting to warrant further study of this agent in these patients. Under the null hypothesis (25% response rate), the probability of early termination is 52%.

At the end of the study, patients will be determined as to whether they are of ABC or GCB subtype, and exploratory evaluations will be made of the response rates in the two subtypes. In addition to evaluating clinical response, progression free survival and overall survival will be estimated using a Kaplan-Meier curve. The results obtained in this study will be compared in an informal manner to those of other previous studies.

Toxicity will also be evaluated and monitored as patients are accrued to the trial. If at any point in the accrual to the trial, there are greater than 1/3 of patients who have an SAE, then the trial will suspend accrual, and the study will either be terminated or have the drug regimen modified to allow a safer outcome. It is anticipated that up to 10-15 patients per year may be enrolled onto this trial. Thus, accrual of up to 44 evaluable patients is expected to require approximately 3-4 years. In order to allow for a small number of unavailable patients, the accrual ceiling will be set at 47.

Effective with Amendment G, version date 05/08/2014, the accrual ceiling will be increased to 52 to allow for continued enrolment of patients with relapsed T cell histiocyte-rich Diffuse Large B cell Lymphoma and advanced Lymphocyte Predominant Hodgkin Lymphoma. This will allow access to this potentially lifesaving treatment, for patients with these two histologies.

9 COLLABORATIVE AGREEMENTS

No collaborative agreements (e.g., MTA, CTA, CRADA, etc.) apply to this protocol at this time.

10 HUMAN SUBJECTS PROTECTION

10.1 RATIONALE FOR SUBJECT SELECTION

Large cell lymphomas and Hodgkin Lymphoma affect all races and genders. However, males are more likely than females to be affected and this will be reflected in the gender distribution of our cases. This trial is directed at assessing Alemtuzumab in combination with EPOCH-R chemotherapy. We have selected patients with relapsed disease and have developed the trial design to recognize that some may be potentially curable and others are not. Additionally, pregnant or nursing mothers are excluded because of the potential teratogenic effects of therapy.

10.2 PARTICIPATION OF CHILDREN

Patients under the age of 18 are excluded because inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this study. Pediatric patients with recurrent large B cell lymphoma are extremely rare and are treated on pediatric studies. In addition, tumor biopsies will be performed.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent were excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is the prospect of direct benefit to subjects from treatment, including ongoing follow-up for detection of early relapse, all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation".

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects meeting the above criteria that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

The potential benefits to the subject are that the patient may achieve a partial or complete remission. The benefits of combinations of chemotherapy and monoclonal antibodies may produce remissions that cannot be achieved with either approach alone. The main danger to participants in this study is drug toxicity. Chemotherapy may produce bleeding or infectious complications as a result of chemotherapy-induced myelosuppression. Alemtuzumab causes immunosuppression which may be made more severe as a result of combining it with chemotherapy.

10.5 Risks/Benefits Analysis

Patients eligible for this protocol may derive benefit from DA-EPOCH-R based on prior research results. However, they will also be subjected to the toxicity associated with EPOCH-rituximab infusional chemotherapy, which include myelosuppression, stomatitis, numbness or tingling in the extremities, motor weakness, and the need for transfusion or hospitalization due to complications of treatment noted. There may be adverse effects due to the combination of agents that is not seen when either is given alone. But this potential toxicity of this combination is reasonable in relation to the potential benefit to this group of patients who have treatable diseases. Additionally, the knowledge that will be gained from this trial is potentially important and may be of direct benefit to patients. Patients will require biopsies both to determine the diagnosis as well as for research purposes. Needle biopsy is minimally invasive and is typically a very safe procedure. The benefits of percutaneous biopsy often far outweigh the risks. However, as with all invasive procedures, certain risks do exist. Depending upon the site being biopsied and the type of biopsy being performed, risks can include infection of the biopsy area, development of a hematoma and bleeding. Rarely more significant complications can occur when structures near the biopsy target are entered with the needle (for example, puncture of lung or bowel). As far as possible surgical biopsies will not be performed unless medically indicated and will not be performed for research purposes. Surgical biopsy has some additional risks versus needle biopsy. Surgical biopsy carries a small risk of mortality (due to risks of anesthesia) and causes moderate chances of bleeding, infection, wound healing problems. General anesthesia will not be performed to obtain biopsies for research purposes but may be necessary when biopsies are medically indicated. However, conscious sedation may be used to obtain research biopsies.

The risks and benefits of participation for adults who become unable to consent during participation are no different than those described for less vulnerable patients.

10.6 CONSENT PROCESS AND DOCUMENTATION

All patients are thoroughly screened for eligibility prior to admission onto this study. All patients age 18 and over, will read and sign the informed consent document prior to enrollment. Members of the protocol team will describe the protocol, alternative therapies, and risks and benefits of each to the individual signing the consent. The specific requirements, objectives, and potential risks and benefits will be discussed. The informed consent document is given to the patient, who is asked to review the document, discuss it with his/her family and write down questions to discuss with the principal investigator or treating physician. The patient is informed that participation is voluntary and that he/she may withdraw at any time without loss of benefits without consequence. The patient or their legal representative must sign the consent document prior to receiving any protocol related treatment. The patient will receive a copy of the signed consent.

10.6.1 Telephone re-consent

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

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

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

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

11 PHARMACEUTICAL INFORMATION

- Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.
- Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.
- The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

11.1 ALEMTUZUMAB

11.1.1 Availability

Available through the Campath Distribution Program (The Sanofi Foundation for North America 1-877-422-6728). Vials are provided through this program upon completion of a patient specific request form. Prior to submission of a drug request the patient must provide authorization for the release of medical information (NIH-527). Refer to the Pharmacy Department or Clinical Pharmacy Specialist for additional details on drug procurement.

11.1.2 Formulation

Alemtuzumab causes the lysis of lymphocytes by fixing to CD52, a highly expressed, non-modulating antigen on the surface of lymphocytes. It mediates the lysis of lymphocytes via complement and antibody-dependent cell-mediated cytotoxicity mechanisms.

Alemtuzumab is supplied as a clear glass vial containing 30 mg Alemtuzumab in 1 mL of solution (8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and 0.187 mg disodium edentate dehydrate). No preservatives are added. Each carton contains three Alemtuzumab vials (NDC 50419-357-03) or one Alemtuzumab vial (NDC 50419-357-01).

11.1.3 Storage & Stability

Vials of Alemtuzumab should be stored at a temperature of 2-8°C and protected from light. The desired dose of 30 mg. should be drawn up into a syringe from the ampoule and further diluted in 250 ml of 0.9% sodium chloride. The vial contains no preservatives and is intended for single use only; the vial should be discarded with any unused portion after 6 hours. An internal NIH Pharmacy (Pharmaceutical Development Section) conducted study demonstrated 24 hour stability of Alemtuzumab when diluted in 0.9% sodium chloride to a concentration range of 6.67 mcg/mL to 120 mcg/mL at room temperature (Goldspiel JT, et.al. Stability of alemtuzumab solutions at room temperature. Am J Health-Syst Pharm, accepted for publication 2012). Alemtuzumab solutions prepared in the concentration range described above may be stored at room temperature (15-30°C) for up to 24 hours. Alemtuzumab solutions should be protected from light.

11.1.4 Administration

The Alemtuzumab solution should be given IV over approximately 12 hours. Patients should be premedicated with 50 mg of diphenhydramine, 650 mg of acetaminophen and two 60 mg/m^2 doses of prednisone as indicated in section 3.2.4 before the infusion. During the alemtuzumab infusion, the patient's vital signs (blood pressure, pulse, respirations, temperature) should be monitored as follows:

- During Cycle 1: every 15 minutes times 4 or until stable and then hourly until the infusion is discontinued.
- During subsequent cycles: one hour after infusion starts; then every 4 hours for the duration of the infusion, as long as patient is stable and has no signs/symptoms of reaction.

11.1.5 Toxicity

The majority of adverse events seen in trial have been administration related and of short duration. Serious adverse events, some of which fatal, have been observed in association with treatment of Alemtuzumab

Infusional reactions occur in most patients. They commonly consist of rigors, fever, headache, nausea, vomiting and diarrhea, rash, pruritus, dyspnea and hypotension. Acute infusional reaction may also include chills, abdominal and back pain, bronchospasm, angioedema, tachyarrhythmia etc. These reactions are most prominent during the first dose of Alemtuzumab administration and improve with subsequent treatments. To reduce the frequency and severity of

the first dose reaction, a step-up dose escalation schedule and proper premedication should be used.

Myelosuppression**:** Anemia, neutropenia, thrombocytopenia, prolonged and profound lymphopenia

Infections: common bacterial (pneumonia and sepsis) or opportunistic infections (e.g. Pneumocystis carinii pneumonia, oral candidiasis, herpes zoster, CMV reactivation, cryptococcosis).

Reported adverse events by organ systems:

- **Body as a whole:** Allergic reaction, rigors, fever, chills, headache, back and abdominal pain, infection, fatigue
- **Cardiovascular**: Hypertension, hypotension, tachycardia, Atrial arrhythmia, ventricular tachycardia, angina and myocardial infarction, peripheral vasoconstriction
- **Digestive**: anorexia, nausea, vomiting, diarrhea, constipation, dyspepsia, liver function abnormality
- **Hematological**: Neutropenia, lymphopenia, thrombocytopenia, anemia, DIC, hemolysis, eosinophil disorder, bleeding (GI, gum, ecchymosis)
- **Muscular Skeletal:** Myalgia, Arthritis, bone pain, hypotonia, tremor
- **Metabolic and nutritional**: Tumor lysis syndrome, acidosis
- **Nervous system**: Dizziness, confusion, somnolence, peripheral neuropathy, Cerebral hemorrhage, speech disorder, mental status changes, paresthesia, syncope, depression, aphasia
- **Pulmonary**: Bronchospasm, cough, Pleural effusion, pulmonary edema, interstitial pneumonitis
- **Skin/subcutaneous**: Angioedema, facial flushing, diaphoresis, pruritis, rash, urticaria, injection site reaction (subcutaneous route)
- **Urogenital**: Hematuria, oliguria, polyuria, urinary retention, urinary tract infection, impotence
- **Tinnitus**

11.2 RITUXIMAB

11.2.1 Availability

The NIH Clinical Center Pharmacy Dept. will purchase rituximab from commercial sources. Rituximab is provided in pharmaceutical grade glass vials containing 10 mL (100 mg) or 50 mL (500 mg) at a concentration of 10 mg of protein per milliliter. Please refer to the FDA-approved package insert for rituximab for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.2.2 Storage & Stability

Rituximab for clinical use should be stored in a secure refrigerator at 2 to 8º C. After dilution, rituximab is stable at 2-8 degrees C (36-46 degrees F) for 24 hours and at room temperature for an additional 24 hours.

11.2.3 Preparation

Rituximab will be diluted to a final volume of 0.9% Sodium Chloride or 5% Dextrose Injection to prepare a standard product with concentration of 2 mg/ml. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody.

11.2.4 Administration

A peripheral or central intravenous line will be established. During rituximab infusion, a patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored according to the standard of care. Medications readily available for the emergency management of anaphylactoid reactions should include: epinephrine (1:1000, 1 mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment.

Prophylaxis against hypersensitivity and infusion-related reactions associated with rituximab will include acetaminophen 650 mg and diphenhydramine hydrochloride 50 mg administered 30 to 60 minutes prior to starting rituximab.

Rituximab will be administered as an intravenous infusion at 375 mg/m2 on day 1 of each cycle of EPOCH-RC just after the Alemtuzumab infusion has completed and immediately prior to starting etoposide + doxorubicin + vincristine administration.

First dose:
The initial dose rate at the time of the first rituximab infusion should be 50mg/hour (25 mL/hr) for the first 30 minutes. If no toxicity is seen, the dose rate may be escalated gradually in 50 mg/hour (25 mL/h) increments at 30 minute intervals) to a maximum of 400 mg/hour (maximum rate = 200 mL/h).

Second and Subsequent Doses (select the appropriate administration timing):

90-minute Administration
If the first dose of rituximab was well tolerated, subsequent doses may be administered over 90 minutes with 20% of the total dose given in the first 30 minutes, and remaining 80% of the total dose administered over the subsequent 60 minutes; e.g.:

Two-Step Rate Escalation	Volume to administer (X mL)
1st portion (0 – 30 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.2 = \text{X mL (over 30 min)}$
2nd portion (30 – 90 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.8 = \text{X mL (over 60 min)}$

Special Note: The 90-minute infusion scheme is not recommended for patients with clinically significant cardiovascular disease or high circulating lymphocyte counts (≥5000/mcL).

Standard Administration for Second & Subsequent Infusions
Patients who tolerate initial treatment without experiencing infusion-related adverse effects but for whom the 90-minute infusion scheme during subsequent treatments is

considered inappropriate, may receive subsequent rituximab doses at the Standard Rate for Subsequent Infusions, which is as follows:

Begin at an initial rate of 100 mg/hour (50 mL/h) for 30 minutes. If administration is well tolerated, the administration rate may be escalated gradually in 100-mg/hour (50-mL/h) at 30-minute intervals to a maximum rate of 400 mg/hour (maximum rate = 200 mL/h).

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

11.2.5 Toxicity

The most severe serious adverse events associated with rituximab include severe infusion reactions, tumor lysis syndrome, and severe mucocutaneous reactions. Severe infusion reactions consisting of hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock may be fatal. Most reported fatal reactions occurred within 24 hours of the first dose of rituximab.

Tumor lysis syndrome resulting in renal failure has been described, and occasional fatalities noted. Tumor lysis syndrome is more likely in patients with high numbers of circulating malignant cells (≥ 25,000/μL).

Severe mucocutaneous reactions associated with rituximab include Stevens-Johnson syndrome and toxic epidermal necrolysis. The onset of these reactions has been from 1-3 weeks.

Less severe infusion reactions are common with rituximab. These include fever, chills, and dyspnea. The mechanism of rituximab infusion reactions is thought to be secondary to release of cytokines. If a reaction occurs, then the infusion should be stopped until the symptoms resolve, and then restarted at a 50% slower rate.

Recent reports describe hepatitis B reactivation with fulminant hepatitis, hepatic failure and death in some patients with hematologic malignancies treated with rituximab. The majority of these patients received rituximab in combination with chemotherapy. The median time to diagnosis of hepatitis was approximately 4 months after starting rituximab and approximately 1 month after the last dose.

11.3 DOXORUBICIN HCL

11.3.1 Availability

Commercially available as a lyophilized powder for reconstitution in 10, 20, 50, and 100mg vials. Also available as a 2 mg/mL solution for injection in 10, 20, 50, and 200mg vials.Please refer to the FDA-approved package insert for doxorubicin for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.3.2 Toxicities

Hematologic: Leukopenia (dose-limiting), thrombocytopenia, anemia. Nadir in 10-14 days with recovery usually in 21 days.

Dermatologic: alopecia (usually complete; reversible) radiation recall reactions; increased sensitivity to sunlight.

Gastrointestinal: nausea and vomiting (doxorubicin is generally considered moderately to highly emetogenic), anorexia, diarrhea, mucositis (stomatitis, esophagitis).

Cardiovascular: cardiomyopathy may occur and is related to total cumulative lifetime dose. The risk for cardiomyopathy increases with total doses > 450 mg/m^2. ECG changes and less often, arrhythmias, are seen. Rarely, sudden death has occurred.

Other: Red discoloration of urine for 24-48 hours after drug administration. Doxorubicin is a vesicant and can cause tissue necrosis if extravasated, especially at the concentration usually employed for bolus injections (i.e., 2 mg/mL).

Fluconazole should not be given during EPOCH infusional chemotherapy because of pharmacologic interactions

11.4 ETOPOSIDE

11.4.1 Availability

Commercially available as a 20 mg/mL solution for injection in 5, 25, and 50 mL vials. Each mL contains 2 mg citric acid, 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg of polyethylene glycol 300, and 30.5% alcohol. Please refer to the FDA-approved package insert for etoposide for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.4.2 Toxicity

Myelosuppression, predominantly neutropenia and thrombocytopenia, is the most common toxicity associated with etoposide. Nausea and vomiting range from mild to severe in severity, depending on the dose. Mucositis is also more common at the dose used in this study. Alopecia is likely. Hypotension is associated with too rapid administration of etoposide. This would be unlikely to occur in this trial.

Fluconazole should not be given during EPOCH infusional chemotherapy because of pharmacologic interactions

11.5 VINCRISTINE SULFATE

11.5.1 Availability

Commercially available as a 1 mg/mL solution for injection in 1, 2, and 5 mL vials. Each ml contains 100 mg mannitol, 1.3 mg methylparaben, and 0.2 mg propylparaben. Drug should be stored at 2-8^{o}C and should be protected from light. Please refer to the FDA-approved package insert for vincristine sulfate for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.5.2 Toxicity

The most common toxicity associated with vincristine is neurotoxicity. Peripheral manifestations of neurotoxicity include: numbness of extremities, paresthesias, loss of deep tendon reflexes, neuropathic pain and muscle weakness. GI manifestations of neurotoxicity include constipation, and adynamic ileus. Cranial nerve manifestations include: diplopia, hoarseness, tinnitus, jaw pain (the latter usually occurring with the first dose of vincristine). Orthostatic hypotension & SIADH may also be seen. Vincristine is a vesicant and may cause tissue necrosis upon extravasation. This is more likely with bolus injections as opposed to dilute infusions.

Fluconazole should not be given during EPOCH infusional chemotherapy because of pharmacologic interactions

11.6 ADMINISTRATION OF VINCRISTINE/DOXORUBICIN/ETOPOSIDE

Stability studies conducted by the Pharmaceutical Development Section, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP (0.9%NS) at concentrations, respectively, of 1, 25, and 125 mcg/mL; 1.4, 35, and 175 mcg/mL; 2, 50, and 250 mcg/mL; and 2.8, 70, 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also, admixtures containing vincristine, doxorubicin, and etoposide concentrations of 1.6, 40, and 200 mcg/mL are stable for at least 30 hours at 32°C.

For this study, etoposide, doxorubicin, and vincristine comprising a daily dose (a 24-hour supply) will be diluted in 0.9%NS. Product containers will be replaced every 24 hours to complete the planned duration of infusional treatment. Product volumes will be determined by the amount of etoposide present in a 24-hour supply of medication. For daily etoposide doses ≤130 mg, admixtures will be diluted in approximately 500 mL 0.9%NS. For daily etoposide doses >130 mg, admixtures will be diluted in approximately 1000 mL 0.9%NS.

Etoposide + doxorubicin + vincristine admixtures will be administered by continuous IV infusion over 96 hours with a suitable rate controller pump via a central venous access device.

11.7 CYCLOPHOSPHAMIDE

11.7.1 Availability

Commercially available as a lyophilized powder for reconstitution in 100 mg, 200 mg, 500 mg, 1 gram, and 2 gram vials. Please refer to the FDA-approved package insert for cyclophosphamide for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.7.2 Preparation

Reconstitute with appropriate amounts of Sodium Chloride Injection (0.9%NS) to produce a solution with final concentration of 20 mg/ml.

11.7.3 Storage & Stability

Intact vials should be stored at room temperature storage (not to exceed 30ºC). Reconstituted solution is stable up to 6 days if refrigerated (2-8ºC). Discard solution after storage for 24 hours at room temperature.

11.7.4 Administration

Cyclophosphamide will be diluted in 100 mL of 5% Dextrose Injection or 0.9%NS and infused over 30 minutes. **Hydration Guidelines:** All patients should receive 0.9%NS at the following volumes (based on cyclophosphamide dose levels) and rates with half the specified volume given before starting cyclophosphamide administration and half the volume given after completion of the cyclophosphamide administration.

Cyclophosphamide Dosage Levels	Fluid Volume and Administration Rate
1 & 2	1000 mL 0.9%NS @ 300 – 500 mL/h
Levels 3, 4, & 5	2000 mL 0.9%NS @ 300 – 500 mL/h
Levels ≥6	2500 mL 0.9%NS @ 300 – 500 mL/h

11.7.5 Toxicity

Myelosuppression, hemorrhagic cystitis (patients must be well-hydrated before, during, and after treatment and have adequate renal function). Syndrome of inappropriate antidiuretic hormone (SIADH), fatigue, alopecia, anorexia, nausea, vomiting, hyperuricemia, azoospermia, amenorrhea, cardiotoxicity (myocardial necrosis) usually at doses higher than those used in this study.

11.7.6 Drug Interactions

Cyclophosphamide undergoes metabolic activation via cytochrome P450 3A4 in the liver and may potentially interact with any drug affecting the same isoenzyme. Inhibitors of 3A4 (e.g., itraconazole) could theoretically inhibit activation and inducers of 3A4 (e.g., phenytoin) could theoretically enhance activation of cyclophosphamide to active alkylating species. For the most part, such interactions have not yet been documented clinically.

11.8 PREDNISONE

11.8.1 Availability

Commercially available in a large number of oral dosage strengths including pills and liquid formulations. Please refer to the FDA-approved package insert for prednisone for product information, and a comprehensive list of adverse events.

11.8.2 Storage & Stability

Tablets, solutions and syrup should be stored in tightly closed containers at temperatures between 15-30ºC.

11.8.3 Administration

- Oral. Prednisone utilization will be simplified by using only 20- and 50-mg tablets to produce individual doses and by stratifying prednisone doses by a patient's body surface area (BSA), as follows:

BSA (m^2)	Each Dose
1.25 – 1.49	80 mg
1.5 – 1.83	100 mg
1.84 – 2.16	120 mg
2.17 – 2.41	140 mg
2.42 – 2.6	150 mg
2.7 – 3	170 mg

11.8.4 Toxicity

Side effects likely to be encountered with intermittent high doses include: GI (dyspepsia, ulceration), insomnia, and hyperglycemia. Occasionally a "withdrawal syndrome" after short-

term high doses, such as in this study, manifest muscle aches and pains. Immunosuppression with risk of infection is also seen.

11.9 FILGRASTIM

11.9.1 Availability

Commercially available as a clear sterile solution in single use vials containing 300 mcg (1 mL vial) and 480 mcg (1.6 mL vial). Please refer to the FDA-approved package insert for filgrastim for product information, extensive preparation instructions, and a comprehensive list of adverse events.

11.9.2 Storage & Stability

Filgrastim will be stored at 2-8ºC and is stable for at least 1 year when maintained under refrigeration. DO NOT FREEZE and DO NOT SHAKE the drug product. Final concentration is 300 mcg/ml.

11.9.3 Administration

Filgrastim will be given by subcutaneous injection; patient or other caregiver will be instructed on proper injection technique.

11.9.4 Toxicity

The most common side effect associated with filgrastim is medullary bone pain. Bone pain is usually reported as mild or moderate and, if necessary, may be treated with non-opioid or opioid analgesics. Rare anaphylactic reactions with the first dose; Local reactions at injection sites; Constitutional symptoms; increased alkaline phosphatase, LDH, uric acid; worsening of pre-existing inflammatory conditions.

12 REFERENCES

1. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002;346:1937-47.

2. Dunleavy K, Pittaluga S, Czuczman MS, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B cell lymphoma. Blood 2009.

3. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med 2008;359:2313-23.

4. Whiteside TL. The role of immune cells in the tumor microenvironment. Cancer Treat Res 2006;130:103-24.

5. Mempel TR, Pittet MJ, Khazaie K, et al. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. Immunity 2006;25:129-41.

6. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71-8.

7. Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 2008;8:618-31.

8. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441:431-6.

9. Greten FR, Eckmann L, Greten TF, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 2004;118:285-96.

10. Bergmann C, Strauss L, Zeidler R, Lang S, Whiteside TL. Expansion and characteristics of human T regulatory type 1 cells in co-cultures simulating tumor microenvironment. Cancer Immunol Immunother 2007;56:1429-42.

11. Bergmann C, Strauss L, Zeidler R, Lang S, Whiteside TL. Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. Cancer Res 2007;67:8865-73.

12. Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. A unique subset of CD4+CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. Clin Cancer Res 2007;13:4345-54.

13. Mantovani A, Schioppa T, Biswas SK, Marchesi F, Allavena P, Sica A. Tumor-associated macrophages and dendritic cells as prototypic type II polarized myeloid populations. Tumori 2003;89:459-68.

14. Nagaraj S, Gupta K, Pisarev V, et al. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat Med 2007;13:828-35.

15. Serafini P, Borrello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol 2006;16:53-65.

16. Ochoa AC, Zea AH, Hernandez C, Rodriguez PC. Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. Clin Cancer Res 2007;13:721s-6s.

17. Rodig SJ, Abramson JS, Pinkus GS, et al. Heterogeneous CD52 expression among hematologic neoplasms: implications for the use of alemtuzumab (CAMPATH-1H). Clin Cancer Res 2006;12:7174-9.

18. Skinnider BF, Mak TW. The role of cytokines in classical Hodgkin lymphoma. Blood 2002;99:4283-97.

19. Aldinucci D, Lorenzon D, Cattaruzza L, et al. Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/Rantes in tumor cell growth and microenvironmental interactions. Int J Cancer 2008;122:769-76.

20. Fischer M, Juremalm M, Olsson N, et al. Expression of CCL5/RANTES by Hodgkin and Reed-Sternberg cells and its possible role in the recruitment of mast cells into lymphomatous tissue. Int J Cancer 2003;107:197-201.

21. Younes A, Carbone A. Clinicopathologic and molecular features of Hodgkin's lymphoma. Cancer Biol Ther 2003;2:500-7.

22. Kapp U, Dux A, Schell-Frederick E, et al. Disseminated growth of Hodgkin's-derived cell lines L540 and L540cy in immune-deficient SCID mice. Ann Oncol 1994;5 Suppl 1:121-6.

23. Carbone A, Gloghini A, Gruss HJ, Pinto A. CD40 ligand is constitutively expressed in a subset of T cell lymphomas and on the microenvironmental reactive T cells of follicular lymphomas and Hodgkin's disease. Am J Pathol 1995;147:912-22.

24. Schwaller J, Went P, Matthes T, et al. Paracrine promotion of tumor development by the TNF ligand APRIL in Hodgkin's Disease. Leukemia 2007;21:1324-7.

25. Molin D, Fischer M, Xiang Z, et al. Mast cells express functional CD30 ligand and are the predominant CD30L-positive cells in Hodgkin's disease. Br J Haematol 2001;114:616-23.

26. Pinto A, Aldinucci D, Gloghini A, et al. Human eosinophils express functional CD30 ligand and stimulate proliferation of a Hodgkin's disease cell line. Blood 1996;88:3299-305.

27. Marshall NA, Christie LE, Munro LR, et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. Blood 2004;103:1755-62.

28. Gandhi MK, Moll G, Smith C, et al. Galectin-1 mediated suppression of Epstein-Barr virus specific T-cell immunity in classic Hodgkin lymphoma. Blood 2007;110:1326-9.

29. Chemnitz JM, Eggle D, Driesen J, et al. RNA fingerprints provide direct evidence for the inhibitory role of TGFbeta and PD-1 on CD4+ T cells in Hodgkin lymphoma. Blood 2007;110:3226-33.

30. Newcom SR, Gu L. Transforming growth factor beta 1 messenger RNA in Reed-Sternberg cells in nodular sclerosing Hodgkin's disease. J Clin Pathol 1995;48:160-3.

31. Janik JE DK, Pittaluga S. A Pilot Trial of Campath-1H and Dose-Adjusted EPOCH in CD52-Expressing Aggressive T-Cell Malignancies. Blood (ASH Annual Meeting Abstracts) 2005;106:3348.

32. Gutierrez M, Chabner BA, Pearson D, et al. Role of a doxorubicin-containing regimen in relapsed and resistant lymphomas: an 8-year follow-up study of EPOCH. J Clin Oncol 2000;18:3633-42.

33. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235-42.

34. Feugier P, Van Hoof A, Sebban C, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2005;23:4117-26.

35. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol 2006;24:3121-7.

36. Jermann M, Jost LM, Taverna C, et al. Rituximab-EPOCH, an effective salvage therapy for relapsed, refractory or transformed B-cell lymphomas: results of a phase II study. Ann Oncol 2004;15:511-6.

37. Wilson WH, Dunleavy K, Pittaluga S, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. J Clin Oncol 2008;26:2717-24.

38. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 2001;194:1861-74.

39. Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001;107:241-6.

40. Elsner J, Hochstetter R, Spiekermann K, Kapp A. Surface and mRNA expression of the CD52 antigen by human eosinophils but not by neutrophils. Blood 1996;88:4684-93.

41. Buggins AG, Mufti GJ, Salisbury J, et al. Peripheral blood but not tissue dendritic cells express CD52 and are depleted by treatment with alemtuzumab. Blood 2002;100:1715-20.

42. Ratzinger G, Reagan JL, Heller G, Busam KJ, Young JW. Differential CD52 expression by distinct myeloid dendritic cell subsets: implications for alemtuzumab activity at the level of antigen presentation in allogeneic graft-host interactions in transplantation. Blood 2003;101:1422-9.

43. Xia MQ, Hale G, Waldmann H. Efficient complement-mediated lysis of cells containing the CAMPATH-1 (CDw52) antigen. Mol Immunol 1993;30:1089-96.

44. Golay J, Manganini M, Rambaldi A, Introna M. Effect of alemtuzumab on neoplastic B cells. Haematologica 2004;89:1476-83.

45. Hale G, Bright S, Chumbley G, et al. Removal of T cells from bone marrow for transplantation: a monoclonal antilymphocyte antibody that fixes human complement. Blood 1983;62:873-82.

46. Hale G, Clark M, Waldmann H. Therapeutic potential of rat monoclonal antibodies: isotype specificity of antibody-dependent cell-mediated cytotoxicity with human lymphocytes. J Immunol 1985;134:3056-61.

47. Crowe JS, Hall VS, Smith MA, Cooper HJ, Tite JP. Humanized monoclonal antibody CAMPATH-1H: myeloma cell expression of genomic constructs, nucleotide sequence of cDNA

constructs and comparison of effector mechanisms of myeloma and Chinese hamster ovary cell-derived material. Clin Exp Immunol 1992;87:105-10.

48. Mone AP, Cheney C, Banks AL, et al. Alemtuzumab induces caspase-independent cell death in human chronic lymphocytic leukemia cells through a lipid raft-dependent mechanism. Leukemia 2006;20:272-9.

49. Stanglmaier M, Reis S, Hallek M. Rituximab and alemtuzumab induce a nonclassic, caspase-independent apoptotic pathway in B-lymphoid cell lines and in chronic lymphocytic leukemia cells. Ann Hematol 2004;83:634-45.

50. Smolewski P, Szmigielska-Kaplon A, Cebula B, et al. Proapoptotic activity of alemtuzumab alone and in combination with rituximab or purine nucleoside analogues in chronic lymphocytic leukemia cells. Leuk Lymphoma 2005;46:87-100.

51. Nuckel H, Frey UH, Roth A, Duhrsen U, Siffert W. Alemtuzumab induces enhanced apoptosis in vitro in B-cells from patients with chronic lymphocytic leukemia by antibody-dependent cellular cytotoxicity. Eur J Pharmacol 2005;514:217-24.

52. Watanabe T, Masuyama J, Sohma Y, et al. CD52 is a novel costimulatory molecule for induction of CD4+ regulatory T cells. Clin Immunol 2006;120:247-59.

53. Dunleavy K BJ, Steinberg S. Phase II study of EPOCH infusional chemotherapy in relapsed or refractory Hodgkin lymphoma. A report on toxicity, efficacy and prognostic indicators of outcome. JCO SCO Annual Meeting Proceedings 2004;22:6598.

54. Younes A, Romaguera J, Hagemeister F, et al. A pilot study of rituximab in patients with recurrent, classic Hodgkin disease. Cancer 2003;98:310-4.

55. Younes A FL, Goy A. Results of rituximab plus ABVD in 65 newly diagnosed patients with classical Hodgkin lymphoma: improvement of event free survival in all international prognostic score groups. Blood 2006;108:2742a.

56. Oki Y, Pro B, Fayad LE, et al. Phase 2 study of gemcitabine in combination with rituximab in patients with recurrent or refractory Hodgkin lymphoma. Cancer 2008;112:831-6.

13 APPENDICES

13.1 APPENDIX 1: EPOCH-RC CHEMOTHERAPY

Drug	Total dose* ($mg/m^2/d$)	Route	Day 1 2 3 4 5 6	22
Alemtuzumab	30 mg	IV	x	
Rituximab	375 mg/m2	IV	x	
Etoposide	50 mg/m2/day	CIV	x-x-x-x-	
Vincristine	0.4 mg/m2/day	CIV	x-x-x-x-	
Doxorubicin	10 mg/m2/day	CIV	x-x-x-x-	
Cyclophosphamide	750 mg/m2	IV	x	
Prednisone	60 mg/m2/day BID	PO	x-x-x-x-x	
Filgrastim 5000/mm3	480 mcg QD	SC	x until ANC recovery ≥	
New cycle begins				x

*First cycle doses. Refer to Section **3.2.2** for dose escalations and Section **3.3.2** for dose modifications

For the first cycle of treatment, Rituximab and the EPOCH infusion may be infused on days 2-6 if the Alemtuzumab infusion is prolonged due to infusion reactions.

13.2 APPENDIX 2: EPOCH ADMIXTURE, PREPARATION: AND ADMINISTRATION

Preparation

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine, *PLUS* 40 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in parenteral product containers and administration set tubing.

Etoposide Dose	**Volume of Fluid Containing a Daily Dose**	**Volume of Overfill** (fluid + drug)	**Total Volume in the Product** (including overfill)
≤ 130 mg	528 mL	40 mL	568 mL
> 130 mg	1056 mL	40 mL	1096 mL

Before dispensing 3-in-1 admixtures, Pharmacy staff will:

[1] Purge all air from the drug product container,
[2] Attach an administration set appropriate for use with a portable pump,
[3] The set will be primed close to its distal tip, and
[4] The set will be capped with a Luer-locking cap.

Pre-printed product labeling will identify the 'Total Volume To Infuse' and the 'Volume of Overfill (fluid + drug)'.

Bags will be exchanged daily for four consecutive days to complete a 96-hour drug infusion (unless treatment is interrupted or discontinued due to un-anticipated events).

Administration

Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see the table, below).

Etoposide Dose	**Total Volume to Infuse per 24 hours**	**Volume of Overfill (drug-containing fluid)***	**Administration Rate**
≤ 130 mg	528 mL	40 mL	22 mL/hour
> 130 mg	1056 mL	40 mL	44 mL/hour

* DO NOT attempt to infuse the overfill.

At the end of an infusion, some residual fluid is expected because overfill (excess fluid and drug) was added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

Example at right: The amount of fluid remaining in a bag after completing a 24-hour infusion (1056 mL delivered).

13.3 APPENDIX 3: PERFORMANCE STATUS CRITERIA

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