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ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

CALGB 50303/CTSU 50303

PHASE III RANDOMIZED STUDY OF R-CHOP V. DOSE-ADJUSTED EPOCH-R WITH MOLECULAR PROFILING IN UNTREATED DE NOVO DIFFUSE LARGE B-CELL LYMPHOMAS

As of Update #14, enrollment on CALGB 50303 will be limited to institutions and patients participating in the required imaging companion study CALGB 580603.

Study Chairs

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Participating Organizations: **ALLIANCE**/Alliance for Clinical Trials in Oncology, **SWOG**/SWOG, **ECOG-ACRIN**/ECOG-ACRIN Cancer Research Group, **NRG**/NRG Oncology

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CAEPR for rituximab appears in Section 15.1

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Medidata RAVE® iMedidata portal

[redacted]

This study is supported by the NCI Cancer Trials Support Unit (CTSU).
Institutions not aligned with the Alliance will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix

- The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org.
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to the CTSU logistical appendix for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to the CTSU logistical appendix for specific instructions and forms to be submitted.
- Data management will be performed by the Alliance. **Case report forms** (with the exception of patient enrollment forms)**, clinical reports, and transmittals** must be sent to the Alliance unless otherwise directed by the protocol. Do not send study data or case report forms to the CTSU Data Operations.
- **Data query and delinquency reports** will be sent directly to the enrolling site by the Alliance (generally via e-mail but may be sent via fax or postal mail). Please send query responses and delinquent data to Alliance and do not copy the CTSU Data Operations. Query responses should be sent to the Alliance via postal mail (no transmittal form needs to accompany response). Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP AMS account contact information current. This will ensure timely communication between the clinical site and the Alliance Statistics and Data Center.

The pharmacogenetic component of this study is conducted as part of the NIH Pharmacogenetics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/Initiatives/PGRN/ for details).

PHASE III RANDOMIZED STUDY OF R-CHOP V. DOSE-ADJUSTED EPOCH-R WITH MOLECULAR PROFILING IN UNTREATED DE NOVO DIFFUSE LARGE B-CELL LYMPHOMAS

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Eligibility Criteria:

Histologically documented de novo stage I mediastinal (thymic) DLBCL or any stage II, III, or IV DLBCL.

Patients must have one of the following WHO histologic subtypes without evidence of indolent histological features in the tissue biopsy or bone marrow:

1. CD20+ diffuse large B-cell lymphoma (includes morphological variants: centroblastic; immunoblastic; T-cell/histiocyte rich; and anaplastic).
2. CD20+ mediastinal (thymic) large B-cell lymphoma
3. CD20+ intravascular large B-cell lymphoma

No prior cytotoxic chemotherapy or rituximab (see Section 4.2)

Age ≥ 18 years.

ECOG Performance Status 0-2.

No active ischemic heart disease or congestive heart failure (see Section 4.5).

No known lymphomatous involvement of the CNS (see Section 4.6).

No known HIV disease (see Section 4.7).

Non pregnant and non-nursing (see Section 4.8).

Patients with active medical processes (e.g., uncontrolled bacterial or viral infection, bleeding) not related to their lymphoma should be excluded.

Required Initial Laboratory Values*

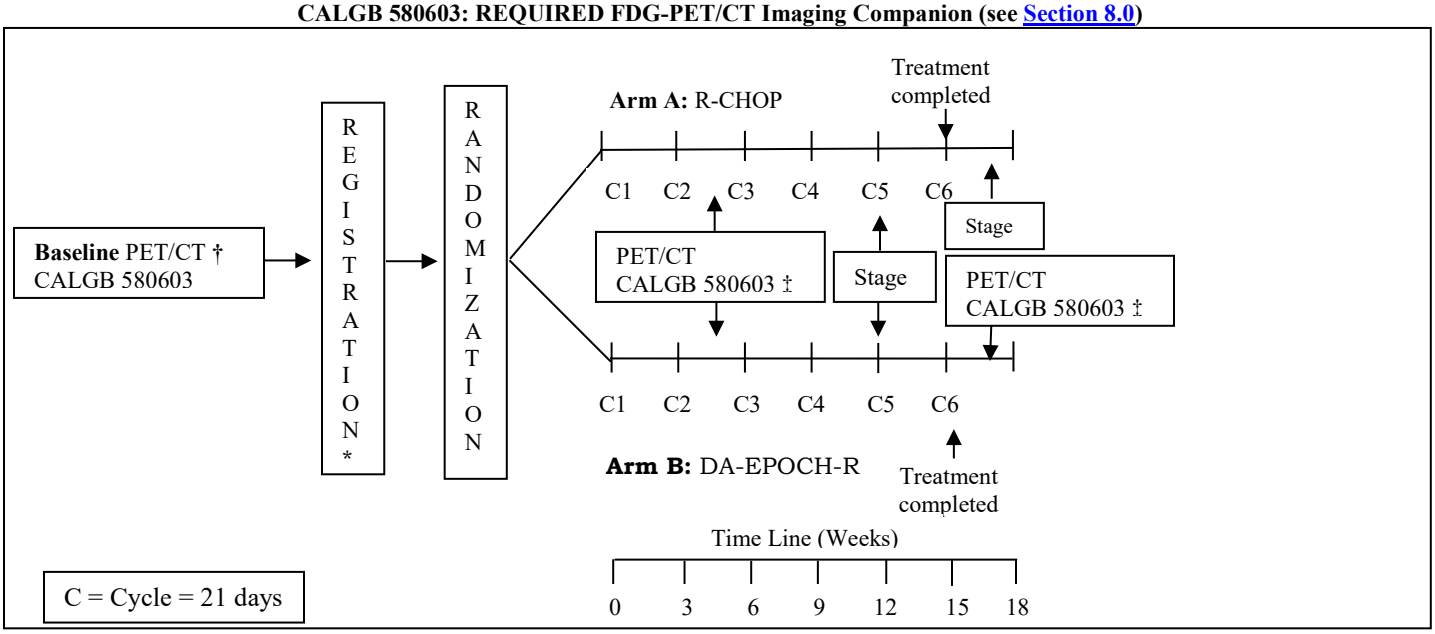
ANC	≥ 1000/µL
Platelets	≥ 100,000/µL
Creatinine	≤ 1.5 mg/dL
or CrCl	≥ 50 cc/min
Total Bilirubin	≤ 2 mg/dL**

**Unless attributable to non-Hodgkin lymphoma*

***Unless attributable to Gilbert's Disease*

TREATMENT OVERVIEW

CALGB 580603: REQUIRED FDG-PET/CT Imaging Companion (see Section 8.0)



* ***A frozen tumor biopsy equivalent to a minimum of 4 at least 16 gauge needle cores is an important component of this study (see repeat tissue biopsy guidance in Section 4.1 and Section 5.4.1). Patients without adequate frozen material should have a biopsy performed to obtain material. If a biopsy is performed and does not yield adequate material, the patient is still eligible for the study. Patients who do not have a tissue site that can be safely biopsied may be enrolled only if permission is granted in writing (e-mail) by the Study Chairs (Drs. Wilson or Zelenetz) or their designees.***

† Baseline PET/CT for patients registered to the optional FDG-PET/CT Imaging Companion (CALGB 580603) is preferred prior to the CALGB 50303 baseline excisional biospsy and ≤ 30 days prior to the start of therapy.

‡ ***For patients registered to the optional FDG-PET/CT Imaging Companion (CALGB 580603), PET/CT of abd/chest/pelvis will be collected at baseline, post-Cycle 2, and post-Cycle 6 (see Section 8.3).***

No concurrent radiation therapy will be permitted on study except for isolated CNS lesions. Patients who receive radiation to any site other than the CNS will be inevaluable. Patients with parenchymal CNS lesions should receive whole brain radiation and will only be considered treatment failures if the systemic disease progresses.

The use of epoetin is allowed at the discretion of the investigator. It is recommended that epoetin not be used in this protocol unless hemoglobin is ≤ 10 g/dL, and that it be held or the dose modified for hemoglobin > 12 g/dL.

PHASE III RANDOMIZED STUDY OF R-CHOP V. DOSE-ADJUSTED EPOCH-R WITH MOLECULAR PROFILING IN UNTREATED DE NOVO DIFFUSE LARGE B-CELL LYMPHOMAS

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Arm A: R-CHOP (see Section 7.1)

Dose reductions for subsequent cycles will be determined by the occurrence of grade 4 neutropenia or febrile neutropenia (see Section 9.1.1).

- Rituximab 375 mg/m^2 IV infusion on Day 1 prior to CHOP chemotherapy.
- Cyclophosphamide 750 mg/m^2 IV on Day 1.
- Doxorubicin 50 mg/m^2 IV on Day 1.
- Vincristine 1.4 mg/m^2 IV (2 mg cap) on Day 1.
- Prednisone 40 mg/m^2/day PO on Days 1-5.
- Begin filgrastim or pegfilgrastim if grade 4 neutropenia (ANC < 500/µL) or febrile neutropenia (ANC < 500/µL + fever ≥ 38.5°C [101°F] sustained for more than one hour) develops on the previous cycle. Administer filgrastim 480 mcg subcutaneous daily from Day 2 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 2-11) if the ANC is not being monitored, or pegfilgrastim 6 mg SubQ on Day 2 (may be delayed up to two days for convenience of scheduling due to holidays, weekend, etc.).

Required ancillary medications administered during all cycles:

- Omeprazole 20 mg/day PO (or equivalent)
- Stimulant laxative/stool softener (e.g., Pericolace®) 2 tablets PO BID and adjusted as necessary for constipation (prevention).
- Lactulose 20 g PO Q 6 hours PRN until resolution or institutional preference for treatment of constipation.
- Acetaminophen 650 mg PO and diphenhydramine 50-100 mg IV or PO 30-60 minutes prior to rituximab.
- Hepatitis B surface Ag+ patients receive lamivudine 100 mg/day PO until 8 weeks past last chemotherapy.

Patients with (1) ≥ 2 extranodal sites and elevated LDH, or (2) bone marrow involvement by large cell lymphoma will receive CNS prophylaxis consisting of intrathecal methotrexate 12 mg on Day 1 (or Day 2) of Cycles 3, 4, 5, and 6.

Cycles will be repeated every 21 days for 6 treatment cycles. Restaging will occur after Cycles 4 and 6.

Arm B: DA-EPOCH-R (see Section 7.2)

Cycle 1 Doses:

- Rituximab 375 mg/m^2 IV infusion on Day 1 prior to EPOCH chemotherapy.
- Doxorubicin 10 mg/m^2/day CIVI on Days 1-4.
- Etoposide 50 mg/m^2/day CIVI on Days 1-4.
- Vincristine 0.4 mg/m^2/day (no cap) CIVI on Days 1-4 (total 1.6 mg/m^2 over 96 hours).
- Cyclophosphamide 750 mg/m^2 IV on Day 5 (following completion of 96 hour infusions).
- Prednisone 60 mg/m^2 PO BID on Days 1-5.
- Administer filgrastim 480 mcg subcutaneous daily from Day 6 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 6-15) if the ANC is not being monitored, **during every** cycle.

Doses for subsequent cycles will be determined by the absolute neutrophil (ANC) or platelet nadir from the previous cycle (see Section 7.2.3).

Required ancillary medications administered during all cycles:

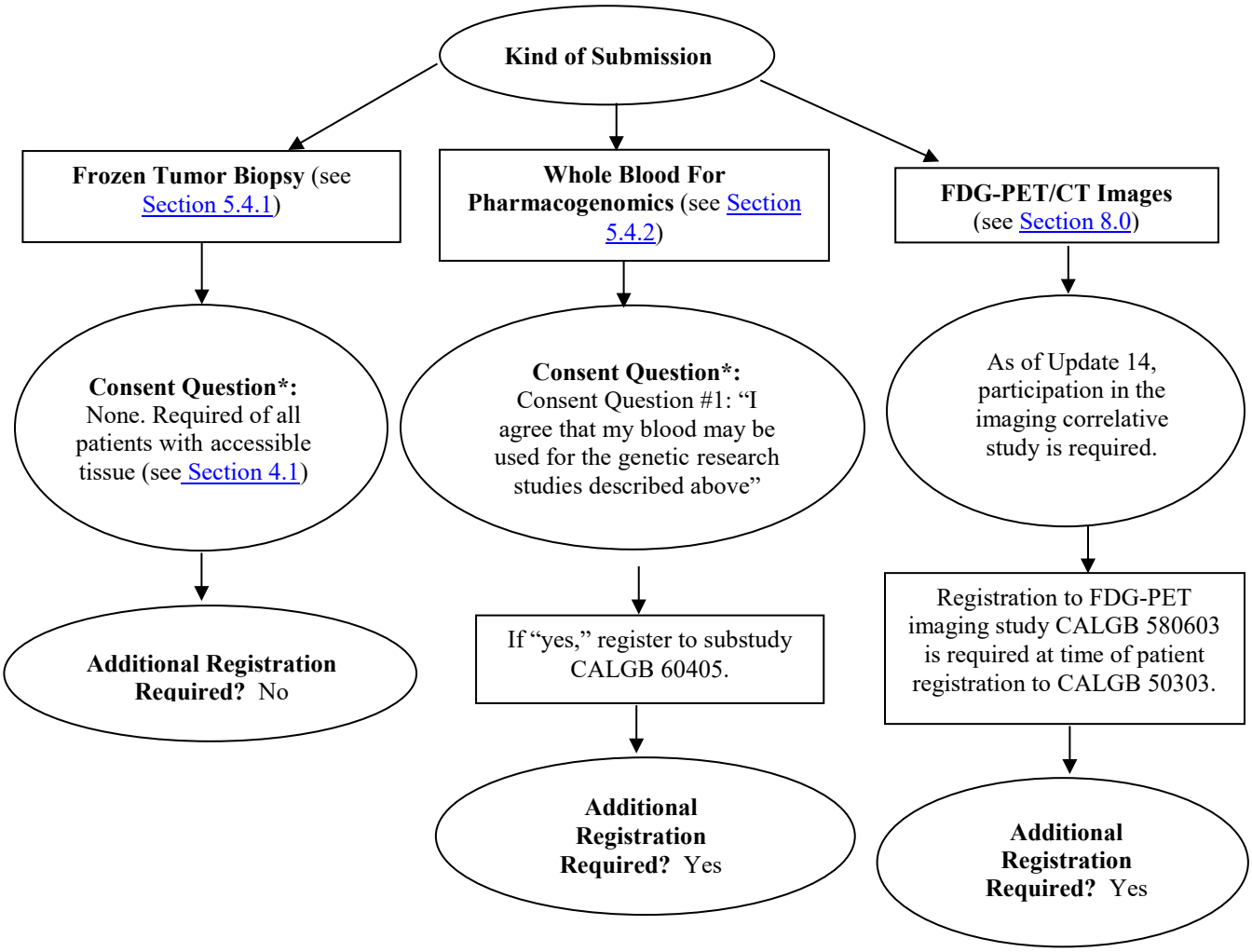
- Cotrimoxazole DS 1 tablet three times weekly (or equivalent if allergic).
- Omeprazole 20 mg/day PO (or equivalent)
- Stimulant laxative/stool softener (e.g., Pericolace®) 2 tablets PO BID and adjusted as necessary for constipation (prevention).
- Lactulose 20 g PO Q6 hours PRN until resolution or institutional preference for treatment of constipation.
- Acetaminophen 650 mg PO and diphenhydramine 50-100 mg IV or PO 30-60 minutes prior to rituximab.
- Hepatitis B surface Ag+ patients receive lamivudine 100 mg/day PO until 8 weeks past last chemotherapy.

Patients with (1) ≥ 2 extranodal sites and elevated LDH, or (2) bone marrow involvement by large cell lymphoma will receive CNS prophylaxis consisting of intrathecal methotrexate 12 mg on Day 1 (or Day 2) of Cycles 3, 4, 5, and 6.

Cycles will be repeated every 21 days for a maximum of 6 cycles. Restaging will occur after Cycles 4 and 6.

Guide to Companion Study Registration

The following table is meant to assist clinical research associates in companion study registration and, if applicable, sample procurement.



* Please note that model informed consent questions are mapped to the CALGB registration system. As a result, it is highly recommended that the wording and order of the model informed consent questions be retained in order to facilitate patient registration.

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1.0 INTRODUCTION

1.1 Background

Approximately 55,000 cases of non-Hodgkin lymphoma (NHL) are diagnosed yearly in the United States. Diffuse large B-cell lymphoma (DLBCL) comprises 25-30% of these cases, and represents a significant public health issue; it is the single most common lymphoma in the United States with over fifteen thousand new cases each year and a rising incidence. Although potentially curable, only 35% of patients achieve a durable complete remission with standard CHOP chemotherapy, and most of the remaining patients die of their disease [1]. Because of the curative potential of DLBCL, advances in treatment will have a significant impact on patient survival and quality of life. Indeed, a 15% increase in cure will save over 2000 additional lives annually with attendant savings in medical costs, social productivity and patient suffering. Investigators have worked to develop alternative regimens that reduce toxicity, permit dose intensification, and circumvent tumor cell resistance. Strategies to improve chemotherapy have been mostly unsuccessful. The second and third generation regimens–m-BACOD, ProMACE-CytaBOM and MACOP-B and high dose therapy with stem cell transplantation–strategies empirically modeled on the Goldie-Coldman and Dose Intensity hypotheses, have not improved survival compared to standard "CHOP" based treatment [1-3].

Several recent reports suggest some advances in the chemotherapy treatment of aggressive lymphomas, but are open to interpretation. A randomized comparison of ACVBP, a high-dose CHOP variant, and standard CHOP in 708 patients aged 61 to 69 years with aggressive NHL and at least one adverse prognostic factor in the age-adjusted international prognostic index (IPI) was recently published [4]. At 5 years, the ACVBP and CHOP groups had an event-free survival (EFS) of 39% and 29% (p=0.005), respectively, and an overall survival of 46% and 38% (p=.036), showing an improved outcome for ACVBP. The results indicate, however, that some of the benefit of ACVBP may lie in its better control of central nervous system (CNS) disease. Because both arms had a similar rate of complete remission (CR) with 58% in the ACVBP and 56% in the CHOP groups, much of the benefit in the ACVBP arm was due to an improvement in disease-free survival (DFS) due in part to a lower rate of CNS progression (9 patients vs. 26 patients) in the ACVBP compared to the CHOP arm (p=.004). Importantly, only patients in the ACVBP group received intrathecal CNS prophylaxis as well as intravenous methotrexate, which likely improved control of occult CNS lymphoma.

The German Lymphoma Study Group recently reported the preliminary results of a 4-arm comparison of 14- and 21-day CHOP and CHOEP in younger and older (> 60 years) patients with untreated aggressive lymphomas [5,6]. In younger patients with low-risk disease, the addition of etoposide (CHOEP) improved overall survival from 74% to 88%, compared to CHOP, whereas in older patients with higher risk disease (i.e., at least 1 adverse IPI factor) CHOP time intensification (i.e., CHOP-14), compared to CHOP-21, was associated with an improved survival (54% v 45%, respectively). One question with these results is the absence of an obvious biological basis for the findings. Further insight will have to await publication of these results.

The absence of significant advances in the treatment of aggressive lymphomas over the past 30 years is a sobering testament to the failure of empirically based clinical research and not necessarily to the absence of more effective chemotherapy platforms. Indeed, chemotherapy remains the centerpiece of curative therapy for DLBCL, although recent studies indicate the importance of targeted agents, such as rituximab. Hence, there is an important need to conduct clinical trials using scientifically engineered therapeutics and to assess biological endpoints.

1.2 Rituximab-CHOP Regimens

Recent studies suggest that the monoclonal antibody, rituximab (R), directed against the CD20 B-cell antigen, may increase the efficacy of chemotherapy. *In vitro*, rituximab has been shown to sensitize drug-resistant lymphoma cell lines to chemotherapy. Recent evidence has shown that rituximab increases the benefit of CHOP in aggressive CD20+ lymphomas. A randomized study of CHOP versus R-CHOP was performed in 197 patients ≥ 60 years old with DLBCL [7]. Patients who received R-CHOP had a higher rate of CR compared to CHOP alone; 76% versus 63%, respectively. With a median follow-up of 2 years, the EFS of R-CHOP and CHOP was 57% and 38%, respectively, and at 3 years, it was approximately 51% and 38%. Results from a phase II study of rituximab and CHOP in untreated aggressive B-cell lymphomas have also been reported by Vose et al. [8]. In this study of 33 patients, 61% achieved CR and 90% of patients were progression-free, with a median follow-up of 26 months. While the 90% PFS is very encouraging, 70% of patients in the series were in the low IPI (0-2) risk group, and at least 21% had evidence of a follicular histological pattern. The preliminary results of a phase III trial of CHOP-R versus CHOP with a second randomization to maintenance rituximab or observation in patients 60 years of age and older with DLBCL were recently presented at the American Society of Hematology meetings in 2003 [9]. Although the second randomization confounded the study interpretation, the results nevertheless suggested that the addition of rituximab improves the outcome of CHOP treatment in DLBCL. Hence, R-CHOP has become the de facto new standard for DLBCL, although longer follow-up will be needed to assess accurately the impact of rituximab. Of note, however, the benefit of rituximab appears to be principally in patients with BCL-2 positive tumors, which is likely a marker for the activated B-cell (ABC) subtype of DLBCL [10].

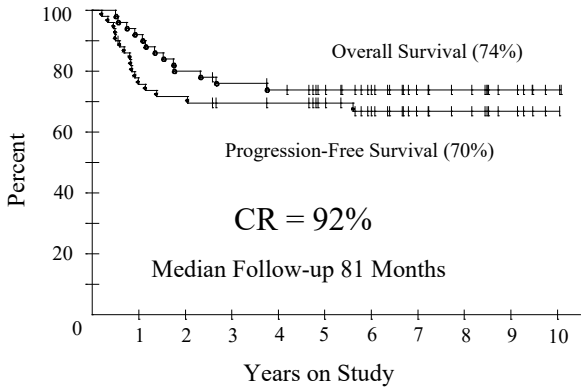
1.3 DA-EPOCH-Rituximab Regimens

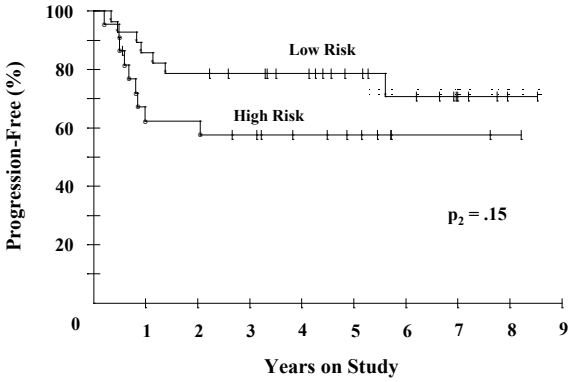
The dose-adjusted (DA) EPOCH regimen was developed based on in vitro and pharmacodynamic principles to help overcome drug resistance [11]. In this regimen, doxorubicin, vincristine, and etoposide are administered as a 96-hour continuous infusion, and cyclophosphamide and prednisone are administered on a bolus schedule. The rationale for the administration schedule derived from the laboratory observation that human tumor cell lines, including those with a multi-drug resistance phenotype, are more sensitive to cytotoxic natural products given for prolonged periods at low concentrations than to the same agents given for brief periods at higher concentrations.

EPOCH was initially developed and tested in 131 patients with relapsed or refractory NHL [12]. The response rate in these heavily pre-treated patients was 74% with 24% of patients achieving complete remission. Furthermore, among the subset of patients with aggressive histologies, 36% achieved complete remissions. Therapy was tolerable with a treatment-related mortality of 2%. A study of EPOCH was undertaken to address the role of EPOCH as initial therapy in DLBCL [13]. In this trial, the doses of doxorubicin, etoposide and cyclophosphamide were escalated 20% on every cycle in which the nadir absolute neutrophil count (ANC) exceeded 500/μL, or reduced 20% if the ANC was less than 500/μL for more than 2 CBC measurements (CBC obtained twice weekly only) or the platelet nadir was < 25,000/μL. The rationale for dose-adjustment in each patient was based on several factors: 1. Dose intensity has been associated with improved outcome in aggressive lymphomas; 2. Small changes in steady state concentrations (Css) for infused agents can significantly affect efficacy (i.e., threshold concentration); 3. Preliminary pharmacokinetics showed up to a 6-fold variation in Css in patients treated with the same dose.

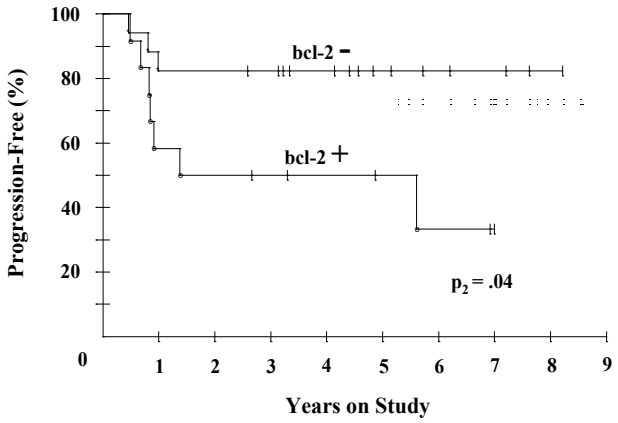
Fifty patients with untreated de novo DLBCL were enrolled. Patients had a median (range) age of 46 (20-88), and IPI distribution of 56% low (0/1/2) and 44% high (3/4/5) risk disease. Complete responses (CR) were obtained in 45 patients (92%) and partial responses in 4 patients.

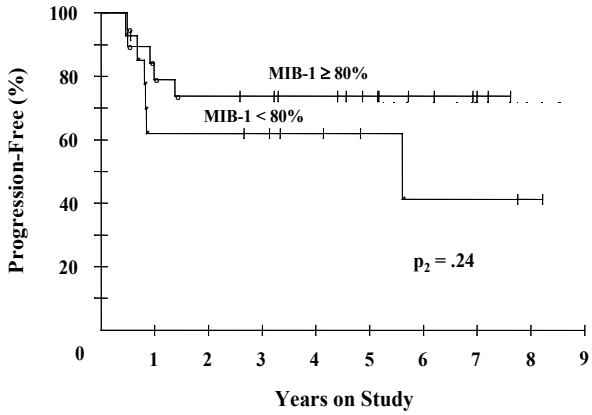
There was no difference in CR rate among the IPI risk groups with 19 of 22 high risk and 26 of 27 low risk patients achieving CR. With a median follow-up of 62 months, the actuarial progression-free survival (PFS) is 70% and overall survival (OS) is 73%. The data have recently been updated to a median follow-up of 81 months, and no relapses have occurred since reported at a median follow-up of 62 months as shown below [14]. The PFS at 62 months for low and high-risk groups are 79% and 58%, respectively, and the OS is 89% and 55%, respectively. Dose-adjusted EPOCH was well tolerated with a 8% incidence of fever and neutropenia.





Potential mechanisms of treatment failure, such as bcl-2 and p53 mutation, which are associated with apoptosis, and tumor proliferation rate (MIB-1), which is associated with drug sensitivity, were assessed by immunohistochemistry [15-18]. Overexpression of bcl-2 was the only factor associated with treatment failure in both univariate and multivariate Cox models, with 82% of bcl-2 negative and only 50% of bcl-2 positive patients progression-free at 52 months (shown below left). Also of interest was the impact of high tumor proliferation on outcome. In contrast to findings with CHOP-based regimens, patients with high tumor proliferation (>80%) were less likely to progress than patients with lower proliferation rates, although the result did not reach statistical significance ($p_2 = 0.09$) (shown below right). These results suggest that dose-adjusted EPOCH may produce greater cell kill than CHOP-based regimens. Patients with p53 overexpression had a higher progression rate, although only survival showed a statistically significant association.

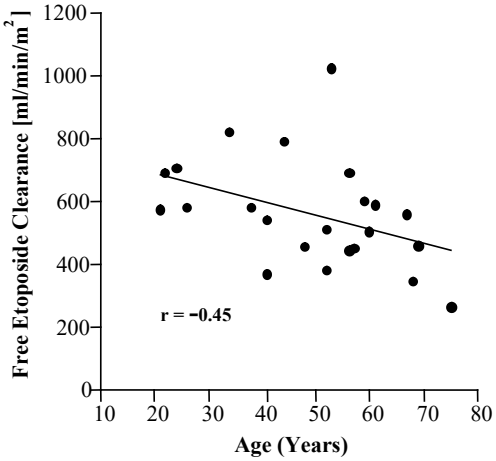


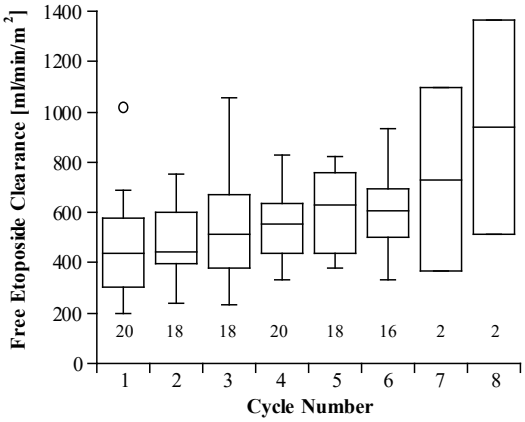


The dose-adjustment paradigm was developed in an attempt to reduce interpatient variation in plasma concentrations of chemotherapy by normalizing dose-rate to neutrophil nadirs. Most patients required dose escalations to achieve a neutrophil nadir, with 29% of cycles at ≥ 144%

of dose level 1 and 5% administered below dose level 1. Of note, the first cycle was more often associated with greater hematopoietic toxicity than cycle 2 with 38% of patients requiring dose escalations on cycle 3. Over all cycles, the mean dose intensification (DI) for the escalated agents were equivalent to the second dose level and were: (mean ± SD: mg/m^2/week) cyclophosphamide 296 ± 59; etoposide 80 ± 14; doxorubicin 16 ± 2.8. The vincristine DI was 96% of the planned dose intensity and was (mean ± SD: mg/m^2/week) 0.51 ± 0.06.

The strategy of normalizing dose rate to the neutrophil nadir allows an opportunity to examine more accurately the influence of clinical factors on DI. This analysis revealed a significant inverse correlation between the DI of all adjusted agents and patient age (etoposide: r = -0.48; $p_2 = 0.0004$), but no correlation with any other IPI risk factor, the IPI index itself, or the outcome measures of PFS or OS (shown below left) (unpublished observations) [13]. The lower DI with age could be explained by decreased hematopoietic tolerance and/or decreased drug metabolism in older patients. Preliminary pharmacokinetics (PK) of etoposide in 14 patients receiving dose-adjusted EPOCH revealed an inverse correlation between age and clearance of free etoposide (r = -0.44; $p_2 = 0.1$), suggesting that older patients may achieve higher serum levels than younger patients at similar dose rates (shown below left). Additionally, in 6 patients with PK on multiple cycles, the clearance of free etoposide increased over each cycle, with an average increase of 58% between cycles 1 and 6 (Hochberg adjustment for multiple comparisons: $p_2 = 0.02$), indicating that, in the absence of dose escalation, the actual etoposide serum levels would decrease in many patients (shown below right).

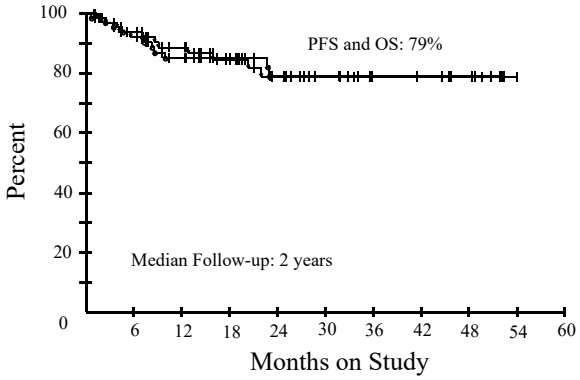




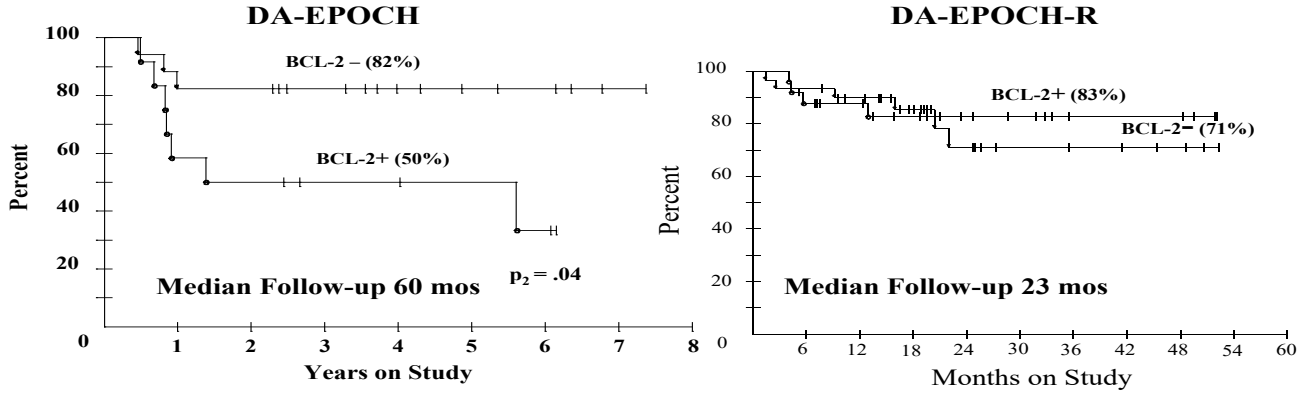
These data suggest that the neutrophil nadir can be used as a surrogate to pharmacokinetics for normalization of Css. However, the neutrophil nadir will also reflect intracellular mechanisms such as drug efflux pumps (e.g., MDR-1), drug metabolism and DNA repair, to name a few. These variables all contribute to the variability of tumor response to chemotherapy, and are influenced by both germline polymorphisms and mutations within the tumor [19]. Hence, a secondary objective of this study will be to investigate the relationship between the pharmacogenomics (genetic polymorphisms) of normal mononuclear cells and clinical outcome (EFS, OS and toxicity) and tumor microarray. Although exploratory, this may provide important insights into the effects of genetic polymorphism on pathways of lymphomagenesis and treatment response and toxicity. This will be the first study to assess pharmacogenomics in DLBCL.

The results with dose-adjusted EPOCH demonstrated that the regimen was well tolerated and suggested that this novel schedule and pharmacodynamic-based regimen may improve upon results achieved with CHOP-based regimens. However, patients with bcl-2+ lymphomas had a significantly worse outcome, compared with bcl-2 negative cases, indicating the need for further

improvement in the regimen. Thus, based upon in vitro evidence showing synergy between rituximab and cytotoxic agents, a phase II study of dose-adjusted EPOCH with rituximab (DA-EPOCH-R) was begun [20]. Preliminary results are available in 76 previously untreated patients with CD20+ DLBCL. The median patient age is 48 (range: 12-85) years, median ECOG performance status is 1 (range: 0-3), and 36% had high-intermediate or high international prognostic index (IPI) scores [14]. Among 71 patients evaluable for response (4 TE; 1 NE), complete responses (CR) were achieved in 92%, which is similar to DA-EPOCH alone. At a median follow-up of 26 months, the progression-free and overall survivals are both 82%.



Based on the hypothesis that rituximab may help overcome molecular causes of drug resistance, it was investigated whether BCL-2 and p53 overexpression are associated with drug resistance in DA-EPOCH-R, as had been shown with DA-EPOCH alone. As shown below, BCL-2 no longer identified an adverse prognostic group in the DA-EPOCH-R regimen, suggesting that rituximab may overcome these pathways of resistance, a result which was confirmed in the GELA study of CHOP v R-CHOP [10]. Biologically, BCL-2 is a marker of the activated B-cell (ABC) molecular subtype of DLBCL, and this subtype may be the actual target of rituximab.



The data on dose-adjusted EPOCH and EPOCH-R suggest these regimens may circumvent the limitations of conventional CHOP chemotherapy, but this needs to be ultimately confirmed in a randomized trial with R-CHOP. Furthermore, the dose-adjustment strategy has made the regimen tolerable in all age ranges and, unlike other approaches, its use is not age dependent.

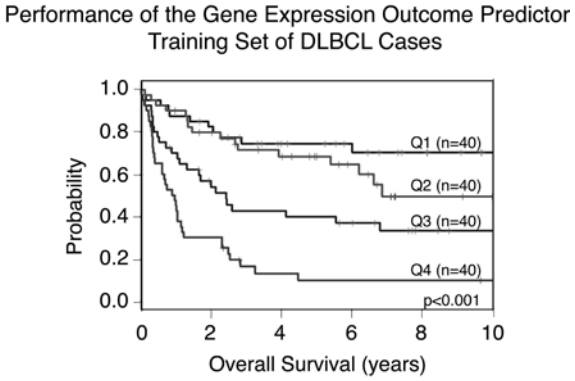
1.4 Molecular Profiling

Historically, treatment studies have been restrained by histological definitions of cancer. Molecular characterization, however, provides the foundation for a biological classification that weighs the relevance of tumor heterogeneity [21,22]. Without molecular characterization, the

clinical evaluation of treatments/drugs may fail to reveal important activity if only a portion of the tumors possess the molecular target(s) or pathways affected by the therapy. The Lymphoma/Leukemia Molecular Profiling Project at the NCI (LLMPP) has used molecular profiling to subdivide the most common type of non-Hodgkin lymphoma, diffuse large B-cell lymphoma, into distinct diseases with different responses to anthracyline-based multi-agent chemotherapy. Diffuse large B-cell lymphoma (DLBCL) presents a unique opportunity to incorporate molecular profiling in a clinical study comparing two treatment strategies that may have different molecular pathways of resistance. This trial will assess a new regimen (DA-EPOCH-R) versus the current standard regimen (R-CHOP) for this common and potentially curable lymphoma. Through molecular profiling, it may be possible to identify molecular subgroups that are more susceptible to one treatment regimen and will allow the development of a molecular prognostic model that may be useful clinically. We believe this study can provide proof of principle for the importance of molecular profiling in prospective clinical trials and hasten the translation of molecular diagnosis into routine clinical management of cancer patients.

Roughly 35% of patients with DLBCL can be cured by conventional anthracycline-based chemotherapy regimens (e.g., CHOP), whereas the others eventually succumb to this disease. This clinical heterogeneity suggested that this lymphoma type might encompass several molecularly distinct subgroups that differ in their responsiveness to therapy. This hypothesis has been confirmed in gene expression profiling studies conducted by the LLMPP, which recently analyzed gene expression in 240 retrospectively obtained biopsy specimens of DLBCL and correlated gene expression profiles of the tumors with clinical outcome of the patients [23,24].

Three broad gene expression subgroups of DLBCL were defined that differed in the expression of over 1,000 genes. These subgroups reflected, in part, the cell of origin of the tumors. One subgroup, termed germinal center B-cell-like (GCB) DLBCL resembled normal germinal center B cells in gene expression whereas another subgroup, termed activated B-cell-like (ABC) DLBCL, resembled mitogenically activated blood B cells. A third minor subgroup, termed Type 3, did not share gene expression features of either GCB or ABC DLBCL and may be heterogeneous. Importantly, this gene expression subgroup distinction identified patients with distinct clinical outcomes: GCB DLBCL patients had a relatively favorable prognosis, with a 5-year survival rate of 60%, whereas ABC and Type 3 DLBCL patients had 5-year survival rates of 35% and 39%, respectively. One mechanism underlying this differential response to chemotherapy may be that GCB DLBCLs, like normal germinal center B-cells, have particularly low activity of the NF-kB pathway. This pathway protects cells from apoptosis induced by chemotherapy and is constitutively active in ABC DLBCLs, perhaps accounting for their relative resistance to CHOP chemotherapy [13].



Additionally, however, further variation in gene expression was found to account for the residual clinical heterogeneity within each of the DLBCL subgroups [24]. A systematic search was undertaken for individual genes with expression patterns that correlated with overall survival. The majority of predictive genes could be classified into four gene expression "signatures" that reflected different aspects of tumor cell biology. Most of the genes that predicted poor outcome belonged to the proliferation gene expression signature that includes genes that are more highly expressed in dividing cells than in quiescent cells. Many genes that predicted good outcome belonged to the germinal center B-cell signature, a finding that reflects the relatively good outcome of GCB DLBCL patients. Two other sets of genes that predicted good outcome suggested that host immune responses might contribute to curative outcomes following chemotherapy. The expression of MHC class II genes in the tumor cells correlated with good outcome, suggesting a role for antigen presentation by the tumor cells to infiltrating T lymphocytes. Another set of genes that predicted good outcome identified tumors with an intense fibrotic reaction characterized by infiltrating macrophage and natural killer cells, and may reflect an innate immune response to the tumor. In addition to these four sets of predictive genes, a single gene, BMP6, was found to be an independent predictor of poor outcome, and its expression may reflect the ability of the lymphoma cells to differentiate towards plasma cells.

A multivariate predictive model of overall survival was created from 17 genes chosen from the different functional groups mentioned above [24]. This gene expression-based outcome predictor was able to stratify patients into risk groups with strikingly distinct overall survival rates. One half of the patients could be placed in a favorable risk group with a 71% 5-year survival rate. One quarter of the patients were in a poor risk group with only a 15% 5-year survival rate and the remaining quarter of patients had a 36% 5-year survival rate. Clearly, these findings demonstrate the need to identify alternative therapies for those patients in the poorest risk groups.

The results of this study will have important implications for future treatment of DLBCL. Clinically, the study will determine if DA-EPOCH-R provides a significant therapeutic advance over R-CHOP, and will determine the therapeutic platform for future trials in DLBCL. Because DA-EPOCH-R incorporates several unique strategies, including infusional scheduling, etoposide use and pharmacodynamics-based dose optimization, the clinical outcome will provide some assessment of these strategies. The microarray tissue analyses and pharmacogenomics will provide important information. This study will be the first to prospectively assess microarray histological diagnosis, which is likely to become an important pathological diagnostic tool. The study will develop a molecular prognostic model for each regimen, which will provide patient prognostic information, identify patients suitable for novel therapy approaches, and will identify potential mechanisms of treatment failure important for the rational development of new treatment strategies. Importantly, this study will be the first to assess molecular mechanisms of treatment failure with a rituximab-based regimen. It will also be the first to prospectively validate the use of microarray in a lymphoma therapeutic trial, thus providing proof of principle, and serve as a model for future trials.

1.5 Radiotherapy consolidation and CNS prophylaxis

A salient question is the role of radiation in the treatment of DLBCL. Radiation has been variably used in clinical trials for both advanced and early stage disease. Although several studies have suggested a benefit for radiation consolidation following CHOP chemotherapy in early stage DLBCL, longer follow-up has indicated a higher rate of late relapses among patients who receive limited chemotherapy and radiation, with an equivalent PFS and OS [25,26]. Additionally, preliminary results of a randomized study of ACVBP versus CHOP followed by radiotherapy in low risk localized aggressive lymphoma showed a statistically superior EFS and OS for the ACVBP arm, and concluded that ACVBP is superior to CHOP + radiation [27]. Radiation has

also been commonly used following chemotherapy in patients with primary mediastinal (thymic) DLBCL (PMLBCL). In a study of 50 patients with PMLBCL initially treated with MACOP-B, 66% had a persistently positive gallium scan, suggesting the presence of active disease [28]. However, following radiation consolidation, only 19% of patients had a positive gallium scan. With a median follow up of 39 months, 80% of all patients were event free. Importantly, all patients with a negative gallium after radiation were event free, whereas 60% of gallium positive patients relapsed. For comparison, none of the patients with PMLBCL treated with DA-EPOCH or DA-EPOCH-R received radiation consolidation. Among 14 PMLBCL patients treated with DA-EPOCH, PFS and OS were 68% and 79%, respectively, at the median follow-up of 81 months; all patients in CR or CRu were gallium negative at the end of treatment. Furthermore, among 13 PMLBCL patients treated with DA-EPOCH-R, PFS and OS is 93% and 100%, respectively, at the median follow-up of 24 months. Although the patient numbers are small, they suggest that rituximab may benefit patients with PMLBCL; a benefit which may also extend to patients receiving CHOP chemotherapy. These studies raise the question of whether radiation consolidation is necessary in patients with PMLBCL. Although radiation appears to benefit patients treated with MACOP-B, this cannot be extended to the DA-EPOCH or ACVBP regimens. It is also important to recognize that radiation consolidation has significant adverse long term side effects, including an increased risk of breast cancer in women under 30 years of age (the most common group with PMLBCL), heart disease and other solid tumors [29]. Hence, inclusion of PMLBCL patients in this phase III study will provide important information on their outcome with rituximab based treatment and in the absence of radiation. To provide a measure of safety, all patients with PMLBCL with residual mediastinal abnormalities after completion of treatment will receive a PET scan. Patients whose PET scan is highly suspicious will undergo biopsy. Patients whose PET scan is equivocal will be followed monthly with a PET and/or CT scan and undergo a biopsy if the scan indicates increasing abnormalities. Only patients with biopsy proven disease following treatment may receive radiation and will be considered treatment failures on the study.

Radiation consolidation has been variably used in patients with more advanced stage DLBCL, but has no clear benefit. The 4-arm studies of CHOP 14/21 and CHOEP 14/21 employed radiotherapy consolidation for those patients with initial masses ≥ 7.5 cm, but the studies were not designed to assess the role of radiation and its inclusion confounds the interpretation of results [5,6]. In contrast, studies employing ACVBP from the GELA group have not included radiation consolidation, including those patients with PMLBCL [4]. Similarly, radiation has not been used in the DA-EPOCH or DA-EPOCH-R studies. In conclusion, these studies do not suggest that radiation consolidation significantly improves the cure of DLBCL. Because the clinical and scientific objectives of this phase III study are to compare the efficacy of R-CHOP and DA-EPOCH-R and to assess mechanisms of treatment failure, inclusion of radiation consolidation would confound the interpretation of results and will not be used in the present study.

Secondary involvement of the central nervous system (CNS) by aggressive B-cell lymphomas is an infrequent but nearly always fatal complication [29,30]. Prophylactic treatment reduces the incidence of CNS relapse, but increases the toxicity of systemic chemotherapy and is only necessary in patients at high risk of CNS spread [4]. This has led to the development of clinical risk paradigms to identify patients who might benefit from CNS prophylaxis, but even among these patients, only a minority ever develop CNS disease [31,32]. For example, in a study of 605 newly diagnosed large cell lymphomas, van Besien et al. reported a Kaplan-Meier estimate of 17.4% probability of CNS recurrence at 1 year in 93 patients at risk according to their multivariate model, which included elevated lactate dehydrogenase (LDH) and more than one extranodal disease site [31]. In a randomized study of ACVBP versus CHOP in poor-prognosis aggressive lymphoma, there was a significantly higher incidence of CNS failures in the CHOP

arm, where CNS prophylaxis was not performed [4]. These studies indicate that patients at high risk of CNS disease may benefit from prophylaxis, and prophylaxis should be used in such patients. For this reason, patients treated on the DA-EPOCH and DA-EPOCH-R studies who were at high risk of CNS disease received CNS prophylaxis with intrathecal methotrexate. Hence, for the phase III study, patients who have a high clinical risk of CNS involvement will receive CNS prophylaxis. Patients will be considered to be high risk based on the risk paradigm developed by van Besien et al. This includes: 1. patients with 2 or more extranodal disease sites and elevated LDH or; 2. any patient with involvement of the bone marrow or testicle by DLBCL.

1.6 Analysis of Candidate Genetic Polymorphisms and Treatment Response

There is increasing evidence suggesting that germline polymorphisms related to the metabolism, transport, therapeutic targets and/or therapeutic pathways of anticancer agents might help predict therapeutic outcomes [33,34]. The major aim of this effort is to determine the influence of candidate gene polymorphisms on toxicity and outcome measures, including event free survival (EFS), with the ultimate goal of individualized cancer therapy.

The primary pharmacogenetic hypothesis will be that the *ABCB1* 3435 CC genotype is associated with decreased EFS in both arms of this study. The *ABCB1* gene (ATP Binding Cassette Beta 1; also called *MDR1*), encodes the P-glycoprotein PGP, a transmembrane protein involved in human cellular transport [35]. PGP achieves the energy dependant efflux of doxorubicin, etoposide, vincristine and prednisone out of target cells [36,37]. While many *ABCB1* polymorphisms have been studied, the synonymous C to T polymorphism at base position 3435 of the *ABCB1* has been most strongly associated with drug treatment outcome [38]. Individuals with the CC genotype at this position exhibit worse outcomes in response to drug therapy for HIV [39], epilepsy [40], and acute myeloid leukemia (receiving an anthracycline based regimen) [41]. We therefore hypothesize that patients with the *ABCB1* 3435 CC genotype will exhibit decreased EFS relative to patients with the 3435 CT and 3435 TT genotypes.

Secondary hypotheses will also be tested to evaluate the association between the presence of variants in candidate genes and measures of both toxicity and outcome in both study arms. Assays for genetic variants will be performed for the following genes: *CD20* (rituximab), *TOP2A* (doxorubicin, etoposide), *TUBB* and *MAP4* (Vincristine), *CYP3A4/3A5* (doxorubicin, vincristine, etoposide, prednisone), *CYP2C19, ADH1,* and *ALDH1A1* (cyclophosphamide) and *NR3C1* (prednisone). These candidate genes have been chosen based on their potential influence on metabolism and activity of the therapeutics used in this study. Additional genes or variants of interest might also be explored as new information relevant to the study emerges.

In addition to specific hypothesis testing for the above candidate genes, this study will also provide the framework for analyzing the relationship between genotypes derived from germline vs. tumor DNA. As excess tumor DNA will be collected in the pharmacogenomics component of this study, we will genotype candidate polymorphisms in DNA derived from both tumor tissue and whole blood. This will allow us to assess the accuracy of germline genotypes for predicting tumor genotypes, and to determine the relative power of tumor vs. germline genotypes for predicting treatment outcome.

1.7 Comprehensive Analysis of Somatic Alterations to the Tumor Genome

Genome sequence characterization is a key to understanding the biology and clinical behavior of cancers and to predict the response to therapy. The present study proposes to perform a comprehensive analysis of somatic alterations to the tumor genome. To understand which genomic alterations are somatically acquired by the tumor and which are encoded in the germ line of the patient, it is necessary to compare the genome of a tumor biopsy sample with that of a matched sample of normal tissue, such as blood. Included in this analysis will be DNA

sequencing of all or part of the tumor and normal cell genomes, which can be achieved by classical DNA sequencing of individual genes or by utilizing high-throughput DNA sequencing technologies. Recently, in diffuse large B cell lymphoma (DLBCL), a number of recurrent oncogenic abnormalities have been uncovered by DNA sequencing. These abnormalities track with known molecular subtypes of this disease. For example, in the ABC subtype of DLBCL, recurrent mutations in CARD11, CD79B, and MYD88 have been described [42-44]. Conversely, the GCB DLBCL subtype is characterized by frequent gain-of-function mutations of EZH2 [45-47].

Other genomic methodologies will be used to assess DNA copy numbers in both tumor and normal DNA using, for example, microarrays containing oligonucleotides representing single nucleotide polymorphisms (SNPs) and other non-polymorphic regions of the genome. These copy number changes also segregate according to the molecular subtype of DLBCL [48]. For example, deletion of the INK4A/Arf tumor suppressor locus occurs in roughly one fifth of ABC DLBCL cases, but never in GCB DLBCL. Moreover, copy number changes have been associated with overall survival in response to CHOP chemotherapy [48]. Specifically, INK4A/Arf locus deletions and trisomy of chromosome 3 were both associated with adverse survival among patients with ABC DLBCL.

The activity of the tumor genome will be assessed by profiling the expression levels of mRNAs and microRNAs in a tumor biopsy sample using microarray and multiplexed PCR methods. The mRNA expression profiling of samples on CALGB 50303 is a primary endpoint of this trial since gene expression signatures have been used to identify molecular subtypes of DLBCL and to predict therapeutic response [49-51]. In addition, new methods have been developed recently to identify new RNA species that are expressed from the genome. For example, digital gene expression is based on the high-throughput resequencing of mRNA, an effort that can uncover previously unannotated genes and new alternatively spliced isoforms of known genes. This effort would be exploratory, aiming to identify new aspects of the biology of DLBCL tumors that are related to therapeutic response. In addition, microRNAs have the capacity to identify molecular subtypes of DLBCL and can yield additional functional information because of their ability to regulate the expression of large repertoire of genes [52-53].

The activity of the genome is also dictated by patterns of cytosine DNA methylation. In acute myelogenous leukemia, molecular subtypes have been identified by their DNA methylation patterns that are biologically and clinically distinct [54-55]. Application to DLBCL revealed the de novo methylation of many targets of the polycomb repression complex PRC2, which may play an important role in DLBCL given the recurrent mutations in EZH2, a component of this complex [56].

The technology platforms that are able to interrogate genomic structure and function are constantly in flux. Therefore, the exact nature of the methodologies that will be employed will be assessed at the time that the samples are collected and ready for analysis. The following are technologies that are currently in use for each task.

Genomic DNA and total RNA will be extracted from tumor and normal samples using a Qiagen All-prep kit. MicroRNAs will be extracted from the samples using a Qiagen miRNeasy Mini Kit.

For individual target genes that are recurrently mutated in DLBCL (CARD11, CD79B, MYD88, EZH2), classical Sanger sequencing will be performed on PCR amplicons, using primers surrounding the known sites of mutation in DLBCL.

To broadly assess mutation, next generation sequencing (e.g. on an Illumina HiSeq 2000 platform) will be employed, using a paired end sequencing strategy of libraries constructed from tumor and matched normal DNA. DNA will either be sequenced in its entirety from a whole genome library or will be first enriched for exonic sequences using the Agilent Sure Select

system, aiming for 30X or 100X average coverage per base, respectively. The sequence fragments will be mapped back to genome using the BWA algorithm [57]. Of sequences overlapping a particular base pair in the genome, the percent mutant cells greater than 20% with a minimum of 25X coverage will be considered as an arbitrary threshold for single nucleotide variants (SNVs). SNVs that are not present in the matched normal sample will be considered candidate somatic mutations.

A related technology, RNA-Seq, utilizes RNA from the tumor specimen to create a cDNA library for high-throughput sequencing. RNA-seq will be performed using Illumina kits followed by high-throughput sequencing on an Illumina HighSeq 2000 machine. The cutoffs for coverage and percent mutant calls mentioned above will also be used to identify putative SNVs. RNA sequencing will also be used to read out digital gene expression across the genome as described [58].

MicroRNAs will be profiled using an Illumina kit to construct a library from tumor RNA samples and will be sequenced using the Illumina HighSeq 2000 platform.

Array comparative genomic hybridization (e.g. on Agilent 240K or Affy SNP 6.0 microarrays) will be used to assess DNA copy number alterations as described, in both tumor and normal genomic DNA [59]. The comparison of these two profiles will yield somatically acquired regions of copy number gain and loss.

Methylated DNA will be immunoprecipitated as described and used to prepare a Illumina genomic DNA library, followed by sequencing using the HighSeq 2000 platform [59].

1.8 FDG-PET Assessment in DLBCL

1.8.1 Oncology Biomarker Qualification Initiative (OBQI)

In February 2006, the Federal Drug Administration (FDA), National Cancer Institute (NCI), and Centers for Medicare and Medicaid Services (CMS) announced the Oncology Biomarker Qualification Initiative (OBQI) – an agreement to collaborate on improving the development of cancer therapies and the outcomes for cancer patients through biomarker development and evaluation. The goal of OBQI is to validate particular biomarkers so that they can be used to evaluate new, promising technologies in a manner that will shorten clinical trials, reduce the time and resources spent during the drug process, improve the linkage between drug approval and drug coverage, and increase the safety and appropriateness of drug choices for cancer patients.

Under the OBQI, biomarker research will be focused in four key areas: standardizing and evaluating imaging technologies to see in more detail how treatments are working; developing scientific bases for diagnostic assays to enable personalized treatments; instituting new trial designs to utilize biomarkers; and pooling data to ensure that key lessons are shared from one trial to another. By working with academic and industry scientists, as well as professional organizations, the OBQI teams can foster the development of key information on biomarkers through clinical trials.

This CALGB imaging companion to the treatment trial study will be carried out under a public-private partnership (PPP) formed by the partners in the OBQI with pharmaceutical manufacturers (represented by the Pharmaceutical Manufacturers' Association [PhRMA]) and administered under the auspices of the Foundation of the National Institutes of Health (FNIH). The project will be overseen by a Lymphoma FDG-PET Working Group comprised of PET experts and oncologists from the NCI, FDA, CALGB, and PhRMA.

1.8.2 FDG-PET Imaging-Derived Biomarkers

In oncology, the gold standard clinical trial endpoint is overall survival, which may require long-term studies and may be confounded by deaths from causes other than the patient's

cancer. Over the years, the oncology community and the FDA have come to rely on other endpoints that from a scientific perspective are regarded as correlates of clinical benefit. These endpoints are objective response (OR), time to progression (TTP), disease-free survival (DFS), and progression free survival (PFS). All are determined by biomarkers measuring the cancer's extent. Anatomic imaging using one- or two-dimensional measurements to characterize cancers has been used traditionally to make these measurements in all aspects of cancer patient management from diagnosis and staging to therapy and disease progression.

However, measurements made using standard anatomic imaging techniques are often inadequate for characterizing the cancer, especially for monitoring the effects of drugs that do not cause tumor shrinkage or for cancers that progress slowly or metastasize diffusely. Newer imaging modalities including volumetric and functional imaging show high promise as the basis for characterizing better biomarkers of cancer (reviewed in [69]). In lymphoma, FDG-PET has the potential to improve patient management, particularly by signaling the need for early therapeutic changes in non-responders (NR) [70-74] and by refining the IWG response criteria to better differentiate complete responses (CR), partial responses (PR), and complete response with residual mass (CRu, unconfirmed due to residual tumor mass) [68, 70, 71, 74-76]. Thus, if the utility of FDG-PET as a biomarker can be confirmed in such settings, unnecessary chemotherapy may be avoided for some patients and the costs of unnecessary long-term follow up reduced. As an early surrogate endpoint for clinical benefit, FDG-PET also has the potential to facilitate oncologic drug development by shortening phase II trials and detecting clinical benefit earlier in phase III investigations.

FDG-PET and FDG-PET/CT imaging are increasingly being used in oncology clinical trials. However, there are as yet no standardized criteria for PET imaging or established procedures detailing transmission, storage, quality assurance, and analysis of PET images. This companion trial is one of the first steps toward developing the standardized protocols and endpoints that will be needed to move forward [77].

1.8.3 CALGB 580603 Companion: Evaluating the Prognostic Utility of PET Scanning in Lymphoma

Considerable evidence supports the association of post-therapy FDG-PET results with outcome in lymphoma patients. Specifically, FDG uptake has been a significant early predictor of residual or recurrent disease and disease progression, as well as PFS and overall survival (OS) [73, 78-83]. FDG-PET is especially useful in differentiating tumor from fibrosis within residual radiographic masses [84, 85]. Such masses are present in half (NHL) to two-thirds (Hodgkin lymphoma (HL)) of patients, of whom only 25% (NHL) to 30% (HL) eventually relapse. In a prospective study of 58 patients (43 with HL) with residual masses following treatment, FDG-PET (SUV ≤ 3) predicted recurrence ($p = 0.004$) and PFS ($p < 0.00001$) [83]. Disease progression was observed after two months in 16/19 versus 3/22 lymphoma patients with positive and negative FDG-PET results, respectively ($p < 0.001$) [81]. FDG-PET results obtained after the first cycle of chemotherapy in 30 NHL and HL patients predicted PFS at 18 months ($p \leq 0.001$) [86]. A recent meta-analysis found that persistence of FDG-avid lesions after therapy predicted relapse, with up to 100% of patients with positive FDG-PET scans experiencing recurrence within two years [87]. Conversely, absence of disease by FDG-PET scan is an indicator of a favorable prognosis. Indeed, the negative predictive value of FDG-PET was 96% in one study of 81 HL patients [88], and several studies have also reported a higher positive predictive value for FDG-PET versus conventional imaging modalities [68, 88-90]. Evidence also supports the integration of FDG-PET with the IWG response criteria to increase the predictability of outcome [70, 71, 74-76].

1.9 Study Rationale and Summary

The proposed study is a phase III randomized comparison of R-CHOP, the de facto standard for DLBCL, to dose-adjusted (DA)-EPOCH-R, a regimen developed from studies on drug schedule and resistance and pharmacokinetics. Importantly, this study will incorporate tumor microarray analysis using Affymetrix genome arrays in all patients to identify molecular mechanisms of drug resistance, develop molecular prognostic models and to assess microarray tissue diagnosis. Microarray is an important new tool, which has altered our understanding of lymphoma biology and has important clinical value. An important aim of the microarray is to identify the next therapeutic hurdles to allow rational treatment design and this is one of the goals of the proposed randomized study. Retrospective microarray results with CHOP-based treatment in DLBCL, for example, has already identified that tumor proliferation and the Activated B cell (ABC) signature are the most important adverse molecular prognostic hurtles [23]. Results with DA-EPOCH show that it can abrogate tumor proliferation as an adverse prognostic factor, making the testing of DA-EPOCH a rational next step approach based on the microarray.

Update #02: Change from 8 Cycles to 6 Cycles of Therapy in Treatment Arms

The necessary number of treatment cycles for R-CHOP and DA-EPOCH-R were based on standard practice. In the case of R-CHOP, 6 or 8 cycles have been routinely administered. Because the present phase III trial was based on the GELA randomized study of CHOP versus R-CHOP for 8 cycles, 8 cycles were selected for the R-CHOP arm of this trial [7, 65]. However, a recent large phase III randomized study of CHOP versus R-CHOP for 6 or 8 cycles have demonstrated no difference in outcome [66]. On an intent-to-treat basis, there was no difference in failure free survival between 6 cycles (n=414) and 8 cycles (n=413) of R-CHOP (p=0.23). Furthermore, an analysis of the GELA study shows the potential for increased cardiac toxicity with R-CHOP, which would be impacted by 8 versus 6 treatment cycles [65]. Specifically, there was a higher incidence of deaths among patients in complete remission that received R-CHOP compared to CHOP. Although the authors attributed this disparity to the higher number of patients in complete remission in the R-CHOP arm that would be at risk of dying, further consideration may be warranted. A general estimate of the risk of dying in remission at 5 years, calculated from the number of patients who died in remission and those who were event free, yields 8% (5/60) and 15% (17/113) for the CHOP and R-CHOP arms, respectively. Furthermore, there was a trend toward a higher incidence of death in remission from cardiac disease among R-CHOP (9/17) compared to CHOP (0/5) patients (p_2=0.054; Fisher's exact test). These findings indicate that 6 cycles of R-CHOP is equivalent to 8 cycles and hence are likely to be associated with lower cardiac toxicity.

DA-EPOCH-R was developed to administer 2 cycles beyond stable disease (defined as < 30% reduction in bi-dimensional measurements between the end of cycles 4 and 6) for a minimum of 6 cycles. Based on this paradigm, over 85% of patients have only required 6 cycles [13,67]. No comparative trial has been performed to determine if the additional 2 cycles are even necessary in this small minority of patients and this paradigm was based on general accepted practices that had not been validated in a randomized study.

Based on the evidence from a randomized study of R-CHOP for 6 versus 8 cycles, there is no evidence that 8 cycles is necessary and may be more toxic. Hence, effective with Update #02, the treatment plan was modified to administer a total of 6 cycles of treatment in both arms of the trial. This change should not affect the study outcome given the low number of patients accrued and have reached > 4 cycles (approximately 5 patients).

1.10 Inclusion of Women and Minorities

It is the intent of the Alliance to enroll patients regardless of gender or race. In the development of this protocol, the possibility of inherent gender or racial/ethnic differences in treatment

response has been considered. Although lymphocytic lymphomas are more common in males than females and the incidence is higher in whites than in blacks, data derived from prior studies involving human subjects, other relevant research, and subjective observation support the conclusion that there is no evidence which would require gender or racial/ethnic subgroupings and a commensurate sample size change. However, detailed analysis of the data will include these as well as other parameters examined for responsiveness to either regimen.

2.0 OBJECTIVES

2.1 Primary Objectives

2.1.1 To compare the event-free survival of R-CHOP versus DA-EPOCH-R chemotherapy in untreated CD20+ diffuse large B-cell lymphomas.

2.1.2 To develop a molecular predictor of outcome of R-CHOP and DA-EPOCH-R chemotherapy using molecular profiling.

2.2 Secondary Objectives

2.2.1 To compare the response rates, overall survival and toxicity of R-CHOP versus DA-EPOCH-R.

2.2.2 To define the pharmacogenomics of untreated DLBCL and correlate clinical parameters (toxicity, response, survival outcomes and laboratory results) with molecular profiling.

2.2.3 To assess the use of molecular profiling for pathological diagnosis.

2.2.4 To identify new therapeutic targets using molecular profiling.

2.2.5 To perform a comprehensive analysis of somatic alterations to the tumor genome and to understand which genomic alterations are somatically acquired by the tumor and which are encoded in the germ line of the patient.

2.2.6 To identify biomarkers of response to chemotherapy by FDG-PET/CT imaging that are predictive of histopathologic remissions and survival in patients with stage I (mediastinal), II, III, or IV untreated DLBCL.

2.2.7 To evaluate the use of semiquantitative measurements of FDG uptake in defining FDG-PET/CT based biomarkers of response to chemotherapy in patients with DLBCL.

2.2.8 To determine whether FDG-PET/CT measurements of tumor response after the second cycle of chemotherapy can predict clinical response.

2.2.9 To establish a standardized protocol for FDG-PET/CT image acquisition.

2.2.10 To determine additional FDG-PET/CT parameters (e.g., the ratio of tumor SUVmax to liver SUVmean; SUVs corrected for body surface area and lean body mass; nuclear medicine physician's assessment) and evaluate their utility in refining FDG-PET/CT based biomarkers of response to therapy.

2.2.11 To evaluate inter-institutional reproducibility of FDG-PET/CT measurements for this indication.

3.0 ON-STUDY GUIDELINES

All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. To maximize patient safety, patients should be treated on this protocol only at centers having ready access to blood product support and adequately

staffed to care for the severely neutropenic patient. Physicians should consider the risks and benefits of any therapy and, therefore, only enroll patients for whom the agents administered are appropriate. Although they will not be considered as formal eligibility criteria, as part of this decision-making process physicians should recognize that the following may increase the risk to the patient entering this protocol:

- Other serious illnesses which would limit survival to < 2 years, or a psychiatric condition which would prevent compliance with treatment or informed consent.
- Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and are considered by their physician to be at less than 30% risk of relapse within one year.
- Patients with severe active medical processes (e.g., active uncontrolled bacterial or viral infection, bleeding, etc.) not related to their lymphoma should be excluded.
- All patients must be screened for hepatitis B infection before starting treatment (see Section 6.0). Those patients who test positive for hepatitis B should be closely monitored for evidence of active HBV infection and hepatitis during and for several months after rituximab treatment, and should be managed as indicated (see Sections 7.1.1 and 7.2.1).

4.0 ELIGIBILITY CRITERIA

As of protocol update #14, the PET/CT imaging companion study CALGB 580603 will be required of all patients enrolling onto the treatment study CALGB 50303. Participation on CALGB 580603 will no longer be optional in order to participate in CALGB 50303.

4.1 Documentation of Disease

Histologically documented de novo CD20+ DLBCL with stage II, III or IV disease. Stage I primary mediastinal (thymic) DLBCL is also eligible. Patients with an underlying low-grade lymphoma, such as a transformed lymphoma or low-grade lymphoma in the bone marrow, are not eligible. Diagnosis should be based on an adequate tissue sample, including open biopsy or core needle biopsy. Needle aspiration for primary diagnosis is unacceptable. Patients must have one of the following WHO [21] classification subtypes:

- **Diffuse large B-cell lymphoma** (includes morphological variants: centroblastic; immunoblastic; T-cell/histiocyte rich; and anaplastic).
- **Mediastinal (thymic) large B-cell lymphoma**
- **Intravascular large B-cell lymphoma**

Note: Failure to submit a pathology block within 60 days of patient registration will be considered a major protocol violation (see Section 5.3).

Fresh (frozen) tumor biopsy must be available or attempted (see repeat tissue biopsy guidance below and Section 5.4). A frozen tumor biopsy equivalent to a minimum of four at least 16 gauge needle cores is an important component of this study. Patients without adequate frozen material should have a biopsy performed to obtain material. If a biopsy is performed and does not yield adequate material, the patient is still eligible for the study. If a biopsy cannot be done safely, the patient may still be eligible for the study if permission is granted in writing (e-mail) by the Study Chairs (Drs. Wilson or Zelenetz) or their designees.

Drs. Wilson or Zelenetz may be consulted to discuss situations involving invasive biopsy procedures that may pose an increased risk to the patient.

NOTE: THIS STUDY DOES NOT ALLOW CONCURRENT RADIATION UNLESS A PATIENT HAS A DOCUMENTED CNS TREATMENT FAILURE WITH NO SYSTEMIC FAILURE. PLEASE DO NOT ENROLL A PATIENT YOU MIGHT WISH TO RADIATE.

4.2 Prior Treatment

No prior cytotoxic chemotherapy or rituximab. Patients may be entered if they have received prior limited field radiation therapy or a short course of glucocorticoids (< 10 days) for an urgent local disease complication at diagnosis (e.g., cord compression, SVC syndrome). Patients who have received chemotherapy for prior malignancies are not eligible.

4.3 Age ≥ 18 years.

4.4 ECOG Performance Status 0-2.

4.5 Cardiac Function

No active ischemic heart disease or congestive heart failure. If there is suspicion of cardiac disease, a cardiac ejection fraction must show LVEF > 45%, but the study is not required.

4.6 Lymphomatous

No known lymphomatous involvement of the CNS. A lumbar puncture prior to study is not required in the absence of neurological symptoms.

4.7 HIV Status

No known HIV disease. Patients with a history of intravenous drug abuse or any other behavior associated with an increased risk of HIV infection should be tested for exposure to the HIV virus. The mechanism of disease in patients with HIV may be different from de novo diffuse large B cell lymphoma. Additionally, the immunocompromised state of patients with HIV infection may result in more extensive dose reductions than intended for the intensive therapeutic regimens used in this study. Therefore, patients who test positive or who are known to be infected are not eligible. An HIV test is not required for entry on protocol, but is required if the patient is perceived to be at risk.

4.8 Pregnancy and Nursing Status

Non pregnant and non-nursing. Treatment would expose an unborn child to significant risks. Women and men of reproductive potential should agree to use an effective form of contraception.

4.9 Active medical processes

Patients with active medical processes (e.g., uncontrolled bacterial or viral infection, bleeding) not related to their lymphoma should be excluded.

4.10 Required Initial Laboratory Values*:

ANC	≥ 1000/μL
Platelets	≥ 100,000/μL
Creatinine	≤ 1.5 mg/dL or creatinine clearance ≥ 50 cc/min.
Total Bilirubin	≤ 2 mg/dL**

* *Unless attributable to non-Hodgkin lymphoma*

** *Unless a history of Gilbert's Disease*

5.0 REGISTRATION/RANDOMIZATION, DATA SUBMISSION, HISTOLOGIC REVIEW AND SPECIMEN SUBMISSION FOR CORRELATIVE STUDIES

5.1 Registration/Randomization

5.1.1 Registration/Randomization Requirements

5.1.1.1 Informed Consent

The patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. Human protection committee approval of this protocol and of its consent form are required.

5.1.1.2 Fresh (frozen) Tumor Biopsy

A fresh (frozen) tumor biopsy must be available or attempted (see guidance for repeat tissue biopsy in Section 4.1). A frozen tumor biopsy equivalent to a minimum of 4 at least 16 gauge needle cores is an important component of this study (see Section 5.4.1). Patients without adequate frozen material should have a biopsy performed to obtain material. If a biopsy is performed and does not yield adequate material, the patient is still eligible for the study. If a biopsy cannot be done safely, the patient may still be eligible for the study if permission is granted in writing (e-mail) by the Study Chairs (Drs. Wilson or Zelenetz) or their designees.

5.1.1.3 Pharmacogenomic Correlative Science Companion

All participating institutions must ask patients for their consent to participate in CALGB 60405 (see Section 5.1.2 "Registration to Companion Studies"), although patient participation is optional. See Section 5.4.2 for instructions for the collection and shipment of samples for pharmacogenomics.

5.1.1.4 FDG-PET/CT Companion Study (CALGB 580603): Institutional Approval Procedures

As of protocol update #14, participation in the PET/CT imaging companion study CALGB 580603 is required of all patients enrolling onto the treatment study CALGB 50303. Starting on August 1, 2012, registration will be limited to patients and institutions participating in CALGB 580603. Participation in CALGB 580603 will no longer be optional in order to participate in CALGB 50303. **Please note:** if a PET/CT scan has already been performed for clinical evaluation, it may be possible to submit the scan as the baseline image for the correlative study CALGB 580603. Please contact the Imaging Co-Chair to determine suitability for submission as a baseline image.

Institutions must be approved to participate in the FDG-PET/CT companion to this protocol (see Section 8.1).

The following documentation is required to participate in CALGB 580603:

1) Submit to the Imaging Core Laboratory:

- PET Instrument Technical Specifications Form (Appendix II)
- Imaging Site Personnel Form (Appendix III)

Institutional approval to participate in this imaging companion is contingent upon receipt of this documentation.

5.1.2 CALGB Registration/Randomization Procedures

Registration and randomization will be accepted only through CALGB Main Member institutions, at-large members, selected affiliate institutions, and CCOPs. Registration and randomization must occur prior to the initiation of therapy.

Confirm eligibility criteria (Section 4.0). Complete the Registration Worksheet. Either fax the worksheet to [redacted] or call the CALGB Registrar ([redacted], Monday-Friday, 9:00 AM – 5:00 PM, Eastern Time) with the following information:

Study
Name of group (CALGB)
Name of institution where patient is being treated
Name of treating physician
Name of treating physician or responsible CRA
Other group patient ID #, if applicable
CALGB patient ID #, if applicable
Patient's initials (last initial, first initial, middle initial)
Patient's Social Security #, date of birth, and hospital ID #
Patient's gender
Patient's race
Patient's height, in centimeters (optional)
Patient's weight, in kilograms (optional)
CTC performance status (if required by CDUS)
Type of insurance (method of payment)
Disease, type and stage, if applicable
Patient's Postal Code, if applicable
Treatment start date
Date of signed consent
Date of HIPAA authorization
Patient demographics, if applicable
Companion studies, if applicable (see below)
Eligibility criteria met (no, yes)

When the patient is registered, a patient identification number will be generated. Please write the number in your records. Registration to any mandatory or optional companion studies will be done at the same time as registration to the treatment study. Registration to both treatment and companion studies will not be completed if eligibility requirements are not met for all selected trials (treatment and companions). **Please see "Registration to Companion Studies" below.**

After registration is complete the patient may be randomized. Once the randomization is complete, note the patient's treatment assignment in your records.

The Main Member Institution and registering institution will receive a Confirmation of Randomization. Please check for errors. Submit corrections in writing to the Alliance Statistics and Data Center, [redacted]

Registration to Companion Studies

Within CALGB 50303 there are two sub-studies. The sub-studies included within CALGB 50303 are:

- Pharmacogenomics in CALGB 50303 (CALGB 60405)

- FDG-PET/CT Imaging in CALGB 50303 (CALGB 580603)

As of protocol update #14, the PET/CT imaging companion study CALGB 580603 will be required of all patients enrolling onto the treatment study CALGB 50303. Starting on August 1, 2012, registration will be limited to patients and institutions participating in CALGB 580603. Participation in CALGB 580603 will no longer be optional in order to participate in CALGB 50303. **Please note:** if a PET/CT scan has already been performed for clinical evaluation, it may be possible to submit the scan as the baseline image for the correlative study CALGB 580603. Please contact the Imaging Co-Chair to determine suitability for submission as a baseline image.

These embedded companion studies must be offered to all patients enrolled on CALGB 50303. Patients may opt not to participate on CALGB 60405, however the imaging companion study 580603 is required.

The rationales for performing the correlative science substudies are discussed in Sections 1.6 and 1.8. If a patient answers "yes" to "I agree that my blood may be used for the genetic research studies described above" (Question #1) in the model consent, they have consented to participate in the correlative science pharmacogenomic substudy. Patients should be registered to CALGB 60405. Although it is preferable that patients be registered to CALGB 60405 at the same time they are registered to CALGB 50303, registration to CALGB 60405 may occur up to 60 days following registration to the treatment trial. Samples should be submitted according to the instructions in Section 5.4.2. If the institution is approved to participate in CALGB 580603 (see Section 8.1 for approval procedures) and the patient consents to participate in the treatment study CALGB 50303, then they have consented to participate in the correlative science FDG-PET/CT imaging correlative study. Patients should be registered to CALGB 580603. Images should be submitted according to the instructions in Section 8.5.

5.2 Data Submission

As of Update #18 to the protocol, this study will use Medidata Rave® for remote data capture (RDC) of all future data collection. All data originally received by the Alliance and Statistics and Data Center (SDC) (either electronically using the "Print and/or Submit to CALGB" button [i.e. Teleform form] or by mail) has been transferred to Medidata Rave ® and can be accessed via the Medidata Rave ® system. If necessary, data originally submitted to the SDC electronically (or by mail) can be amended via the Medidata Rave ® system.

The Rave system can be accessed through the iMedidata portal at [redacted] For additional information regarding account setup or training, please visit the training section of the Alliance website. Forms should be submitted in compliance with the table below, and a copy of the All Forms Packet can be downloaded from the Alliance and CTSU websites.

Site personnel with Rave roles assigned on the appropriate roster may receive a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login [redacted] using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. Personnel who did not receive an invitation should contact the Alliance Service Center.

Users who have not previously activated their iMedidata/Rave account at the time of an initial site registration approval for a study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website's Rave tab under the Rave Resource Materials heading (Medidata Account Activation

and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at [redacted] or by e-mail at [redacted].

Form		**Submission Schedule**
C-1319	CALGB 50303 On-Study Form	
C-1321	Lymphoma Measurement Form	
C-1332	Tumor Biopsy Specimen Submission Form	Within one week of registration.
C-1333	Pharmacogenomic Sample Submission Form*	
	Pathology Reports	
C-1331	**Copy of C-1331 & Block to Alliance Biorepository at OSU**	**Within 60 days of registration. See Section 5.3.**
C-1323	Chemotherapy Dose Form	Every cycle while on therapy.
C-1627	50303 PET and CT Measurement Form**	**As of protocol update #14, submission is required:** Prior to therapy; post-Cycle 2; and post-Cycle 6 per Section 8.3.
C-1657	50303 Imaging Adjunctive Data Sheet**	Prior to therapy; post-Cycle 2; and post-Cycle 6 per Section 8.3.
C-1322	CALGB 50303 Follow-Up Form	Every 2 cycles while on therapy, then q3 months for 2 years, then q6 months for 5 years from study registration, at relapse, and at death.
C-1320	CALGB 50303 Treatment Form	Every 2 cycles while on therapy.
C-1324	CALGB 50303 Adverse Event Form	
C-1321	Lymphoma Measurement Form	Post-Cycle 4; post-Cycle 6; q3 months for 2 years, then q6 months for 5 years from study registration, and at relapse.
C-1001	New Primary Malignancy Form	At time of new primary malignancy.
C-1742	CALGB: Confirmation of Lost to Follow-up Form	Follow form directions.
C-2089	CALGB 50303 Consent for Additional Genomic Research Studies ††	Only for patients already enrolled on study prior to Update #12 (4/15/2011); submit once patient has read the Addendum to the Model Consent and answered Question #6.

† Submit legible copies of all institutional pathology, immunophenotyping, and baseline CT scan reports documenting diagnosis.

‡ Submit legible copies of all pathology, immunophenotyping, and CT scan reports documenting response and/or progression.

* Patient may be enrolled on pharmacogenomic correlative science study (CALGB 60405) as many as 60 days after enrollment on CALGB 50303.

** Send forms to the Imaging Core Laboratory and the Alliance Statistics and Data Center. See Section 8.5 for image submission.

†† Submit only for patients already enrolled on study prior to Update #12 (4/15/2011), NOT those who are new patients on study. For patients already enrolled on study, submit C-2089 once the patient has read the Addendum to the Model Consent, answered Question #6, and provided a signature. (New patients being enrolled onto CALGB 50303 will also receive the Addendum to the Model Consent, answer Question #6, and provide a signature, but their answers to Question #6 are to be recorded within the registration system.)

5.3 Histologic Review

Submission of either a nodal or extranodal biopsy from the original diagnosis is required. An adequate specimen of tissue must be available for central review of morphology, architecture and appropriate immunohistochemical stains to confirm the diagnosis. Diagnosis should be based on an adequate tissue sample, including open biopsy or core needle biopsy. Needle aspiration for primary diagnosis is unacceptable.

Within 60 days of registration, send a formalin-fixed, paraffin-embedded block of well-fixed lymphoma tissue containing adequate material for histologic confirmation of diagnosis. Please label the block with the CALGB patient ID number, institution, date of acquisition, protocol number, and tissue source. A block at least 2 cm x 1 cm x 0.5 cm is preferable, although smaller is acceptable if no other block is suitable. If only one block exists, and the tissue is sufficiently large, it is acceptable to split the block into two and submit one. Contact Dr. Eric Hsi (216-444-5230) with questions. Submit the labeled block and a copy of the pathology report (include consultative pathology reports, if available), CT scan report(s), and a copy of the completed Lymphoma Pathology Submission Form C-1331 to the Alliance Biorepository at Ohio State University (ABOSU).

Alliance Biorepository at Ohio State University

[redacted]

Failure to submit a pathology block **within 60 days** of patient registration will be considered a major protocol violation. Send the **original** C-1331 form to the Alliance Statistics and Data Center.

The Alliance has instituted special considerations for the small percentage of institutions whose policies prohibit the release of any blocks. If, due to institutional policy, a block cannot be sent, please call the Alliance Biorepository at OSU at [redacted] to obtain a protocol for submission of representative tissue from your institution.

5.4 Specimen Submission for Correlative Studies

A frozen tumor biopsy equivalent to a minimum of 4 at least 16 gauge needle cores is an important component of this study. **Patients without adequate frozen material should have a biopsy performed to obtain material. If a biopsy is performed and does not yield adequate material, the patient is still eligible for the study. If a biopsy cannot be done safely, the patient may still be eligible for the study <u>if permission is granted</u> in writing (e-mail) by the Study Chairs (Drs. Wilson or Zelenetz) or their designees.**

All participating institutions must ask patients for their consent to participate in the additional pharmacogenomic companion substudy in CALGB 50303, although patient participation in the pharmacogenomic study is optional (see Section 5.4.2). Instructions for the collection and shipment of samples are detailed below.

Samples shipped from Alliance institutions should be logged and shipped via the CALGB LabTrak application. All samples should be labeled with the CALGB patient ID, CALGB protocol number (CALGB 50303), institution, date of acquisition, and tissue source. Instructions for the procurement and shipping of samples are included below.

5.4.1 Fresh (Frozen) Tumor Biopsy Sample (ALL PATIENTS)

- **Fresh (Frozen) Tumor Biopsy Sample Procurement**

  Contact one of the following individuals to obtain shipping packages:

  [redacted] [redacted] [redacted]

  Shipping packages will be sent to each participating institution. To ensure timely arrival of shipping packages, place a request for multiple packages (at least 2 more than is needed) at the time of local IRB review. Packages will consist of the following items:

  (1) Fresh (Frozen) Tumor Biopsy Sample Procurement Instructions
  (1) Styrofoam Box
  (1) Cardboard Box
  (1) Biohazard Labeled Ziplock Bag
  (10) 2 mL cryopreservation screw cap tube

  Each package should be used to mail only one set of samples from a single patient. **DO NOT CONSOLIDATE SAMPLES FROM MULTIPLE PATIENTS INTO A SINGLE BOX.**

  Remove the collection tubes from the styrofoam container. Label all tubes with the CALGB patient ID number, CALGB protocol number, institution, date of acquisition, and tissue source.

  Use the 2 mL cryotubes to collect tumor samples. A minimum of a hundred milligrams (100 mg) of tissue should be provided. This corresponds to a minimum of 4 core needle biopsy specimens **(16 gauge needle at least)** or an excisional biopsy that is at least 100 cubic mm in volume (i.e., the size of a pea). Fill the Nunc tubes 2/3 full and use as many Nunc tubes as necessary. **REMOVE ALL LIQUID FROM SAMPLE BEFORE FREEZING. Immediately preserve by freezing in dry ice or liquid nitrogen and transfer them from the liquid nitrogen or dry ice container, to a -70°/-80° C freezer (preferable), or to a -20°C freezer for storage until time of shipment.**

- **Fresh (Frozen) Tumor Biopsy Sample Shipment**

  Note: Must have patient identification number before shipping sample. Place all tubes with samples into the styrofoam box filled with at least 2 lbs. of dry ice. Position the tubes so that they are roughly in the center of the container (i.e., surrounded by dry ice). Remember: ONE PATIENT SAMPLE SET PER "ZIPLOCK" BAG/BOX. Complete the Tumor Biopsy Specimen Submission Form (C-1332) and place in the document compartment of the waterproof "Ziplock" plastic bag. Place in the styrofoam box. Send a copy of Form C-1332 to the Alliance Statistics and Data Center.

  Tape the styrofoam box. Put the box inside the provided cardboard box. Close with tape. Affix a FedEx label. Make a note of the air bill number on the FedEx label. **Please mail overnight on a Monday through Thursday so shipment will arrive during the week.** After mailing, send an e-mail to: [redacted] with the subject: "URGENT: CALGB 50303 Fresh/Frozen Biopsy Sample Sent" indicating the **DAY, TIME, INSTITUTION, CALGB PATIENT ID NUMBER AND AIR BILL NUMBER OF THE PACKAGE** in which the sample set was mailed. Mail the samples to:

  [redacted]

[redacted]

Questions or problems with sample procurement or shipment should be directed to the following individuals:

[redacted]

Institutions without access to CALGB LabTrak application for shipping samples should send an e-mail to [redacted] with the subject "URGENT. CALGB 50303 Fresh/Frozen Biopsy Sample Sent" indicating the day, time, institution, CALGB patient ID number, and the airbill number of the package in which the sample was sent. Questions or problems with sample procurement may be directed to the individuals identified above.

Please note: it is necessary to submit CALGB form C-1332 in order to receive reimbursement, regardless of whether biopsy was successful. In this instance, complete form C-1332 and indicate that biopsy specimen does not accompany the form, and fax it to [redacted] as well as to the Alliance Statistics and Data Center, Data Operations. Reimbursement for sample procurement will not be made for patients who are exempted from the biopsy requirement.

5.4.2 Pharmacogenomic Sample (Must be offered to all patients enrolled on CALGB 50303)

- **Pharmacogenomic Sample Procurement**

  Prior to initiation of therapy obtain 2 EDTA (purple top) tubes of peripheral blood (each containing approximately 10 mL) for pharmacogenetic studies. A mailing label should be completed that contains the CALGB patient ID number, CALGB protocol number, institution, date of sample acquisition, sample collection time point (prior to initiation of therapy), tissue source, and sample purpose (i.e., CALGB 50303 Pharmacogenetic Sample).

- **Pharmacogenomic Sample Shipment**

  Samples should be shipped on a cold/gel pack in an approved container using an express courier. Ship samples to:

  [redacted]

  A copy of the 50303 Pharmacogenomic Specimen Submission Form (C-1333) should accompany all samples sent to the ABOSU. **Note: If specimen is sent on Friday, CHECK SATURDAY DELIVERY on the FEDERAL EXPRESS INVOICE.** Also, send a copy of the completed forms to the Alliance Statistics and Data Center at the time of shipment.

6.0 REQUIRED DATA

Guidelines for Pre-Study Testing

To be completed within 16 DAYS before registration:

- All blood work
- History and physical

To be completed within 28 DAYS before registration:

- Any X-ray, scan of any type or ultrasound which is utilized for tumor measurement

To be completed within 42 DAYS before registration:

- Any baseline exams used for screening, i.e., EKG, LVEF or MUGA
- Any X-ray, scan of any type or ultrasound of uninvolved organs which is not utilized for tumor measurement; bone marrow biopsy and aspirate.

Tests & Observations	Prior to Study	Between Cycles	Day 1 of Each Cycle‡	At Time of Restaging*	Post-Treatment Follow-up**
History & Progress Notes	X		X	X	X
Physical Examination	X		X	X	X
Pulse, Blood Pressure	X		X	X	X
Height/Weight/Body Surface Area	X		A		
Performance Status (ECOG)	X		X	X	X
Tumor Measurements	X		B	X	X
Drug Toxicity Assessment			X	X	X
Studies					
CBC, Diff, Platelets	X	2x/week†	X	X	X
Serum Creatinine, BUN	X		X		
AST, Alk Phos, Bilirubin, LDH	X		X	X	
Total Protein, Albumin	X				
HIV serology (if necessary, see Section 4.7)	X				
Serum of urine ☐HCG	G				
EKG	X				
LVEF (echo or MUGA) (if necessary, see Section 4.5)	X				
HBSAg and serology	X,E				
Fresh Tumor Biopsy (Section 5.4.1)	X				
Pharmacogenomic Sample (see Section 5.4.2)	X				
Staging					
CT/MRI Scan Chest, Abd, Pelvis	X			X	X
Bone Marrow Aspirate & Biopsy	X			C	
Head scan (MRI preferred) & CSF exam, if risk factors	F				
PET Scan (CALGB 50303)				D	D
Histologic Review	X				

‡ Pre-registration tests, observations, and lab studies completed within 7 days prior to the first day of Cycle 1 treatment need not be repeated. Labs and physical exam may be obtained within 48 hours prior to treatment for all other cycles.

* **In Arms A and B,** restage after Cycles 4 and 6.

** Every 3 months for 2 years, then q6 months until 5 years from study registration or relapse (whichever occurs first).

† Twice weekly (e.g., on Monday & Thursday or Tuesday & Friday) to assure that counts are checked every three-four days. **The twice-weekly monitoring is required for Arm B (DA-EPOCH-R) only.**

A The weight at Cycle 1 should be used for dose calculations for all cycles, unless there is a ≥ 10% change over a period of one cycle. If a weight change ≥10% occurs, doses should be recalculated and the new dose used from that time forward.

B If accessible to physical examination, please record measurements on Day 1 of each cycle.

C If bone marrow initially positive, repeat to confirm a complete response.

D **PET scan is recommended at end of treatment in patients who achieve CRu. If positive, biopsy site. If borderline positive, biopsy site OR follow closely with repeat PET and CT scan monthly as indicated to assess sites. Contact Study Chair for guidance concerning "borderline" PET positivity. If scans show progression, perform a biopsy. NO patient will be considered to have failed treatment based on a positive PET scan alone.**

E All patients must be screened for hepatitis B infection before starting treatment. Carriers of hepatitis B should be closely monitored for evidence of active HBV infection and hepatitis during and for several months after rituximab treatment.

F Patients with (1) ≥ 2 extranodal sites and elevated LDH, or (2) bone marrow involvement by large cell lymphoma will receive CNS prophylaxis according to Sections 7.1.3 (Arm A) or 7.2.3 (Arm B).

G In women of childbearing potential

6.1 CALGB 580603 FDG-PET/CT Imaging Requirements

As of protocol update #14, the PET/CT imaging companion study CALGB 580603 will be required of all patients enrolling onto the treatment study CALGB 50303. Participation on CALGB 580603 will no longer be optional in order to participate in CALGB 50303. **Please note:** if a PET/CT scan has already been performed for clinical evaluation, it may be possible to submit the scan as the baseline image for the correlative study CALGB 580603. Please contact the Imaging Co-Chair to determine suitability for submission as a baseline image.

Tests & Observations	Prior to Study	Day of FDG-PET/CT Imaging	Post-Cycle 2	Post-Cycle 6
Blood glucose	X	X		
Height/Weight/Body Surface Area	X	X		
Staging and Imaging				
PET/CT Scan‡	X	X	A	B
Biopsy				C

‡ Baseline PET/CT scan preferred prior to the CALGB 50303 baseline excisional biopsy and≤ 30 days prior to start of therapy (see Section 8.3). If a PET/CT scan has already been performed for clinical evaluation, please contact the Imaging Co-Chair to determine suitability for submission as a baseline image.

A To be performed 17-21 days post-Cycle 2 therapy and 0-4 days prior to start of Cycle 3.

B To be performed post-Cycle 6 (3-8 weeks after last dose of chemotherapy). At Cycle 6, if PET is positive, but CT negative, another PET/CT scan will be performed after 2-3 months.

C If PET remains positive after the second post-Cycle 6 PET/CT scan, the site should be biopsied to determine histopathology. If a PET/CT scan shows progression, results will be provided to treating physician to assist in further evaluation of the patient, and the site should be biopsied to document active disease. Patients with a 4+ positive PET scan after Cycle 6 may undergo biopsy immediately at treating physician's discretion. No patient will be considered to have failed treatment based on a positive PET scan alone.

7.0 TREATMENT PLAN

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's lymphoma.

No radiation therapy will be permitted on study except for isolated CNS lesions. Patients who receive radiation to any site other than the CNS will be inevaluable. Patients with parenchymal CNS lesions should receive whole brain radiation and will only be considered treatment failures if the systemic disease progresses.

Patients must begin treatment within seven days of registration/randomization. Patients will be randomized to either Arm A (R-CHOP) or Arm B (DA-EPOCH-R).

All patients must be screened for hepatitis B infection before starting treatment. Those patients who test positive for hepatitis B should be closely monitored for evidence of active HBV infection and hepatitis during and for several months after rituximab treatment, and should be managed as indicated (see Sections 7.1.1 and 7.2.1).

Institutions must be approved to participate in the optional FDG-PET/CT companion CALGB 580603 (see Section 8.1).

7.1 Arm A (R-CHOP Treatment [7])

7.1.1 Required Ancillary Medications

- Omeprazole 20 mg/day PO (or equivalent).
- Stimulant laxative/stool softener (e.g., Pericolace®) 2 tablets PO BID (adjusted as necessary) or institutional preference for prevention of constipation.
- Lactulose 20 g PO Q 6 hours PRN until resolution or institutional preference for treatment of constipation.
- Acetaminophen 650 mg PO and diphenhydramine 50-100 mg IV or PO 30 to 60 minutes prior to starting each rituximab infusion.
- Hepatitis B surface Ag+ patients receive lamivudine 100 mg/day PO until 8 weeks past last chemotherapy.

7.1.2 R-CHOP Chemotherapy

- Rituximab 375 mg/m^2 IV infusion on Day 1 prior to CHOP chemotherapy. See Section 10.10 for dose rate information.
- Cyclophosphamide 750 mg/m^2 IV Day 1
- Doxorubicin 50 mg/m^2 IV Day 1
- Vincristine 1.4 mg/m^2 (2 mg cap) IV Day 1
- Prednisone 40 mg/m^2/day PO on Days 1-5
- Begin filgrastim or pegfilgrastim if grade 4 neutropenia (ANC < 500/µL) or febrile neutropenia (ANC < 500/µL + fever 38.5° (101°F) lasting for more than one hour) developed on the previous cycle. Administer filgrastim 480 mcg subcutaneous daily from Day 2 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 2-11) if the ANC is not being monitored, or pegfilgrastim 6 mg subcutaneous on Day 2 (may be delayed up to two days for convenience of scheduling due to holidays, weekend, etc.).
- Repeat cycles every 21 days. To begin a cycle, ANC must be ≥ 1500/µL and platelets ≥ 100,000/µL. Delay cycle by up to 2 weeks if these values are not met on Day 1 of cycle. Contact the Study Chair if delay is greater than two weeks.

7.1.3 CNS Prophylaxis and Treatment

7.1.3.1 CNS Prophylaxis

Patients with (1) ≥ 2 extranodal sites and elevated LDH, or (2) bone marrow involvement by large cell lymphoma will receive CNS prophylaxis consisting of intrathecal methotrexate 12 mg on day 1 (or day 2) of Cycles 3, 4, 5, and 6.

7.1.3.2 Treatment of CNS Disease

- **Treatment of CNS Disease**

  If the CSF is cytologically positive at the time CNS prophylaxis is scheduled to begin (Cycle 3), the patient should receive active treatment of the CSF with methotrexate (see below). If this treatment either is not tolerated or found to be not effective, then cytarabine alone or in combination with methotrexate may be used. Additionally, a brain MRI should be obtained and radiation consolidation administered at the end of chemotherapy if there are parenchymal lesions.

- **CNS Induction Treatment**

  CNS induction treatment will be administered twice per week for two weeks beyond negative cytology with a minimum of 4 weeks of treatment. CNS induction may consist of one of the following:

  - Intrathecal methotrexate 12 mg or intraventricular (via Ommaya) methotrexate 6 mg

  OR

  - Intrathecal or intraventricular (via Ommaya) cytarabine 70 mg

  OR

  - Intrathecal methotrexate 12 mg + cytarabine 30 mg + hydrocortisone 15 mg **or** intraventricular (via Ommaya) methotrexate 6 mg + cytarabine 30 mg + hydrocortisone 15 mg

- **CNS Consolidation Treatment**

  CNS consolidation will consist of the same treatment as induction, but administered weekly x 6.

- **CNS Maintenance Treatment**

  CNS maintenance will consist of the same treatment as induction, but administered monthly x 4.

  If CNS treatment must be modified due to unforeseen circumstances or investigator preference, please consult with the Alliance Study Chair.

7.1.4 Restaging and Treatment Duration

Perform PET/CT scan post-Cycle 2 and after Cycles 4 and 6. Repeat CT/MRI scans at site(s) of initial involvement. If initial bone marrow biopsy is positive, a bone marrow examination should be repeated at each restaging point until it is negative and then no further biopsy is necessary. **Patients will receive a maximum of 6 cycles.**

Submit PET/CT scan documentation post-Cycle 6. If patient is PET positive but CT negative, then repeat PET/CT scan 2-3 months later and submit documentation (see Section 8.3).

7.2 Arm B (DA-EPOCH-R Treatment [20])

7.2.1 Required Ancillary Medications

- Cotrimoxazole DS 1 tablet three times weekly (or equivalent if allergic).
- Omeprazole 20 mg/day PO (or equivalent).
- Stimulant laxative/stool softener (e.g., Pericolace®) 2 tablets PO BID (adjusted as necessary) or institutional preference for prevention of constipation.
- Lactulose 20 g PO Q6 hours PRN until resolution or institutional preference for treatment of constipation.
- Acetaminophen 650 mg PO and diphenhydramine 50-100 mg IV or PO 30 to 60 minutes prior to starting each rituximab infusion.
- Hepatitis B surface Ag+ patients receive lamivudine 100 mg/day PO until 8 weeks past last chemotherapy.

7.2.2 Dose-Adjusted EPOCH-R Chemotherapy

All patients initiate therapy at Dose Level 1 of DA-EPOCH-R shown below. Subsequent treatment doses are determined by hematological toxicity experienced on the previous cycle according to the dose-adjustment paradigm in Section 9.2. **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

- Rituximab 375 mg/m^2 IV infusion on Day 1 prior to EPOCH chemotherapy. See Section 10.10 for dose rate information.
- Doxorubicin 10 mg/m^2/day CIVI on Days 1-4 (total 40 mg/m^2 over 96 hours)
- Etoposide 50 mg/m^2/day CIVI on Days 1-4 (total 200 mg/m^2 over 96 hours)
- Vincristine 0.4 mg/m^2/day **(no cap)** CIVI on Days 1-4 (total 1.6 mg/m^2 over 96 hours)
- Cyclophosphamide 750 mg/m^2 IV on Day 5 (following completion of 96 hour infusions)
- Prednisone 60 mg/m^2 PO BID on Days 1-5
- Administer filgrastim 480 mcg subcutaneous daily from Day 6 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 6-15) if the treating physician is not actively monitoring the required twice-weekly CBC/differential/platelets, **during every** cycle.

  Note: DA-EPOCH-R dosing has only been validated using filgrastim. Pegfilgrastim has different kinetics of neutrophil recovery and its use may adversely affect dosing and reduce disease cure rates. Use of pegfilgrastim (6 mg subcutaneous Day 6) must be approved by the Study Chair and will only be approved if the patient cannot receive filgrastim.
- Repeat cycles every 21 days. To begin a cycle, ANC must be ≥ 1000/μL and platelets ≥ 100,000/μL. Delay cycle by up to 2 weeks if these values are not met on Day 1 of cycle. Filgrastim may be used for several days to increase ANC. Contact the Study Chair if delay is greater than two weeks.

7.2.3 CNS Prophylaxis and Treatment

7.2.3.1 CNS Prophylaxis

Patients with (1) ≥ 2 extranodal sites and elevated LDH, or (2) bone marrow involvement by large cell lymphoma will receive CNS prophylaxis consisting of intrathecal methotrexate 12 mg on day 1 (or day 2) of Cycles 3, 4, 5, and 6.

7.2.3.2 Treatment of CNS Disease

- **Treatment of CNS Disease**

  If the CSF is cytologically positive at the time CNS prophylaxis is scheduled to begin (Cycle 3), the patient should receive active treatment of the CSF with methotrexate (see below). If this treatment either is not tolerated or found to be not effective, then cytarabine alone or in combination with methotrexate may be used. Additionally, a brain MRI should be obtained and radiation consolidation administered at the end of chemotherapy if there are parenchymal lesions.

- **CNS Induction Treatment**

  CNS induction treatment will be administered twice per week for two weeks beyond negative cytology with a minimum of 4 weeks of treatment. CNS induction may consist of one of the following:

  - Intrathecal methotrexate 12 mg or intraventricular (via Ommaya) methotrexate 6 mg

  OR

  - Intrathecal or intraventricular (via Ommaya) cytarabine 70 mg

  OR

  - Intrathecal methotrexate 12 mg + cytarabine 30 mg + hydrocortisone 15 mg **or** intraventricular (via Ommaya) methotrexate 6 mg + cytarabine 30 mg + hydrocortisone 15 mg

- **CNS Consolidation Treatment**

  CNS consolidation will consist of the same treatment as induction, but administered weekly x 6.

- **CNS Maintenance Treatment**

  CNS maintenance will consist of the same treatment as induction, but administered monthly x 4.

  If CNS treatment must be modified due to unforeseen circumstances or investigator preference, please consult with the Alliance Study Chair.

7.2.4 Restaging and Treatment Duration for DA-EPOCH-R

If institution and patient are participating in the optional imaging companion study (CALGB 580603), perform PET/CT scan post-Cycle 2. Otherwise, restage patients after Cycles 4 and 6. **Patients will receive a maximum of 6 cycles.** Repeat CT/MRI scans at site(s) of initial involvement. If initial bone marrow biopsy is positive, a bone marrow examination should be repeated at each restaging point until it is negative, and then no further biopsy is necessary.

If institution and patient are participating in the optional imaging companion study, submit PET/CT scan documentation post-Cycle 6. If patient is PET positive but CT negative, then repeat PET/CT scan 2-3 months later and submit documentation (see Section 8.3).

8.0 FDG-PET/CT IMAGING COMPANION (CALGB 580603)

As of protocol update #14, the PET/CT imaging companion study CALGB 580603 will be required of all patients enrolling onto the treatment study CALGB 50303. Participation on CALGB 580603 will no longer be optional in order to participate in CALGB 50303. **Please note:** if a PET/CT scan has already been performed for clinical evaluation, it may be possible to submit the scan as the baseline image for the correlative study CALGB 580603. Please contact the Imaging Co-Chair to determine suitability for submission as a baseline image.

If the CT scan for the PET/CT imaging companion (CALGB 580603) is performed with (a) both oral and IV contrast with contrast enhancement in the arterial and/or portal venous phase; (b) is at least a 2-slice CT; and (c) is acquired with at least 40-60 mAs and CT scans are obtained with contiguous sections with a maximum of 5 mm slice thickness, then the pre-study and post-Cycle 6 PET/CT scan alone will suffice for patients enrolled on both CALGB 50303 and the PET/CT imaging companion.

8.1 Institution Approval Procedures for FDG-PET/CT Imaging Companion

Prior to enrollment of patients, institutions must be approved by the Alliance Imaging Core Lab at IROC Ohio. Institutions will be responsible for evaluating PET/CT scans as they do for clinical care, while the Alliance Imaging Core Laboratory will be responsible for the standardized central imaging reads and the quantitative SUV analyses.

8.1.1 FDG-PET/CT Equipment

All institutions participating in the FDG-PET/CT imaging companion are required to have a hybrid PET/CT scanner. All PET/CT scanners manufactured by General Electric, Philips, and Siemens meet the technical specifications of this companion. If an institution uses a scanner not listed above, the Imaging Core Laboratory must approve the equipment. For information regarding whether the PET/CT scanner to be used complies with this protocol, contact the Alliance Imaging Core Laboratory. **Institutional approval for participation in the imaging companion is contingent upon submission of the PET/CT Instrument Technical Specifications Form (Appendix II) to the Imaging Core Laboratory (see Section 5.1.1.4).**

8.1.2 Institution FDG-PET/CT Imaging Approval

The Alliance Imaging Core Lab at IROC Ohio will adhere to the ACRIN criteria for PET imaging approval procedures. In order for an institution to be approved to participate in this FDG-PET/CT companion, they are required to submit two de-identified test patient studies, as well as a submission of a uniform phantom image for technical and quality review by the Alliance Imaging Core Laboratory (see Section 8.1.3 below). After approval, the participating institution will be provided with an institution-specific (equipment-specific) instruction manual that details all components of the image acquisition and reconstruction throughout the study. The Imaging Core Laboratory will continue to verify that the acquisition and reconstruction performed at the participating sites are in compliance with set guidelines.

Institutional approval for participation in the imaging companion is contingent upon submission of the PET/CT Instrument Technical Specifications Form (Appendix II) to the Imaging Core Laboratory.

8.1.3 Approval Procedures for PET/CT Imaging

Approval requirements for the optional imaging companion CALGB 580603 are equivalent to ACRIN credentialing procedures for PET/CT imaging.

Each institution must submit FOR ALL PET/CT instruments utilized: 1) Two test patient studies; 2) Uniform phantom data (with the SUV measurement of the phantom); and 3) PET/CT Instrument Technical Specifications Form (Appendix II). **Each institution must also submit the Imaging Site Personnel Form (Appendix III) to the Imaging Core Laboratory.**

1) Two test patient studies

   Images of two unidentified patients shall consist of three volume or multi-slice files as follows: a) Whole body CT from PET/CT scanner; b) Whole body (torso) emission with attenuation correction (A/C); and c) Whole body (torso) emission without A/C.

2) Uniform phantom images

   Water-fillable uniform phantom: The phantom must be filled with water, and a known amount of F-18 (either as fluoride or as FDG) should be injected into the phantom. The activity injected should be determined by measurement of the syringe before and after the injection in a properly calibrated dose calibrator. The injected activity should be chosen to result in an activity concentration similar to that encountered in clinical FDG imaging (i.e., 1-1.5 mCi of F-18 should be added to the 6,283 mL phantom; 2 mCi for the 9,293 mL phantom). After thoroughly mixing the phantom, the phantom must be scanned with the same protocol used for the patient imaging. The images also must be reconstructed with the same algorithm and filters used for patient imaging. A circular or elliptical region of interest (ROI) covering most of the interior of the phantom must be drawn over all slices, and the average SUV and standard deviation must be measured and reported in the PET/CT Instrument Technical Specifications Form (Appendix II). The expected SUV for the uniform phantom is 1.0 and the acceptable range is 0.9 to 1.1.

   (Alternatively) Ge-68/Ga-68 calibration phantom: This phantom can readily be scanned with the same protocol used for patient imaging. The assay date and activity from the calibration certificate of this phantom must be reported on the PET/CT Instrument Technical Specifications Form (Appendix II). The images must be reconstructed with the same algorithm and filters used for patient imaging. A circular or elliptical ROI covering most of the interior of the phantom must be drawn over all slices, and the average SUV and standard deviation must be measured and reported in the PET Instrument Technical Specification form. The expected SUV for the uniform phantom is 1.0 and the acceptable range is 0.9 to 1.1.

3) PET/CT Instrument Technical Specifications Form

   The PET/CT Instrument Technical Specifications Form may be found in Appendix II. Please fax, e-mail, or send this form to:

8.2 Procedures for FDG-PET/CT Imaging

8.2.1 Patient Preparation

Patients should avoid any strenuous exercise for 24 hours prior to the PET/CT scan. Patients with poorly controlled diabetes (based on HbA1C) will be excluded. Patients must fast for ≥ 4 hours prior to the injection of FDG, and must have a fasting blood glucose level ≤ 200 mg/dL prior to FDG injection. However, an attempt will be initially made to control the blood glucose level, which will require the rescheduling of the injection. If the glucose level cannot be controlled (i.e., the blood glucose still exceeds 200 mg/dL), the patient will not be included in the FDG-PET/CT imaging companion. Patients should be adequately hydrated with plain water. Weight (kg), height (cm), and blood glucose (mg/dL) will be measured and recorded prior to the injection of FDG.

Sedation (alprazolam, 0.5-1 mg, po, 30 minutes pre-FDG injection) is allowed, but not mandatory and should be used consistently by the patient for all scans. Filgrastim, pegfilgrastim, and epoetin are known to affect FDG uptake, and so their use by the patient should be recorded.

Patients should wait in a warm room to avoid false positive brown fat FDG uptake. Patients will rest comfortably in a quiet room during the FDG uptake period.

FDG will be synthesized and prepared in accordance with the institution's standard procedures or obtained from a commercial supplier.

8.2.2 FDG Dosing and Administration

The administered activity of FDG should be based on the PET/CT scanner manufacturer's recommendation. The recommended FDG dose is 0.14-0.21 mCi/kg. The actual FDG dose should be a bolus of 8-20 mCi, followed by a saline flush (per institutional procedure). A dose at the higher end of the range is recommended, if feasible, with appropriate reduction in the per kilogram dose for heavier patients (in accordance with the manufacturer's recommendation). The effective FDG dose injected and time of injection should be documented on CALGB form C-1657 (50303 Imaging Adjunctive Data Sheet).

8.3 FDG-PET/CT Imaging Acquisition

The FDG-PET/CT Imaging protocol must be followed exactly for every examination and for every patient. Any changes must be approved in advance by the Imaging Core Laboratory [redacted] and changes will be documented in a revised Technical PET Site Manual. It is highly suggested that the baseline exam is available for comparison at the time of the follow-up examination.

The following PET/CT images will be collected digitally for evaluation and archival:

- Baseline (preferred ≤ 30 days prior to start of therapy and before baseline excisional biopsy). If an eligible patient has a baseline PET/CT scan after the baseline excisional biopsy, the biopsied site must be excluded from quantitative evaluation. Please contact the Imaging Core Laboratory with questions. For qualified local sites participating in the evaluation of semi-quantitative PET parameters, study-specific baseline scans are required;
- 17-21 days after the beginning of Cycle 2 therapy and 0-4 days prior to start of Cycle 3;
- Post-Cycle 6 (3-8 weeks after last dose of chemotherapy);
- Any PET/CT image obtained post-Cycle 6.

Whole body emission acquisition at both pre-therapy (baseline) and post-therapy (Cycles 2 and 6) MUST start 60 (± 10) minutes after FDG injection. The starting time will be recorded. Oral and/or IV contrast is optional. The field of view is to encompass the region between the base of the skull and the mid-thighs, but the entire head or distal lower extremities should be included

if these are known sites of disease at baseline. Patient will be scanned supine with arms positioned comfortably above the head, if possible. **It is critical that follow-up emission scans be performed in an identical way to the baseline scan, with the same scanner, same scan direction (skull to thighs or thighs to skull), and consistent arm positioning (arms up or arms down).** The interval between FDG injection and initiation of emission scanning should be the same as the baseline scan (no more than a ± 10 minute difference). All emission scans should be started between 50-70 minutes after injection of FDG. Preferably, schedule the patient for both baseline and follow-up scans at the same time of day (AM or PM) to improve reproducibility. Patient should empty his/her bladder immediately before the acquisition of images.

For baseline scans, if the start time of the PET emission acquisition is outside of the protocol time window (60 ± 10 minutes post-injection), a non-compliance notification will be sent to the institution and the Alliance Imaging Committee. The Imaging Core Laboratory will coordinate with the Imaging Committee to decide whether or not (a) the patient should be included in follow-up studies, and if yes, (b) if the follow-up emission scans should be performed identically to the baseline scan in which the uptake time departed from the protocol, or if an adjustment will be necessary. The Imaging Core Laboratory will notify the institution of the decision and the case-specific procedure required for follow-up exams on that specific patient.

If emission scans in follow-up exams are not performed identically to the baseline scan according to the protocol or to the decision by the Alliance Imaging Committee in a case of an accepted deviation, the same procedure outlined in the paragraph above will be used to clarify the acceptance of such an examination.

For the emission scanning, the acquisition should be performed in 2D or 3D mode in accordance with the manufacturer's recommendations defined in the Technical PET Site Manual. The emission scanning must be consistent between examinations and bed positions. The duration of acquisition for emission data should be in accordance with the manufacturer's recommendations and the data must be corrected for scatter, random events, and dead-time losses using the manufacturer's recommended procedure defined in the Technical PET Site Manual. Images should be attenuation corrected using CT data (note that if IV contrast is used, blood FDG SUV may be altered; therefore attenuation correction must be done without contrast).

The patient will empty his/her bladder again immediately following PET/CT imaging.

8.4 Post-Treatment Study PET/CT Scans (Cycles 2 and 6)

The post-treatment PET/CT scans are to be done according to the same specifications described in Section 8.0. The post-treatment PET/CT scans should be done on the same scanner within the same qualified institution used for the baseline PET. If there is a need to change the scanner, the institution must notify the Imaging Core Laboratory.

The first post-treatment PET/CT scan will be done 17-21 days after completion of Cycle 2 of chemotherapy, and 0-4 days prior to initiation of Cycle 3 chemotherapy.

The final post-treatment PET scan will be done at 3-8 weeks after the completion of Cycle 6 in order to allow for resolution of post-treatment inflammatory effects that can cause false positive PET scan results. If the patient is unable to complete six cycles of chemotherapy, a PET/CT scan should be performed at a similar interval after the last treatment.

Each post-treatment PET/CT scan will be read and interpreted together with the baseline PET/CT scan and knowledge of other relevant clinical information. There will be a separate central review by the Imaging Core Laboratory.

In addition to the tumor SUV and background SUV determinations described below (see Section 8.7.4), an overall PET response scale will be reported for each patient, and the presence of any new lesions will be recorded and a confidence assessment indicated. The scale is as follows:

Negative

0 No abnormal activity (tumor cold compared with background)

1+ Minimal activity (tumor less than background)

2+ Equivocal (tumor = background)

Positive

3+ Moderately increased activity (tumor greater than background; i.e., greater than ABP)

4+ Markedly increased activity (tumor much greater than background)

After completion of standard chemotherapy,

(1) Are there new foci of FDG activity (yes/no)?

(2) Are these judged to represent lymphoma (yes/no)?

8.5 FDG-PET/CT Data Archiving, Storage, and Submission

The complete PET/CT scan will be submitted to the Imaging Core Laboratory in digital DICOM format as defined in the Technical PET Site Manual. **BMP files, JPG files, or hard copies (films) are not acceptable.** The raw data of the entire study should be saved until the scan is accepted by the Imaging Core Laboratory. De-identify the patient data using institutional procedures to remove patient name and medical record number while preserving the CALGB ID number and protocol number. The de-identified digital images may be burned to a CD or transferred to a PC based system. **The following complete data sets must be sent:** 1) Transmission CT data; 2) emission data with attenuation correction; and 3) emission data without attenuation correction.

Data may be transferred by 1) FTP transfer or 2) mail/express courier transfer.

1) FTP Transfer: Any FTP software can be used to initiate access to the secure FTP Server of the Imaging Core Laboratory. The standard FTP access information will be provided separately through the specific trial e-mail [redacted], per the request by participating sites before their first data submission.

Once you have access to the main data directory of the FTP server at the Imaging Core Laboratory, create a folder using the CALGB 50303 Subject Identifier assigned to the image data set you wish to FTP (upload). Then create another sub-folder with the date of the exam (YYYYMMDD), followed by the abbreviation of the study performed (PET, CT). Upload (copy) the imaging files to the appropriate sub-directory (the imaging files can be exported as a single file series). Once the upload is complete, **you must send an e-mail to the Imaging Core Laboratory at [redacted] to inform them that the images have been uploaded from your institution**. Please include the CALGB patient ID number and the date of the scan.

2) Shipment/Mail Transfer: If FTP data transfers cannot be achieved, the de-identified images in digital DICOM format should be burned to a CD and mailed to the Imaging Core Laboratory. Please submit only one patient's images per CD, with the patient's CALGB ID number, study types (i.e., PET/CT baseline, post-Cycle 2, post-Cycle 6, or any other follow-up study), date of scans, date of first study treatment, and name of submitting institution. Alternatively, where institutional information systems allow, the images can be e-mailed to the Alliance Imaging Core Laboratory at the Ohio State University.

Submit these data to: Alliance Imaging Core Laboratory

Once the image acquisition has been completed, the entire study (complete PET/CT data in digital DICOM format), along with CALGB form C-1657 (50303 Imaging Adjunctive Data Sheet), must be submitted to the Imaging Core Laboratory within no more than 3 business days. Institutions must send C-1657 to the Alliance Statistics and Data Center, Data Operations. **Please note** that it is optional to submit the CALGB form C-1627 (50303 PET and CT Measurement Form). If C-1627 is submitted, it should be submitted to the Imaging Core Laboratory and the Alliance Statistics and Data Center, Data Operations at the same time as CALGB form C-1657 (50303 Imaging Adjunctive Data Sheet).

If there are any difficulties or questions concerning the FDG-PET/CT data submission, please contact the Imaging Core Laboratory.

The Alliance Imaging Core Laboratory will acknowledge receipt of the imaging data via e-mail confirmation to the institution within one business day of receipt. The data will be reviewed for quality (see Section 8.7.2). After completion of the quality check, another e-mail assessing the protocol compliance will be sent to the institution.

8.6 Training for FDG-PET/CT Data Acquisition and Handling

As part of the approval process, the Imaging Core Laboratory will train the technologists and responsible imaging physicians for the institutions in all aspects of patient handling, image acquisition, and image transfer. The Imaging Core Laboratory will use a virtual site visit concept based on WebEx that can facilitate communication on any computer that has access to the Internet. Outlined below is the WebEx conference information that will be included in the institution-specific manual.

WebEx conferencing allows the simultaneous display of images/text/help instructions (desktop presentations/desktop applications, such as Power Point) in a secure manner to participating sites to any computer with Internet access. For a WebEx conference, computers must have broadband Internet access and users must have the authority to download a WebEx client. For another level of safety and quality, the Imaging Core Laboratory will use a CALGB call-in number that should preferably be used from a conference phone if there is more than one participant from your location.

The Imaging Core Laboratory will create a video stream that includes didactic information and training for technologists on any of the institution-specific issues. This information can be accessed at any time and/or downloaded at the institution for viewing. Furthermore, the Imaging Core Laboratory will offer a training course/workshop on a needed basis that can be attended by site personnel.

The Imaging Core Laboratory will train intra- and inter-institutional image readers using other independent data sets including imaging data import/export using the Workstations; image integrity/completeness check with the support of software tools; PET/CT fusion; ROI/VOI placements; SUV calculation.

The readers will be benchmarked against a gold standard established for those images by a consensus assessment of the experienced nuclear medicine physicians selected by the Imaging Committee, to include the protocol Imaging Co-Chair. Readers will be required to assess their consistency in assessment at least every six months. Furthermore, a web-based training module for nuclear medicine attendings reading trial studies will be developed. The Imaging Core Laboratory will not provide basic skills, but a dedicated training on all assessment aspects of this trial.

8.7 CALGB Imaging Core Lab Processing and Storage

8.7.1 HIPAA Compliance

The image data sets must be sent in a HIPAA-compliant manner. Upon receipt, the Imaging Core Laboratory will verify that the images are de-identified and contain the trial and CALGB patient ID. In the case that they do contain patient private information, the Imaging Core Laboratory will issue a non-compliance statement to the institution and will de-identify the submitted data sets. The Imaging Core Laboratory will work in a completely de-identified manner and will use study and patient identification alpha-numerics according to CALGB procedures.

8.7.2 Quality Assurance and Quality Control of PET/CT Images

During institutional approval, institution-specific manuals will be established. All submitted PET/CT images will be reviewed regarding technical specifications such as dosage, timing, acquisition, and reconstruction, and that they are compliant with the protocol. Any non-compliance or discrepancies will be reported in the quality assessment and compliance review. This procedure will be performed for every submitted data set and will occur within 72 hours upon receipt of the data set at the Alliance Imaging Core Lab at IROC Ohio. The quality of the PET/CT systems will be verified on a daily and monthly basis. The review of the institution- and equipment-specific quality management, including daily quality assurance set-up and compliance to the policies, will be established at the approval assessment and verified on an ongoing basis.

If, at the approval review of the institution, the Imaging Core Laboratory determines that there are insufficient quality standard operating procedures established for the site, it will propose that established procedures for the specific equipment type, based upon the manufacturer's recommendation and established guidelines, be used. If at any time the institution is deemed to be non-compliant, a non-compliance statement will be issued and a remedy procedure suggested. If an institution does not remedy quality compliance concerns, the Imaging Core Laboratory will revoke the approval of the institution. The institution would then become ineligible for participation in the FDG-PET/CT companion until resolution of the defined issues. Imaging Core Laboratory guidelines will be in alignment with the ACRIN guidelines.

The quality of each PET/CT image will be assessed in three steps: 1) confirmation that images have been received and are electronically accessible; 2) image review for completeness and image quality by an experienced research associate or technologist who will have access to staff nuclear medicine physicians in case of questions; and 3) review of images and post-processing results by an experienced staff member (nuclear medicine physician or radiologist). All ROI and calculation steps will be electronically documented and the quantitative data transferred into the trial database.

There are two components regarding readability of images. One is the technical readability (e.g., the images are in a DICOM format, which is a requirement, or in a data format of any of the three major equipment vendors: General Electric, Philips, and Siemens). The other aspect is the image quality. As described above, every data set received will be reviewed

regarding compliance to the acquisition and reconstruction protocol. Patient images that show artifacts due to patient motion, extravasation of radiotracer, or other technical issues, including non-compliance of the timeline, will be identified as such during the quality compliance review and reported as compliant/non-compliant or acceptable/unacceptable to the institution.

Submitted images will be classified as "optimal" if they are compliant with all acquisition, reconstruction, and quality criteria without showing any artifacts. The classification "readable but not optimal" will be reserved for those images which are compliant in the essential components, but are sub-optimal due to patient motion or non-detrimental deviation from the expected performance. The classification "not readable" will be used for any other cases that cannot be characterized in the other two categories (i.e., no evaluable lesions; too much motion; wrong body location; data broken; images incomplete; non-DICOM image formats, such as JPEG images, bitmap images, screen-captured images, scanned films or hardcopies, etc.) and that are non-compliant.

An initial QA check will be performed within 72 hours by the Imaging Core Laboratory upon receipt of each imaging study from the sites. The imaging studies will be stratified as (1) optimal, (2) readable but not optimal, and (3) not readable, based upon predetermined rules. Imaging studies that are not readable will be communicated to the site and Imaging Co-Chair by the Imaging Core Laboratory and will be encouraged to be immediately repeated. Imaging studies that are readable but not optimal will be discussed with the Imaging Co-Chair to decide upon status in the analysis. It may be that these studies are included in the qualitative analysis, but not semiquantitative analysis.

Strict enforcement of compliance and quality assessment is necessary in order to ensure the highest quality of the data collected in this trial. The assessment of every submitted study and image within 72 hours of submission to the Imaging Core Laboratory regarding compliance and quality enables not only the appropriate third party review, but also a timely notification about non-compliance to the institution and the Alliance. The Imaging Core Laboratory will copy non-compliance notices to the Alliance Imaging Committee and the Alliance Statistics and Data Center, and it will support the institution to ensure that compliance is achieved through appropriate measures. The Imaging Core Laboratory will propose remedy options that will include expected timelines and will revoke the institutional approval if compliance is not achieved within reasonable timelines. Any such action will be coordinated with the Alliance Imaging Committee.

For non-compliant images: If images received by the Imaging Core Laboratory are not able to be analyzed (i.e., no evaluable lesions; data broken; images incomplete; non-DICOM image formats, such as JPEG images, bitmap images, screen-captured images, scanned films or hardcopies) and these potential problems cannot be resolved, then the following scenarios may apply:

a) If the problem occurs with the baseline images, then an imaging quality check report will be sent to both the institution and the Alliance Imaging Committee Leadership, in which the Imaging Core Laboratory will suggest that the baseline scans be rescheduled/reperformed, as well as that the imaging data be retransmitted. Otherwise, if the patient has been in follow-up studies, then the Alliance Imaging Committee Leadership will decide whether and how the patient can be included in the analyses.

b) If the problem occurs with the Cycle 2 images, but the baseline images are compliant, then an inquiry will be sent to the Alliance Imaging Committee Leadership to decide if the patient will be included in follow-up studies. If so, an imaging quality check report will be sent to both the institution and the Alliance

Imaging Committee Leadership in which the Imaging Core Laboratory will remind the institution to avoid these problems in the follow-up scans and to provide DICOM-formatted imaging data for Cycle 6 and post-Cycle 6 images.

c) If the problem occurs with the Cycle 6 images, but both baseline and Cycle 2 images are compliant, then an inquiry will be sent to the Alliance Imaging Committee Leadership to decide if the patient will be included in any follow-up studies. If so, then an imaging quality check report will be sent to both the institution and the Alliance Imaging Committee Leadership in which the Imaging Core Laboratory will remind the institution to avoid these problems in post-Cycle 6 scans. Otherwise, if the Cycle 2 images were also non-compliant, then an imaging quality check report will be sent to both the institution and the Alliance Imaging Committee Leadership, and the Alliance Imaging Committee Leadership will decide if the patient will be excluded from further post-Cycle 6 PET/CT scans.

If the images received are analyzable, then an imaging quality check report will be sent to both the institution and the Alliance Imaging Committee Leadership, and the Alliance Imaging Committee Leadership will decide if the analysis for the patient is to be finished by the Imaging Core Laboratory.

Summary Reports: The Imaging Core Laboratory will generate a monthly summary report for each institution that will report on the number of cases submitted, specification of images received, quality assurance failures and queries outstanding, and details of reconciliation processes in terms of images received/acquired, the quality/compliance assessment, etc. In addition to these institution-specific reports, the Imaging Core Laboratory will create summary reports for the entire trial, including recruitment quality status and number of non-compliance queries. The Imaging Core Laboratory will submit quarterly and yearly reports to the Lymphoma FDG-PET Working Group (including experts from the NCI, FDA, Alliance, and PhRMA), the Imaging Co-Chair, and the Alliance Statistics and Data Center.

8.7.3 Imaging Core Laboratory Image Reconstruction and Analysis

Image reconstruction (i.e., an interactive reconstruction method with preference for OSEM reconstruction, 8 subsets, 2 iterations, followed by smoothing with a 6 mm 3-D Gaussian kernel) will be performed using the manufacturer-recommended parameters and specified in the Technical PET Site Manual. Both visual/qualitative and semi-quantitative (SUV) PET data analysis will be performed.

Both visual/qualitative and semi-quantitative (SUV) PET/CT data analysis will be performed locally by the sites, as well as by the Alliance Imaging Committee in collaboration with the Imaging Core Laboratory. Data analysis of the study endpoints will be based on readings by three qualified independent nuclear medicine physicians/radiologists, selected by the Imaging Committee. Readings will be performed according to procedures outlined by the FDA imaging reports and will consist of two independent reads. Any discordance (defined by endpoints in the imaging analysis plan) will be adjudicated by a third independent nuclear medicine physician/radiologist. The adjudicator can only select one of the two initial independent reads, as outlined by FDA independent read documentation. Quantitative assessments (e.g., tumor location, tumor size, ROI/VOI information, SUV, etc.) will be electronically documented (screen captured from the Workstation) independently and used for determining consensus of readings, as well as inter-observer variability. Both Cycle 6 and Cycle 2 endpoints are dichotomous, and so will be determined by consensus of two or three readings. If an eligible patient has a baseline PET/CT scan after the baseline excisional biopsy, the biopsied site must be excluded from quantitative evaluation. Please contact the Imaging Core Laboratory with questions.

8.7.4 Institutional & Imaging Core Laboratory Determination of Tumors and SUVs

Tumor Selection: Up to six tumor masses > 2 cm will be selected for analysis at baseline, and their locations and size (length and width) will be defined. Tumor dimensions will be determined in a single plane on the transverse CT image where the tumor appears largest. The maximum dimension (length) should be measured, followed by the width, which should be perpendicular to the length. Tumor #1 will be the tumor site with highest SUVmax; the others will be selected in descending order of SUV. Location and dimensions of the index tumors will be determined at baseline and at all subsequent PET/CT scans. For post-treatment assessment of lesions other than the six index lesions, see Section 8.8.

SUVs: SUVs for the study endpoints will be calculated using decay-corrected administered dose and uncorrected body weight. SUVs will be calculated at baseline and at all subsequent PET/CT scans. These procedures are described briefly below.

Tumor SUVmax: For the purposes of this study, the primary SUVs for calculation and reporting will be the SUVmax within each of the index (up to 6) tumor volumes. This will be determined by the nuclear medicine physician visually identifying the region or regions on the PET images that qualitatively appear to have the most intense FDG uptake and that correspond to known tumor based on other data (e.g., CT scan). SUVmax should be determined on the transverse slice where the tumor visually appears the brightest, as well as on several adjacent slices above and below, to be certain that the reported maximum is correct. Determinations will be made on a 128 x 128 image matrix size image. VOIs can be placed where the tumor visually appears the brightest by 3-D ellipse/spherical or 3-D isosurface tools.

Prints of the image and the SUV cursor should be acquired and retained for source documentation. As noted above, for the purposes of this protocol, up to six lesions will be analyzed. The one with the highest SUVmax will be reported as lesion #1; the others should be identified in descending order of SUV.

ABP SUVmean: Place the ROI on a transverse image or the spherical VOI with a 1 cm oval in the middle third of the aorta, well away from the edge. Take the mean SUV of the ROI/VOI.

Liver SUVmean: Place the ROI on a transverse image (a 3-5 cm long by 2 cm irregular region) or the spherical VOI (a 3-5 cm long by 2 cm irregular volume) through the mid liver with relative uniform intensities, which excludes the central hilum of the liver. Take the mean SUV of the ROI/VOI.

Other SUV data will be determined. As noted above, SUVs obtained and used for the endpoints of this study will be based on body weight and will not be corrected for body surface area or other measures of patient size/shape. However, patient height data will be collected, and exploratory analysis of SUV corrected for body surface area and lean body mass will be performed to determine whether this correction provides more useful data than conventional, uncorrected SUV.

8.8 Interpretation of Positive Post-Treatment Scans and Implications of PET Images for Progressive Disease

At Cycle 6, if a scan is PET positive but CT negative, another PET/CT scan will be performed after 2-3 months. If PET remains positive, the PET-positive site(s) should be biopsied to determine histopathology, if feasible, or additional follow-up PET scans should be performed until the activity resolves or is stable for six months or longer. If a PET scan shows progression, results will be provided to the treating physician to assist in further evaluation of the patient. No patient will be considered to have failed treatment based on a positive PET scan alone.

PET/CT results obtained post-Cycle 2 will not be provided to treating physicians during the course of the trial, since these data are exploratory in nature and their utility in patient management is unknown. Exceptions are the rare occurrences of any results suggesting the presence of progression or life-threatening disease. These data will be provided to the treating physician as soon as possible after they are obtained to aid the further evaluation of the patient.

8.9 PET/CT Scan Reimbursement

The patient and/or his/her health plan/insurance company will need to pay for the costs of the PET/CT scans performed at prior to treatment, 3-8 weeks post-Cycle 6, and (if necessary) 2-3 months after the post-Cycle 6 scan. The costs for as many as two PET/CT scans – a repeat baseline PET/CT scan if the original is non-compliant (i.e., not performed per protocol specifications), and the PET/CT scan performed at post-Cycle 2 – will be reimbursed by funding provided by the Cancer Trials Support Unit, funded by the National Cancer Institute. The reimbursement form can be found on the 50303 study page on the Alliance Web site, www.allianceforclinicaltrialsinoncology.org.

9.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

9.1 Arm A (R-CHOP) Dose Modifications

9.1.1 Hematologic Toxicity

9.1.1.1 Neutropenia

- To begin a cycle, ANC must be ≥ 1500/μL and platelets ≥ 100,000/μL. Delay cycle by up to 2 weeks if these values are not met on Day 1 of cycle. If delay is greater than two weeks. then contact Study Chair.
- If a patient develops grade 4 neutropenia (ANC < 500/μL) or any febrile neutropenia (defined as ANC < 500/μL + fever ≥ 38.5°C (101°F) sustained for more than one hour), filgrastim or pegfilgrastim should be administered for all subsequent cycles. Administration of colony stimulating factors at time of neutropenia is at the discretion of treating physician.
- If febrile neutropenia (defined as ANC < 500/μL + temperature ≥ 38.5°C (101°F) sustained for more than one hour) occurs despite filgrastim or pegfilgrastim support, reduce doses of cyclophosphamide and doxorubicin by 25% for subsequent cycles. If toxicity recurs, cyclophosphamide and doxorubicin doses should be reduced by an additional 25% for subsequent cycles.
- If grade 4 neutropenia or febrile neutropenia persists despite two dose reductions, remove patient from protocol therapy.

9.1.1.2 Thrombocytopenia

- If grade 3 (platelets < 50,000/μL and ≥ 25,000/μL) or 4 thrombocytopenia (platelets < 25,000/μL) occurs, reduce doses of cyclophosphamide and doxorubicin by 25% for subsequent cycles. If toxicity recurs, cyclophosphamide and doxorubicin doses should be reduced by an additional 25% for subsequent cycles.
- If grade 3 (platelets < 50,000/μL and ≥ 25,000/μL) or 4 thrombocytopenia (platelets < 25,000/μL) recurs despite two dose reductions, remove patient from protocol therapy.

9.1.2 Ileus and Constipation

Symptomatic ileus/constipation may occur with vincristine. If ileus or constipation require hospitalization, the next dose of vincristine should be reduced by 25%. If symptoms do not

recur at the reduced dose, then the vincristine dose may be re-escalated on subsequent cycles.

9.1.3 Neuropathy

Sensory and motor neuropathy may occur with vincristine. Vincristine doses should only be reduced if the patient develops neuropathy that interferes with activities of daily living. Most vincristine associated neuropathy resolves after completion of treatment.

- **Sensory neuropathy**: If grade 3 toxicity develops, reduce vincristine 25%. If symptoms improve, doses may be increased to previous levels. If patient experiences grade 4 toxicity, discontinue vincristine.
- **Motor neuropathy**: If patient experiences grade 2 toxicity, reduce vincristine 25%. If patient experiences grade 3 toxicity, reduce vincristine 50%. If symptoms improve, doses may be increased to previous levels. If patient experiences grade 4 toxicity, discontinue vincristine.

9.1.4 Hyperbilirubinemia

- **Doxorubicin**

  No doxorubicin dose modifications will be made for increased bilirubin ≤ 7 mg/dL. Doxorubicin should be held for bilirubin > 7 mg/dL, until it returns to ≤ 7 mg/dL. Pharmacokinetic data from Dr. Wilson et al. (unpublished data) has shown no significant effect of bilirubin on doxorubicin clearance. Specifically, doxorubicin pharmacokinetics were measured in 47 patients during multiple cycles of DA-EPOCH in which doxorubicin was administered as a continuous infusion. Seven patients presented with bilirubin levels above normal. Although doxorubicin clearance was delayed in cycle one in these seven patients, the clearance values were within the range of those seen in patients with normal bilirubin, and no patients experienced excessive toxicity. Significant reductions in doxorubicin clearance were observed primarily in patients with bilirubin levels ≥ 2 mg/dL. Bilirubin levels above 2 mg/dL did not demonstrate a quantitative relationship with doxorubicin clearance. Clinically, all of these patients received full dose doxorubicin, and no patient experienced excessive toxicity. Additionally, these patients showed significant increases in doxorubicin clearance in cycle 2.

- **Vincristine**

  Vincristine dose will be decreased for elevated bilirubin **only** secondary to lymphoma as follows:

Bilirubin (mg/dL)	Vincristine Dose
> 1.5 - < 3	decrease by 25%*
≥ 3	decrease by 50%*

*** Vincristine dose will be re-escalated as hyperbilirubinemia improves.**

9.2 Arm B (DA-EPOCH-R) Dose Modifications

9.2.1 Hematologic Toxicity

Delay cycle by up to 2 weeks if ANC < 1000/μL or platelets < 100,000/μL. Filgrastim may be used for several days to increase ANC. If no recovery after 2 weeks, contact the Study Chair for guidance.

Doses for doxorubicin, etoposide and cyclophosphamide will be based on measurements of the previous cycle ANC or platelet nadir whichever is lower **(i.e., twice weekly starting 3-4 days after completion of chemotherapy).**

If ANC ≥ 500/μL on all measurements:	↑ one dose level*
If ANC < 500/μL on 1 or 2 measurements (3-4 days apart):	maintain dose level
If ANC < 500/μL ≥ 3 measurements: (3-4 days apart):	↓ one dose level**
OR	
If platelet < 25,000/μL on ≥ 1 measurement:	↓ one dose level

* to a maximum of Dose Level 7.

** to a minimum of Dose Level –2. Recurrence of a toxicity requiring a dose reduction below Dose Level –2 will result in removal of patient from protocol therapy.

9.2.2 DA-EPOCH Dose Levels

Dose adjustments for hematologic toxicity apply only to etoposide, doxorubicin and cyclophosphamide. Only cyclophosphamide is reduced in Dose Levels –1 through –3.

	Dose Levels									
Adjusted Agents	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Doxorubicin (mg/m^2/day)	10	10	10	**10**	12	14.4	17.3	20.7	24.8	29.8
Etoposide (mg/m^2/day)	50	50	50	**50**	60	72	86.4	103.7	124.4	149.3
Cyclophosphamide (mg/m^2)	384	480	600	**750**	900	1080	1296	1555	1866	2239
Non-Adjusted Agents										
Rituximab (mg/m^2)	375	375	375	**375**	375	375	375	375	375	375
Vincristine (mg/m^2/day) (No cap)	0.4	0.4	0.4	**0.4**	0.4	0.4	0.4	0.4	0.4	0.4
Prednisone (mg/m^2 BID)	60	60	60	**60**	60	60	60	60	60	60

9.2.3 Non-Hematologic Toxicity

9.2.3.1 Ileus and Constipation

Symptomatic ileus/constipation may occur with vincristine. If ileus or constipation requires hospitalization, the next dose of vincristine should be reduced by 25%. If symptoms do not recur at the reduced dose, then the vincristine dose may be re-escalated on subsequent cycles.

9.2.3.2 Neuropathy

Sensory and motor neuropathy may occur with vincristine. Vincristine doses should only be reduced if the patient develops neuropathy that interferes with activities of

daily living. Most vincristine associated neuropathy resolves after completion of treatment.

- **Sensory neuropathy:** If grade 3 toxicity develops, reduce vincristine 25%. If symptoms improve, doses may be increased to previous levels. If a patient experiences grade 4 toxicity, discontinue vincristine.
- **Motor neuropathy:** If grade 2 toxicity develops, reduce vincristine 25%. If a patient experiences grade 3 toxicity, reduce vincristine 50%. If symptoms improve, doses may be increased to previous levels. If a patient experiences grade 4 toxicity, discontinue vincristine.

9.2.3.3 Hyperbilirubinemia

- **Doxorubicin**

  No doxorubicin dose modifications will be made for increased bilirubin ≤ 7 mg/dL. Doxorubicin should be held for bilirubin > 7 mg/dL, until it returns to ≤ 7 mg/dL.. Pharmacokinetic data from Dr. Wilson et al. (unpublished data) has shown no significant effect of bilirubin on doxorubicin clearance. Specifically, doxorubicin pharmacokinetics were measured in 47 patients during multiple cycles of DA-EPOCH in which doxorubicin was administered as a continuous infusion. Seven patients presented with bilirubin levels above normal. Although doxorubicin clearance was delayed in cycle one in these seven patients, the clearance values were within the range of those seen in patients with normal bilirubin, and no patients experienced excessive toxicity. Significant reductions in doxorubicin clearance were observed primarily in patients with bilirubin levels ≥ 2 mg/dL. Bilirubin levels above 2 mg/dL did not demonstrate a quantitative relationship with doxorubicin clearance. Clinically, all of these patients received full dose doxorubicin, and no patient experienced excessive toxicity. Additionally, these patients showed significant increases in doxorubicin clearance in cycle 2.

- **Vincristine**

  Vincristine dose will be decreased for elevated bilirubin **only** secondary to lymphoma as follows:

Bilirubin (mg/dL)	Vincristine Dose
> 1.5- < 3	decrease by 25%*
≥ 3	decrease by 50%*

*** Vincristine dose will be re-escalated as hyperbilirubinemia improves.**

9.2.3.4 Dose Modifications Based on Creatinine Clearance Secondary to Lymphoma

No etoposide dose modifications will be made for decreased creatinine clearance or increase creatinine. It is anticipated that decreased clearance or increased creatinine will improve after the first treatment cycle.

9.3 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. Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on Alliance protocols.

10.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

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.

The weight at Cycle 1 should be used for dose calculations for all cycles unless there is a ≥10% change over a period of one cycle. If a weight change ≥ 10% occurs, then doses should be recalculated and the new dose used from that time forward.

10.1 Doxorubicin HCL (Adriamycin PFS™, Adriamycin RFS™, Rubex®)

AVAILABILITY

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

STORAGE & STABILITY

Intact vials of doxorubicin solution should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multidose vials.

Compatibility and stability studies were conducted by the Pharmaceutical Development Service [60], Pharmacy Department, NIH Clinical Center, simulating concentrations of each drug that would be applicable to this trial. Admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, in polyolefin-lined IV bags were stable for up to 72 hours at room temperature, provided that the concentration of etoposide was < 250 mcg/ml.

PREPARATION

Reconstitute the vials of doxorubicin powder with 5, 10, 25, or 50 mL, respectively, of sodium chloride for injection, USP, resulting in a concentration of 2 mg/mL.

In Arm A (CHOP-R), withdraw the desired volume into a syringe for IV injection.

In Arm B (EPOCH-R), doxorubicin will be admixed in 0.9% sodium chloride, along with vincristine IV for infusion over 24, 48 or 72 hours. Etoposide may be mixed with doxorubicin and vincristine, or it may be infused separately. See reference 49 for specific compatibility and stability information base on concentration of each agent.

ADMINISTRATION

In Arm A (CHOP-R), doxorubicin is administered intravenously over 3-5 minutes. Avoid extravasation, as severe local tissue necrosis may result.

In Arm B (EPOCH-R), doxorubicin will be administered as a 96-hour continuous IV infusion, along with vincristine and etoposide in the same infusion solution (see "Storage and Stability" above). The chemotherapy will be administered with a suitable infusion pump via a central venous access device. Temporary PICC lines or permanent lines may be used. Extravasation of

these diluted agents has not caused local tissue damage due to their low concentrations in the solution.

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).

10.2 Etoposide (VePesid®, Epidophyllotoxin, VP-16)

AVAILABILITY

Etoposide is commercially available as a solution for injection in 5 mL, 7.5 mL, 25 mL, and 50 mL vials containing 20 mg/mL. Please refer to the FDA-approved package insert for etoposide for product information, extensive preparation instructions, and a comprehensive list of adverse events.

STORAGE & STABILITY

Intact vials of etoposide for injection should be stored at room temperature and protected from light.

Compatibility and stability studies were conducted by the Pharmaceutical Development Service [60], Pharmacy Department, NIH Clinical Center, simulating concentrations of each drug that would be applicable to this trial. Admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, in polyolefin-lined IV bags were stable for up to 72 hours at room temperature, provided that the concentration of etoposide was < 250 mcg/ml.

PREPARATION

Etoposide will be admixed in 0.9% sodium chloride for IV infusion over 24, 48 or 72 hours. Etoposide may be mixed with doxorubicin and vincristine or it may be infused separately. See reference 49 for specific compatibility and stability information based on concentration of each agent. Infusion solutions should be changed every 24 hours. The volume of the infusion solution will be determined by the 24 hour etoposide dose. If etoposide ≤ 150 mg per 24 hours, then dilute drugs in 500 mL 0.9% sodium chloride; if etoposide > 150 mg per 24 hours, then dilute drugs in 1000 mL 0.9% sodium chloride.

ADMINISTRATION

Etoposide is administered in Arm B only. Etoposide will be administered as a 96-hour continuous IV infusion, along with vincristine and doxorubicin (see "Storage & Stability" above). The chemotherapy will be administered with a suitable infusion pump via a central venous access device. Temporary PICC lines or permanent lines may be used. Extravasation of these diluted agents has not caused local tissue damage due to their low concentrations in the solution.

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. At the dose used in this study, etoposide is moderately highly emetogenic. 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.

10.3 Vincristine sulfate (VCR, Leurocristine sulfate, Oncovin®, Vincasar PFS)

AVAILABILITY

Vincristine is commercially available in 1 mL, 2 mL, and 5 mL vials in a concentration of 1 mg/mL. Please refer to the FDA-approved package insert for vincristine sulfate for product information, extensive preparation instructions, and a comprehensive list of adverse events.

STORAGE & STABILITY

Unopened vials should be stored under refrigeration and proteced from light. Commercially available solutions labeled as such are intended to be multidose vials.

Compatibility and stability studies were conducted by the Pharmaceutical Development Service [49], Pharmacy Department, NIH Clinical Center, simulating concentrations of each drug that would be applicable to this trial. Admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, in polyolefin-lined IV bags were stable for up to 72 hours at room temperature, provided that the concentration of etoposide was < 250 mcg/ml.

PREPARATION

In Arm A (CHOP-R), withdraw the desired volume into a syringe for IV injection.

In Arm B (EPOCH-R), vincristine will be admixed in 0.9% sodium chloride for IV infusion over 24, 48 or 72 hours. Etoposide may be mixed with doxorubicin and vincristine or it may be infused separately. See reference 49 for specific compatibility and stability information based on concentration of each agent. Infusion solutions should be changed every 24 hours. The volume of the infusion solution will be determined by the 24 hour etoposide dose. If etoposide ≤ 150 mg per 24 hours, then dilute drugs in 500 mL 0.9% sodium chloride; if etoposide > 150 mg per 24 hours, then dilute drugs in 1000 mL 0.9% sodium chloride. Infusion solutions should be protected from light.

ADMINISTRATION

In Arm A (CHOP-R), administer without further dilution by slow IV push. **Precaution: Maximum single dose is 2 mg.**

In Arm B (EPOCH-R), vincristine will be administered as a 96-hour continuous IV infusion, along with etoposide and doxorubicin (see "Storage & Stability" above). The chemotherapy will then be administered with a suitable infusion pump via a central venous access device. Temporary PICC lines or permanent lines may be used. Extravasation of these diluted agents has not caused local tissue damage due to their low concentrations in the solution.

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.

10.4 Cyclophosphamide (Cytoxan®; Neosar®)

AVAILABILITY

Commercially available as a 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.

PREPARATION

Reconstitute 100 mg, 200 mg, 500 mg, 1 gram and 2 gram vials with 5, 10, 25, 50, or 100 mL of sterile water for injection or normal saline to give a final concentration of 20 mg/mL. Vigorous shaking and/or gentle warming may be necessary for non–lyophilized preparations. Bacteriostatic water for injection (paraben preserved only) may be used; benzyl alcohol derivatives may NOT be used.

STORAGE & STABILITY

Intact vials should be stored at room temperature. Reconstituted and diluted solutions are stable for 24 hours at room temperature and 6 days if refrigerated.

ADMINISTRATION

The total dose of cyclophosphamide will be administered by IV.

All patients should receive hydration with normal saline at the following volumes (based on cyclophosphamide dose levels) and rates with half administered before and half administered after cyclophosphamide.

R-CHOP and DA-EPOCH-R Levels 1-2 (see § 9.2.2):	1 liter NS @ 300-500 cc/hr
DA-EPOCH-R Levels 3-5 (see § 9.2.2):	2 liter NS @ 300-500 cc/hr
DA-EPOCH-R Level ≥ 6 (see § 9.2.2):	2.5 liter NS @ 300-500 cc/hr

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, azospermia, amenorrhea, cardiotoxicity (myocardial necrosis) usually at doses higher than those used in this study.

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.

10.5 Prednisone (Deltasone, miscellaneous)

AVAILABILITY

Commercially available in 1, 2.5, 5, 10, 20, 25, and 50 mg tablets, or as an oral solution or syrup - 5 mg/5 ml (in 5% alcohol); solution concentrate - 5 mg/ml (with 30% alcohol). Please refer to the FDA-approved package insert for prednisone for product information, and a comprehensive list of adverse events.

STORAGE & STABILITY

Store tablets, solutions and syrup in tightly closed containers at room temperature.

ADMINISTRATION

Oral.

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.

10.6 Rituximab

AVAILABILITY

Rituximab is commercially available in 10 mL and 50 mL single-use vials containing 100 mg or 500 mg rituximab solution, respectively, at a concentration of 10 mg/mL. Please refer to the FDA-approved package insert for rituximab for product information, extensive preparation instructions, and a comprehensive list of adverse events.

STORAGE & STABILITY

Intact vials should be stored under refrigeration. Dilute solutions for infusion (1-4 mg/mL) are stable for 24 hours under refrigeration, and for an additional 24 hours at room temperature.

PREPARATION

The desired dose of rituximab should be diluted in 0.9% NaCl or D_5W to a final concentration of 1-4 mg/mL. The solution should be mixed by gently inverting the bag.

ADMINISTRATION

Oral pre-medication 650 mg of acetaminophen and 50-100 mg diphenhydramine hydrochloride will be administered 30 to 60 minutes prior to starting each infusion of rituximab. Rituximab will be administered as an intravenous infusion at 375 mg/m^2 on day 1 of each cycle of R-CHOP or DA-EPOCH-R, immediately prior to the start of chemotherapy. The first rituximab infusion should be started at 50 mg/hr, and increased in 50-mg/hr increments every 30 minutes to a maximum rate of 400 mg/hr. If this rate of escalation is well tolerated the second and subsequent infusions can begin at a rate of 100 mg/hr and increase in 100 mg/hr increments every 30 minutes to a maximum of 400 mg/hr. CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

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 ($\geq$ 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.

Exacerbation or reactivation of other viral infections has also been reported with rituximab. Recent reports describe JC virus reactivation leading to progressive multifocal leukoencephalopathy (PML) in patients who were receiving rituximab. Patients presenting with new neurologic findings (e.g., major changes in vision, unusual eye movements, loss of balance or coordination, confusion) should be evaluated for PML.

10.7 Filgrastim (G-CSF; r-met HuG-CSF; Granulocyte Colony Stimulating Factor; Neupogen®)

AVAILABILITY

Commercial filgrastim is available in 1 mL and 1.6 mL vials containing 300 mcg and 480 mcg filgrastim, and in prefilled syringes containing 300 mcg/0.5 mL or 480mcg/0.8 mL. Please refer to the FDA-approved package insert for filgrastim for product information, extensive preparation instructions, and a comprehensive list of adverse events.

STORAGE & STABILITY

Intact vials and prefilled syringes should be stored in the refrigerator at 2-8° Centigrade (36-46° Fahrenheit). Do not freeze.

ADMINISTRATION

Filgrastim will be administered as a subcutaneous injection. In both arms, the daily dose will be 480 mcg.

In Arm A (CHOP-R), filgrastim (or pegfilgrastim) will be administered after subsequent cycles following the occurrence of grade 4 neutropenia or febrile neutropenia. Filgrastim will be given daily from Day 2 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 2-11) if the ANC is not being monitored.

In Arm B (EPOCH-R), filgrastim will be given daily from Day 6 until ANC > 5000 after the nadir (nadir usually between Days 10-12) or for 10 days (Days 6-15) if the ANC is not being monitored, **in all cycles.**

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.

10.8 Pegfilgrastim (Neulasta®)

AVAILABILITY

Pegfilgrastim is commercially available prefilled single-dose syringes containing 6 mg/0.6 mL of pegfilgrastim (10 mg/mL). Please refer to the FDA-approved package insert for pegfilgrastim for product information, extensive preparation instructions, and a comprehensive list of adverse events.

STORAGE & STABILITY

Intact syringes should be stored under refrigeration and protected from light. Pegfilgrastim syringes are reportedly stable at room temperature for up to 48 hours prior to injection, if protected from light. Syringes that are frozen unintentionally may be allowed to thaw in the refrigerator. Pegfilgrastim syringes that have been frozen a second time should be discarded.

ADMINISTRATION

The dosage of pegfilgrastim is a single subcutaneous injection of 6 mg administered between 24 and 36 hours after the completion of the cyclophosphamide infusion. Pegfilgrastim will be administered as a subcutaneous injection. In Arm A, pegfilgrastim (or filgrastim) will be administered after subsequent cycles following the occurrence of grade 4 neutropenia or febrile neutropenia. Pegfilgrastim will be administered at a dose of 6 mg one time per cycle. If pegfilgrastim is given, the patient should not receive additional filgrastim.

Note: DA-EPOCH-R dosing has only been validated using filgrastim. Pegfilgrastim has different kinetics of neutrophil recovery and its use may adversely affect dosing and reduce disease cure rates. Use of pegfilgrastim must be approved by the Study Chair and will only be approved if the patient cannot receive filgrastim.

TOXICITY

The most common side effect associated with pegfilgrastim is bone pain. Bone pain is usually reported as mild or moderate, and, if necessary, may be treated with non-opioid or opioid analgesics.

Other reported side effects include reversible elevations in LDH, alkaline phosphatase, and uric acid. These laboratory abnormalities are not usually of clinical significance and do not require any intervention.

10.9 Methotrexate (MTX, Folex)

AVAILABILITY

Methotrexate is commercially available in 2 mL (25 mg/mL) vials or 20 mg, 25, mg, 50 mg, or 100 mg vials for reconstitution. Read the manufacturer's package labeling carefully for solution concentrations. Please refer to the agent's package insert for additional information.

STORAGE & STABILITY

Unopened vials of methotrexate should be stored at room temperature, and protected from light.

PREPARATION

For intrathecal injection: reconstitute to a concentration of 1-5 mg/mL with an appropriate sterile, preservative free medium such as Sodium Chloride Injection, USP.

ADMINISTRATION

In this study, methotrexate will be administered as an intrathecal injection of 12 mg. Methotrexate will be given to select patients in Arm A and Arm B, as described in Section 7.1.3 and 7.2.3.

TOXICITY

Toxicities associated with intrathecal administration: arachnoiditis, ataxia, coma, confusion, dementia, encephalopathy, headache, paresis, seizures.

DRUG INTERACTIONS

The use of NSAIDs, probenecid, salicylates, sulfonamides may prolong methotrexate levels and enhance toxicity.

11.0 ANCILLARY THERAPY

11.1 Supportive Care

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on CALGB Form C-260 (Remarks Addenda).

11.2 Hormones and Other Chemotherapeutic Agents

Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure or hormones administered for non-disease-related conditions (e.g., insulin for diabetes) Use of dexamethasone and other steroidal anti-emetics is prohibited in this protocol.

11.3 Epoetin

The use of epoetin is allowed at the discretion of the investigator. It is recommended that epoetin not be used in this protocol unless hemoglobin is ≤ 10 g/dL, and that it be held or the dose modified for hemoglobin > 12 g/dL.

12.0 Criteria For Response, Progression, And Relapse

R-CHOP patients will be re-evaluated after Cycles 4 and 6. DA-EPOCH-R patients will be re-evaluated after Cycles 4 and 6.

12.1 Definitions of Response [61]

12.1.1 Complete Response (CR)

- Complete disappearance of all detectable clinical and radiographic evidence of target lesions and disappearance of all disease-related symptoms if present prior to therapy, as well as normalization of those biochemical abnormalities (e.g., LDH, etc.) definitely assignable to DLBCL.
- All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm prior to therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter prior to treatment must have decreased to ≤ 1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of their greatest transverse diameters (SPD).
- The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. (No normal size can be specified, however, because of the difficulties in evaluating splenic and hepatic size.) Any macroscopic nodules in any organs detectable on imaging studies should no longer be present. Similarly, other organs considered to be enlarged prior to therapy due to involvement of lymphoma (i.e., kidneys, liver, etc.) must have decreased in size.
- If the bone marrow was involved by lymphoma prior to treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of same site.

12.1.2 Complete Response Unconfirmed (CRu)

Complete response/unconfirmed will include those patients who have met the criteria in Section 12.1.1 bullet points 1 and 3, but with one or more of the following:

- A residual node > 1.5 cm in greatest transverse diameter that has regressed more than 75% in the product of its diameters. Individual nodes that were previously confluent must have regressed more than 75% in the product of their diameters compared with the size of the original mass. **PET is recommended at the end of treatment in patients with CRu. If positive, follow with repeat PET or biopsy.**
- Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

12.1.3 Partial Response (PR)

- A decrease of ≥ 50% in the SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved. **PET is recommended at the end of treatment in patients with PR. If positive, follow with repeat PET or biopsy.**
- No increase in the size of other nodes, liver, or spleen.
- Splenic and hepatic nodules must regress by at least 50% in SPD.
- With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.
- Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive the cell type should be specified in the report, e.g., large-cell lymphoma.
- No new sites of disease.

12.1.4 Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest SPD since the treatment started.

12.1.5 Progression (PD) or Relapse

- ≥ 50% increase from nadir in the SPD of any previously identified abnormal node for PRs or nonresponders.
- Appearance of any new lesion during or after completion of therapy.
- PET+ is not a criterion for progressive disease. Patients only with PET+ findings must have evidence of progression on CT or biopsy proven.

12.2 Response Assessment Table

Response Category	Lymph Nodes	Splenic or Extranodal Sites	Bone Marrow
CR	Normal size	Negative	Negative for tumor
CRu	> 75% decrease and PET negative. If PET equivocal, observe or biopsy. PET + is not a criterion for treatment failure.	Measurable sites > 75% decrease and PET negative. If PET equivocal, observe or biopsy. PET + is not a criterion for treatment failure. Non-measurable (e.g. bone), MRI and CT improved and PET negative.	Negative for tumor
PR	50-75% decrease or CRu with PET positive or biopsy positive.	Measurable sites 50-75% decrease or CRu with PET positive or biopsy positive.	If positive for tumor, must have PR in measurable disease sites.
SD	No Change (Section 12.1.4)	No change (Section 12.1.4)	No change (Section 12.1.4)
PD/Relapse	≥ 50% increase from nadir or new site. PET + alone is not a criterion for treatment failure.	Measurable sites ≥ 50% increase from nadir or new site. Non-measurable (e.g. bone), evidence of progressive worsening on MRI/CT and PET positive. Strongly suggest biopsy.	Newly positive for tumor.

12.3 Criteria for FDG-PET Response

12.3.1 Primary Endpoint: IWG Criteria + FDG-PET Response

The primary FDG-PET response endpoint is defined by visual reading of FDG uptake after Cycle 6 of chemotherapy. For responders, tumor FDG uptake is ≤ ABP FDG uptake in all tumors, and no new avid lesions are seen.

In a recent study, 54 patients with aggressive NHL underwent CT and PET exams 1-16 weeks after completing four to eight cycles of CHOP therapy. Each patient was assessed for one of the following using the standard IWG criteria or the IWG criteria with PET: a complete response (CR), an unconfirmed complete response (CRu), a partial response (PR), stable disease (SD), and progressive disease (PD). Progression-free survival was also compared between IWG criteria and IWG criteria + PET [70].

The study found that with IWG alone, 17 patients had a CR, seven had a CRu, 19 had a PR, nine had SD, and two had PD. By comparison, IWG + PET produced a significantly higher response rate: 35 patients had a CR, 12 had a PR, six had SD, one had PD, and zero had CRu. Additionally, extensive analysis revealed that when the response rates were measured by IWG criteria alone and compared to IWG criteria + PET, the IWG + PET assessments provided a more accurate response classification in patients with aggressive NHL.

For this imaging companion study, the FDG-PET endpoint will be combined with IWG criteria to further assess and refine the improved response classification seen in the 54-patient study.

12.3.2 Secondary Endpoints

As secondary endpoints, metabolic response after Cycles 6 and 2 is defined by decreases in tumor SUV in the index tumors. In responders after Cycle 6, the ratio of tumor SUVmax to ABP SUVmean is ≤ 1.25 in all index tumors, and no FDG-PET uptake greater than background is seen in any other lesion. In responders after Cycle 2, tumor SUVmax is decreased ≥ 85% from baseline for index tumors, or the ratio of tumor SUVmax to ABP SUVmean is ≤ 1.75 in all index tumors, and no new avid lesions are seen.

Because of uncertainty regarding the optimal values of tumor SUVmax decrement and tumor SUVmax to ABP SUVmean ratio, ROC curves will be calculated to determine the best values of these measurements as correlates of PFS and other measurements of clinical outcome.

13.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

13.1 Duration of Treatment

13.1.1 CR/CRu & PR: Complete all required treatment cycles.

13.1.2 Disease Progression

Remove from protocol therapy any patient with rapid disease progression during treatment. PET positivity is not a criterion for treatment failure. Document details, including tumor measurements, on CALGB Form C-260 (Remarks Addenda). Patients with isolated disease progression in the CNS without evidence of systemic progression will remain on study and receive CNS directed treatment.

13.2 Extraordinary Medical Circumstances

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.

- Document the reason(s) for discontinuation of therapy on CALGB Form C-260 (Remarks Addenda).
- Continue to follow the patient for response, progression, toxicity, survival and secondary malignancy, as indicated in Section 5.2.

14.0 STATISTICAL CONSIDERATIONS

14.1 Clinical Endpoints

14.1.1 Sample Size and Power Estimates

The primary clinical endpoint will be event-free survival (EFS) measured from randomization until documented progression or death from any cause. Sample size is determined based on this endpoint. Patients will be randomized with equal probability to treatment with R-CHOP or DA-EPOCH-R. Patients will be stratified according to the International Prognostic Index.

Fifty-five percent (55%) of patients treated with R-CHOP are expected to be event-free at three years. This corresponds under exponential survival to median EFS of 3.5 years. With 430 patients (96 patients enrolled over 4.5 years and followed for 3 years) a hazard ratio (HR) of 1.53 for R-CHOP versus DA-EPOCH-R is detectable with 90% power (2-sided logrank test, α=0.05). Two hundred forty-two (242) events are expected at the time of the final analysis. The targeted sample size will be inflated to 478 patients to allow for a 10% pathologic ineligibility rate. Accrual is expected to be approximately 106 patients per year (approximately 96 eligible). Analysis will be "intent-to-treat" as randomized for eligible patients (i.e., with an histologic diagnosis of diffuse large B cell lymphoma).

As of protocol update #14, the following change has been made:

Sample Size Increase: Given the fewer than predicted PET scans and biopsies on 50303, accrual will be extended to 45 additional patients, for a total of 523 (=478+45) patients. Extended accrual will be limited to institutions and patients participating the required imaging companion study CALGB 580603.

As of protocol update #17, the following change has been made:

Revised final analysis timing due to low event rate: At the protocol specified interim analysis at the time of the May 2015 Alliance DSMB meeting, the pooled event rate was much lower than that specified by the study design. The observed number of events at this time was 158 (based on protocol assumptions this would have been the time for final analysis with 242 events). In order to provide a timely final analysis, the final analysis plan was revised to reduce the study power from 90% to 80%. With 178 events, we have 80% power if the hazard ratio between the two arms is 1.53 as specified by the original design. Based on the estimated pooled event rates, the final data analysis is now expected in May 2016. By the current Kaplan-Meier estimate, there are no events after 6 years, so that we do not expect more events after 6 years of additional follow-up. So, the maximum number of events expected based on the current survival estimate is 194, which will occur about May 2019 (i.e. after 6 years of additional follow-up).

14.1.2 Secondary Analysis

Secondary endpoints will be response rate, overall survival (OS), and toxicity. Overall survival will be measured from randomization until death from any cause.

Different treatment effects are expected in different biological subgroups. In a secondary analysis, the two arms will be compared adjusting for the subgroups, ABC and GCB. The interactions between treatment and the subgroups will be tested using a Cox regression model. We will also conduct the log-rank test for the treatment effect within each subset.

14.2 Interim Analysis

14.2.1 Primary Endpoint

Ten interim analyses are planned, five during the accrual period and five during the follow-up period. Interim analyses for the primary endpoint of EFS will be conducted beginning when 18% of the expected total number of events (n=242) are observed. This should occur at approximately 2.5 years, during the accrual period. After this time, interim analyses will be conducted every six months corresponding with scheduled meetings of the Alliance Data and Safety Monitoring Board (DSMB). It is estimated that these analyses will occur when 0.18, 0.25, 0.33, 0.42, 0.52, 0.61, 0.70, 0.78, 0.86, and 0.93 of the expected total number of events have been observed. The Lan-DeMets analogue of the O'Brien-Fleming boundaries will be used (1-sided α=0.05) truncating at 3.5. These boundaries are as follows: 3.5, 3.5, 3.5, 3.28, 2.93, 2.70, 2.50, 2.40, 2.26, and 2.18. The final analysis will be conducted at the 2.10 boundary.

14.2.2 Futility

Futility testing will be conducted at the times of interim analyses. The futility statistics calculated for HR=1.53 will be compared with the critical values with 1-sided α=0.0025 at the first 5 interim analyses and with 1-sided α=0.005 at the following 5 interim analyses. This futility rule has about 86% of probability to reject DA-EPOCH-R when the two regimens have similar efficacy. If survival on one treatment arm is found to be superior at an interim analysis conducted during the accrual period and determination is made to curtail enrollment to the inferior treatment arm, enrollment will continue on the superior regimen until the targeted sample size of 215 patients is reached.

14.2.3 Toxicity

Toxicity data will be provided to the DSMB from the first meeting after patient accrual. If there exists significant evidence that one regimen has 10% or higher grade 5 toxicity from any cause at two-sided α=0.01 level, the study will be reviewed as grounds for amendment or early stopping. Grade 3 or 4 neutropenia/thrombocytopenia themselves should not be grounds for stopping the trial as they are goals of EPOCH-R to give the maximum deliverable doses of the chemotherapy by escalating with each course. However, if there are serious sequelae, such as an increase in hospitalizations due to toxicities, serious/life-threatening infections (pneumonia, sepsis, or bleeding, in excess of 20% in one regimen over the other at two-sided α=0.01 level), then the study will be reviewed for amendment or early stopping.

14.2.4 Accrual Monitoring

Accrual to this Phase III trial will be monitored according to the NCI guidelines. In addition, the proportion of eligible patients with usable biopsies will be monitored. If at any time the proportion of patients with usable biopsies falls significantly below the assumed value of 0.75, consideration will be given to further modifying eligibility requirements or to increasing the targeted sample size.

14.3 Molecular Characterization Studies

The Staudt laboratory has recently profiled 197 DLBCLs using Affymetrix U133 A and B arrays, and this dataset has enabled us to perform power calculations to estimate the number of DLBCL tumors that we would need to analyze in order to achieve the molecular profiling goals of the trial.

An endpoint of the trial is to test whether the gene expression signatures that were previously associated with survival following CHOP therapy [62] are associated with survival in either of the treatment arms of the prospective trial. The four gene expression signatures that were found to be correlated with survival were the germinal center B cell signature, the proliferation

signature, the MHC class II signature and the lymph node signature [44]. These gene expression signatures were identified in the Affymetrix data set and their associations with survival are shown in Table 1:

Signature	Survival association relative risk per 2-fold change in expression	# events/arm needed to detect survival association (p<0.05) with 90% power	# of patients required/arm assuming75% biopsy rate and 30% event rate	Power to detect survival assocation assuming 215 patients/arm
Germinal Center B Cell	0.36	27	119	0.99
Proliferation	0.54	28	123	0.99
MHC class II	3.42	37	163	0.96
Lymph node	0.53	43	189	0.93
GCB vs ABC DLBCL	1.54	56	248	0.86

Table 1 shows the number of biopsies that we would need to be profiled in order to detect a significant association of these signatures with survival ($p<0.05$) in a new set of DLBCL patients with 90% power. These numbers were used to calculate how many eligible patients would need to be enrolled in the trial in order to detect these associations in each treatment arm assuming a 75% success rate in obtaining biopsies and a 30% event rate. In addition, the survival difference between the GCB and ABC DLBCL subgroups in response to CHOP treatment [62,63] was used to calculate the number of biopsies that would be needed in order to observe the association with survival ($p<0.05$) with 90% power in the prospective clinical trial. As can be seen from this Table, a trial size of 430 eligible patients would allow all of these prognostic features to be evaluated in each treatment arm of the prospective trial with at least 86% power. A second goal of the trial is to develop new molecular predictors of survival for both of the therapeutic regimens being evaluated. Previous gene expression profiling in DLBCL involved patients treated with CHOP chemotherapy alone. Both therapeutic regimens being evaluated in the proposed prospective trial differ from CHOP chemotherapy in important respects: one regimen adds rituximab to CHOP and the other regimen adds etoposide to CHOP and is delivered by continuous infusion using a dose escalation scheme. Therefore, the previous predictors of survival following CHOP chemotherapy may not necessarily apply to these new regimens.

The survival data from the proposed trial will be used to discover and validate new molecular predictors of survival for each therapeutic regimen. For such an analysis, both arms of the trial will be divided into a training set and a validation set of cases, as was done previously [62]. The training set will be used to discover associations between gene expression and survival. The validation set will be used to test the prognostic models that are generated using the training set data. Using this procedure, the danger of over fitting the statistical models of survival to the data will be avoided.

We will not know beforehand which gene expression signatures will be associated with survival in each arm. However, we can model the discovery of such signatures and their validation based on our experience with molecular profiling in the context of CHOP chemotherapy. Let us assume that we wish to discover and validate a new gene expression signature that is associated with survival to the same degree as the proliferation signature. To discover an association of this magnitude with a statistical significance of $p=0.01$ and with 90% power, we would need to include 113 tumor biopsies in the training set. A p-value cutoff of 0.01 was chosen for the discovery phase based on our previous experience in creating a gene expression-based outcome

predictor for DLBCL [62]. At this significance cutoff, all of the gene expression signatures in Table 1 were identified as associated with survival, but spurious associations between gene expression and survival were largely avoided. In the validation phase, we wish to verify the association of a gene expression signature with survival ($p<0.05$) with 80% power. It is expected that multiple gene expression signatures will be discovered in the training set that influence survival. Therefore, a power of 80% will insure that most of these univariate predictors of survival will be validated within this prospective trial. Using the example of the proliferation signature, we would need to include 47 tumor biopsies in the validation set. Based on these modeling assumptions, 160 biopsies would need to be profiled in each arm of the clinical trial. This translates into a total trial accrual of 426, approximately 430 eligible patients, assuming a 75% biopsy success rate.

In summary, an accrual of 349 eligible patients to the prospective trial would be required to test the importance of previously identified molecular predictors of outcome in DLBCL. An accrual of approximately 430 eligible patients would be required to discover and validate new molecular predictors of survival for both treatment regimens in the prospective trial.

The intent of the genomic characterization is exploratory, aiming to identify somatic DNA mutations, copy number alterations, microRNA expression patterns, and DNA methylation events that are associated with biological or clinical attributes of the DLBCL tumors. Recurrent genomic abnormalities that are present in at least 5% of patients will be identified and prioritized for analysis. DLBCL cases will be divided into two subgroups based on the presence or absence of the abnormality.

The data will be preprocessed for statistical analysis using the computing software appropriate for the used technology platforms. Associations with progression-free and overall survival will be assessed using the Kaplan-Meyer method. A Fisher's exact test will be used to determine the association between these two patient subgroups and components of the International Prognostic Index (IPI) as categorical variables (age > 60; ECOG performance status > 1; Ann Arbor stage > II; Number of extranodal sites > 1; LDH > 1X normal) as well as the IPI score (low (0-1), intermediate (2-3), high (4-5)). A Fisher's exact test will also be used to determine the association between genomic abnormalities and previously defined molecular subtypes of DLBCL (ABC vs. GCB subtypes).

Joint prediction model will be developed using the gradient lasso method which provides variable selection and prediction model fitting [95, 96]. The developed prediction models will be validated using a cross-validation method (e.g. 10-fold cross validation). The possible over-fitting bias in the cross-validation result will be adjusted by using permutation method. Kaplan-Meier curves will be estimated for the high- and low-risk groups as defined by the fitted prediction model and the IPI score groups and the log-rank test will be applied to compare the survival distributions among the groups to investigate if the discovered genomic markers are independent prognostic factors for PFS and OS.

14.4 Study Size Requirements to Achieve Pharmacogenetic Goals

The primary objective of the pharmacogenetic portion of this study is the assessment of the discrepancy in event free survival (EFS), within both treatment arms, between the two genotypic groups (Group 1 and Group 2). For notational simplicity, let us denote individuals with *ABCB1* 3454 CC genotypes by group 1 and individuals with CT or TT genotypes as group 2. The hypotheses of interest can be canonically presented as testing H_0: $\Delta=1$ versus H_1: $\Delta>1$, where Δ is the hazard ratio between group 1 and group 2 within an arm.

Assuming that within each arm, the EFS in both groups follow exponential laws, with potentially different means, the hazard ratio is given by $\Delta_i=M_{i,2}/M_{i,1}$, where $M_{i,1}$ and $M_{i,2}$ denote median EFS for groups 1 and 2 in treatment arm i (1= R-CHOP and 2= DA-EPOCH-R) respectively.

Out of the 430 eligible patients accrued to the study, we expect to have about 344 (roughly 80%), which corresponds to n=172 per treatment arm, available for pharmacogenetic sampling. The putative prevalence rates for groups 1 and 2 are 0.30 and 0.70 respectively [64]. This corresponds to n_1=52 and n_2=120 patients accrued to groups 1 and 2 in each arm.

Needless to say, given the small sample size only large discrepancies (as quantified by the hazard ratio, will be detectable with large power. The accrual and follow-up periods are 4.5 and 3 years respectively. Under the given setup, we will consider the power of two-sided log-rank test, assuming exponential survival for both groups, for testing H_0: Δ_i=1 versus the local alternative H_1: Δ_i=Δ, for Δ>1, at a level of significance of α=0.025 (as a crude adjustment for testing the hypothesis for each of the two arms, we have used 0.05/2), for various choices of $M_{1,i}$ and Δ. Table 2 illustrates, for $M_{1,i}$ ε (2.5,3.0,3.5,4.) years, the smallest hazard ratio Δ that is detectable with at least a power of 0.6 and 0.7:

Table 2 Power analysis of pharmacogenetic studies

$M_{1,i}$	ß	$M_{2,i}$	Power
2.5	1.66	4.12	0.60
2.5	1.76	4.40	0.70
3.0	1.70	5.10	0.60
3.0	1.81	5.43	0.70
3.5	1.75	6.13	0.60
3.5	1.87	6.55	0.70
4.0	1.80	7.20	0.60
4.0	1.93	7.72	0.70

Secondary Objectives: We will also look at the above questions in the context of other genotypes in additional candidate genes of putative importance. The pharmacogenetic analysis related to these candidate genes will be conducted as a secondary exploratory analysis of gene-toxicity, gene-response, and gene-survival relationships. The association between genes implicated in metabolism, transporter and clinical phenotype (systemic toxicity, response, and survival) will be performed by construction of hazard ratios or other appropriate non-parametric techniques.

14.5 Overview of FDG-PET/CT Study (CALGB 580603)

This is a multi-center, prospective study in which 170 patients who consent will undergo PET/CT scans at baseline, post-Cycle 2, and post-Cycle 6. Scans will be read without the baseline scan, and then again with the baseline scan and knowledge of other relevant clinical information. Response (both PET and CT) will be assessed by consensus readings made in the context of the baseline scan and knowledge of clinical data. To be classified as a responder, all index tumors of the patient must meet the criteria for response and no other evidence of lymphoma progression should be present. The accrual goal of 300 patients was changed to 170 patients because of an unexpectedly low accrual rate to CALGB 580603 among the patients who had entered into CALGB 50303 by November 2008. The revised sample size of 170 patients is based on the assumption that the FDG-PET study can accrue about 55% of patients that will ultimately accrue to CALGB 50303.

The primary focus of this FDG-PET/CT companion will be a comparison of the ability of PET plus IWG criteria after Cycle 6 to predict occurrence of an event or no event within three years to that of IWG criteria alone. An event is defined here as progression or death from any cause. Secondary endpoints include the predictive ability, sensitivity and specificity of PET after Cycle 2. Sensitivity and specificity describe the ability to distinguish between those who have and

those who do not have an event within three years. ROC curves will also be constructed as secondary endpoints for results that are on a continuous scale.

14.5.1 FDG-PET/CT Imaging Endpoints and Analyses

14.5.1.1 Test of Sensitivity and Specificity of PET + IWG Criteria vs IWG Criteria Alone

Let for a patient, D=1 if the event time is shorter than 3 years and D=0 otherwise, and X=1 if the patient is a non-responder by an imaging method (PET + IWG or IWG) and X=0 otherwise. Responders are those with a complete response (CR) or complete response unconfirmed (CRu). Sensitivity of the imaging is defined as $p=(X=1 \mid D=1)$ and specificity is defined as $q=(X=0 \mid D=0)$.

Response with PET + IWG criteria evaluated will be defined according to the criteria outlined by Juweid et al. [75]. The consensus read will be used as the primary assessment of response.

Only patients who have both PET and IWG criteria evaluated will be used for the analyses in this companion study. The study is designed to establish (1) non-inferiority of the sensitivity for PET + IWG criteria, and (2) the superiority of the specificity of PET + IWG criteria compared to that of IWG criteria alone.

14.5.1.2 Semi-Quantitative Measurements of FDG Uptake

The predictive ability and accuracy of SUV-based measurements will be estimated. Sensitivity/specificity and responder and non-responder predictive values, $P(D=0 \mid X=0)$ and $P(D=1 \mid X=1)$, respectively, will be estimated, with response defined as the ratio of tumor SUVmax to ABP SUVmean for all index tumors ≤ 1.25 (and no evidence of lymphoma by visual reading of total scan). ROC curves will be estimated using SUVmax decrement and the tumor SUVmax ratio to ABP SUVmean ratio.

14.5.1.3 Accuracy of Cycle 2 PET Scan

The predictive ability and accuracy of SUV-based measurements after Cycle 2 will be estimated. Here, response is defined as a tumor SUVmax decrease of $\geq 85\%$ from baseline or a ratio of tumor SUVmax to ABP SUVmean ≤ 1.75.

Chi-square test(s) will be conducted to determine if Cycle 2 PET/CT measurements can predict clinical response, and EFS and OS using a cutoff of 3 years. The log-rank test will be used to compare the raw data of EFS and OS between Cycle 2 PET+ and PET- patient groups.

Response at Cycle 2 will be compared to that at Cycle 6 and patient status at three years post-enrollment (i.e., progression free or not progression free at three years). ROC curves will also be estimated to compare Cycle 2 PET outcome with Cycle 6 PET outcome in terms of EFS at 3 years using the methods of Delong et al. [92] if there is no censoring within 3 years or Heagerty et al. [93] in the presence of censoring within 3 years. The correlation between Cycle 2 PET outcome and Cycle 6 PET outcome will be tested by using 2x2 Chi-square test(s) for binary variables and by using regression methods for continuous variables.

14.5.1.4 Evaluating Treatment Effect

Regression analysis will consider the effect of treatment on the predictive values. This will be evaluated with either regression models for predictive values [91] or with a Cox model. A multivariate analysis using a Cox regression model will be conducted

including PET + IWG criteria, IWG criteria, treatment, and other predictors to see if any of the imaging criteria are independent predictors.

14.5.1.5 Evaluating Inter-Institutional Reproducibility of PET/CT Measurements

Concordance rate will be estimated between the reading from each institution and that from the Core Lab. The variance of the estimated concordance rate will be adjusted for possible clustering effect among different institutions (e.g., Jung and Ahn) [94].

14.5.2 Sample Size Considerations

A total of m=170 patients will be accrued to this FDG-PET/CT companion study. The addition of PET to IWG is expected to improve the specificity, but not the sensitivity. So, the power analyses below are based on the paired two-sample tests for (1) the non-inferiority of PET + IWG in sensitivity, H_0:p_1=p_2-□ versus H_1:p_1>p_2-□, and (2) the superiority of PET + IWG in specificity, H_0:q_1=q_2 versus H_1:q_1>q_2. Here, p_1 and p_2 (q_1 and q_2) are the sensitivities (specificities) of PET + IWG and IWG, respectively. We assume that a difference of □=0.1 or smaller in sensitivity is considered to be within the non-inferiority limit.

Paired two-sample tests are used for the superiority and non-inferiority tests. For example, for the patient i(=1,…,m) whose event time is shorter than 3 years (D_i=1), the non-inferiority test rejects H_0:p_1=p_2-□ if $m^{1/2}(P_1-P_2)/S$ is larger than $z_{1-\alpha}$, where P_k is the sample estimate of p_k and S^2 is the mean sum of squares of X_{1i}-X_{2i}+ □. The power of these tests depends on the correlation coefficient between X_{1i} and X_{2i}. Preliminary data are based on Juweid et al. [75]. The relevant tables are provided below:

Three-year EFS tables

Progression or death by year 3 (D=1):

		IWG Criteria	
		Non-responder	Responder
PET + IWG Criteria	Non-responder	13	1
	Responder	2	4

No progression (or death) by year 3 (D=0):

		IWG Criteria	
		Non-responder	Responder
PET + IWG Criteria	Non-responder	4	1
	Responder	11	18

The estimated sensitivities are P_1=0.7 and P_2=0.75 from the first table (D=1), and the estimated specificities are Q_1=0.85 and Q_2=0.56 from the second table (D=0). From both tables, the correlation coefficient between X_{1i} and X_{2i} is about 0.3. Assuming 55% are event free at 3 years, about 77 of N=170 patients will be in the D=1 group and about 93 will be in the D=0 group. In the following power calculations, we assume one-sided α=0.05 and a correlation coefficient of 0.3. With 76 patients in the D=1 group, the non-inferiority test of PET + IWG in sensitivity has 55% power to detect an alternative hypothesis of H_1:p_1=p_2=0.75, and 84% power to detect H_1:p_1=0.8, p_2=0.75. Note that, because of the small

sample size, the non-inferiority test will not have enough power unless PET + IWG has a larger sensitivity than IWG (i.e. $p_1>p_2$).

With 93 patients in the D=0 group, the superiority test of PET + IWG in specificity has 82% power to detect an alternative hypothesis of H_1:q_1=0.7, q_2=0.55, and 93% power to detect H_1:q_1=0.75, q_2=0.55.

15.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site [redacted] All reactions determined to be "reportable" in an expedited manner must be reported using the CTEP Adverse Event Reporting System (CTEP-AERS). Reporting of cases of secondary AML/MDS should be done using CTEP-AERS. New primary malignancies should be reported using Study Form C-1001.

The Alliance requires investigators to route all adverse event reports (AERs) through the Central Office for Alliance-coordinated studies.

CALGB 50303 Adverse Event Reporting Requirements

Phase 2 and 3 Trials: CTEP-AERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of Treatment

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	With hospitali-zation	Without hospitali-zation	With hospitali-zation	Without hospitali-zation	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hrs; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment require reporting as follows:**

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:

- "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
- "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Additional Instructions or Exclusions to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials:

- Grade 3 or 4 myelosuppression and hospitalization resulting from grade 3 or 4 myelosuppression do not require CTEP-AERS, but should be submitted as part of study results.
- Grade 3 constipation and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- Grade 3 sensory or motor neuropathy and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- Grade 3 nausea or vomiting and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- A list of specific expected adverse events can be found in Section 10.0 (Drug Formulation, Availability, and Preparation).
- For the purposes of expedited adverse event reporting, the CAEPR for rituximab may be found in Section 15.1 below.
- Exacerbation or reactivation of serious viral infections (e.g., hepatitis, JC) as described in Section 10.10 should be reported via CTEP-AERS within 10 calendar days of the investigator learning of the event.
- CTEP-AERS reports are to be submitted electronically [redacted]
- The reporting of adverse events described in the table above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see Section 5.2 for required CALGB forms).
- All adverse events reported via CTEP-AERS (i.e., serious adverse events) should also be forwarded to your local IRB.

15.1 Comprehensive Adverse Events and Potential Risks List (CAEPR) for Rituximab (NSC 687451)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' [redacted] for further clarification. *Frequency is provided based on 986 patients.* Below is the CAEPR for Rituximab.

Version 2.1, March 19, 2010[1]

Adverse Events with Possible Relationship to Rituximab (CTCAE 4.0 Term) [n= 986]			**EXPECTED AEs FOR CTEP-AERS REPORTING Agent Specific Adverse Event List (ASAEL)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	***Expected***
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia***
	Blood and lymphatic system disorders - Other (Hyperviscosity: Waldenstrom's)		***Blood and lymphatic system disorders - Other (Hyperviscosity: Waldenstrom's)***
	Febrile neutropenia		***Febrile neutropenia***
CARDIAC DISORDERS			
	Myocardial infarction		***Myocardial infarction***
	Sinus tachycardia		***Sinus tachycardia***
	Supraventricular tachycardia		***Supraventricular tachycardia***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain***
	Diarrhea		***Diarrhea***
	Nausea		***Nausea***
	Vomiting		***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Chills			***Chills***
	Edema limbs		
	Fatigue		***Fatigue***
Fever			***Fever***
Infusion related reaction			***Infusion related reaction***
	Pain		
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction***
		Anaphylaxis	
	Serum sickness		***Serum sickness***
INFECTIONS AND INFESTATIONS			

	Infection[2]		***Infection[2]***
	Infections and infestations - Other (Activation of Hepatitis B, C, CMV, parvovirus B19, JC virus, varicella zoster, herpes simplex, West Nile virus)		***Infections and infestations - Other (Activation of Hepatitis B, C, CMV, parvovirus B19, JC virus, varicella zoster, herpes simplex, West Nile virus)***
	Infections and infestations - Other (Infection in HIV Positive Patients)		
INVESTIGATIONS			
Lymphocyte count decreased			***Lymphocyte count decreased***
	Neutrophil count decreased		***Neutrophil count decreased***
	Platelet count decreased		***Platelet count decreased***
	White blood cell decreased		***White blood cell decreased***
METABOLISM AND NUTRITION DISORDERS			
	Hyperglycemia		
	Hypocalcemia		***Hypocalcemia***
	Hypokalemia		
		Tumor lysis syndrome	***Tumor lysis syndrome***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia***
	Back pain		
	Myalgia		***Myalgia***
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Tumor pain		***Tumor pain***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness***
	Headache		***Headache***
	Lethargy		
		Nervous system disorders - Other (progressive multifocal leukoencephalopathy)	
	Seizure		***Seizure***
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome	
	Allergic rhinitis		
	Bronchospasm		***Bronchospasm***
	Cough		***Cough***
	Dyspnea		***Dyspnea***
	Hypoxia		***Hypoxia***
	Pneumonitis		***Pneumonitis***
	Sore throat		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			

		Erythema multiforme	***Erythema multiforme***
	Hyperhidrosis		***Hyperhidrosis***
	Pruritus		***Pruritus***
	Rash maculo-papular		***Rash maculo-papular***
	Skin and subcutaneous tissue disorders - Other (angioedema)		
		Stevens-Johnson syndrome	***Stevens-Johnson syndrome***
		Toxic epidermal necrolysis	***Toxic epidermal necrolysis***
	Urticaria		***Urticaria***
VASCULAR DISORDERS			
	Flushing		***Flushing***
	Hypertension		***Hypertension***
	Hypotension		***Hypotension***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting [redacted] Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Gastrointestinal obstruction includes Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Obstruction gastric, Rectal obstruction, and Small intestinal obstruction under the GASTROINTESTINAL DISORDERS SOC.

[4]Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Bone marrow hypocellular; Hemolysis
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Cardiac disorders - Other (cyanosis); Left ventricular systolic dysfunction; Sinus bradycardia; Ventricular fibrillation
EYE DISORDERS - Conjunctivitis; Eye disorders - Other (ocular edema); Uveitis; Watering eyes
GASTROINTESTINAL DISORDERS - Constipation; Dyspepsia; Dysphagia; Gastrointestinal obstruction[3]; Gastrointestinal perforation[4]; Mucositis oral
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (Opportunistic infection associated with >=Grade 2 Lymphopenia)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture
INVESTIGATIONS - Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Cardiac troponin I increased; Cardiac troponin T increased; Creatinine increased; Investigations - Other (hyperphosphatemia); Investigations - Other (LDH increased); Weight loss
METABOLISM AND NUTRITION DISORDERS - Anorexia; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hyperuricemia; Hypoglycemia; Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis
NERVOUS SYSTEM DISORDERS - Nervous system disorders - Other (Cranial Neuropathy NOS); Peripheral motor neuropathy; Peripheral sensory neuropathy; Pyramidal tract syndrome; Reversible posterior leukoencephalopathy syndrome; Syncope
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Depression; Insomnia
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Epistaxis; Pharyngolaryngeal pain; Pleural effusion; Pulmonary edema; Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans)

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Skin and subcutaneous tissue disorders - Other (paraneoplastic pemphigus)
VASCULAR DISORDERS - Phlebitis; Thromboembolic event; Vasculitis

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

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APPENDIX I CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION:

To submit site registration documents:

[redacted]

For patient enrollments:

[redacted]

Hours: 9:00AM – 5:30PM Eastern Time, Monday–Friday (excluding holidays)

[Registrations received after 5:00PM ET will be handled the next business day. For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call [redacted] between 9:00AM and 5:30PM.]

Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:

[redacted]

Sites should submit Teleforms via Fax or Mail. See section 5.2 Data Submission Section for details on forms submission.

Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility or treatment related questions: Contact the CALGB Study Chair.

For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail:

CTSU General Information Line: [redacted] All calls and correspondence will be triaged to the appropriate CTSU representative.

The CTSU Web site is located at: [redacted]

Registration/Randomization

Prior to the recruitment of a patient for this study, investigators must be registered members of the CTSU. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU registered member Web site or by calling the PMB at [redacted] Monday through Friday between 8:30AM and 4:30PM Eastern time.

Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member Web site at [redacted].

All forms and documents associated with this study can be downloaded from the CALGB 50303 Web page on the CTSU registered member Web site [redacted] Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as "approved" in the CTSU RSS.

Requirements for CALGB 50303 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

Pre-study requirements for patient enrollment on CALGB 50303:

- Patient must meet all inclusion criteria, and no exclusion criteria should apply.
- Patient has signed and dated all applicable consents and authorization forms.
- All baseline laboratory tests and pre-study evaluations performed within the time period specified in the protocol and fresh (frozen) tumor biopsy must be available or attempted.
- As of protocol update #14, and starting August 1, 2012, registration to the companion study CALGB 580603 is required to register to the treatment study CALGB 50303, and will be performed at the same time as registration to the treatment study. Registration to CALGB 580603 must be done at time of patient enrollment. Sites must submit the institutional approval letter provided by the Imaging Core Laboratory to the CTSU with each patient enrollment for CALGB 50303 and 580603.
- As of protocol update #14, and starting August 1, 2012, all sites that enroll patients must be approved (or have previously been approved) by the Alliance Imaging Core Laboratory at the Ohio State University Medical Center. See the "Special Materials and Substudies" section below for further information on procedures.

CTSU PROCEDURES FOR PATIENT ENROLLMENT

1. Contact the CTSU Patient Registration Office by calling [redacted] between 9:00AM and 5:30PM Eastern Time, Monday-Friday. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g., within one hour, call the registrar cell phone at [redacted]
2. Complete the following forms:
   - CTSU Patient Enrollment Transmittal Form
   - CALGB 50303 Eligibility Checklist
   - CALGB Registration Worksheet (indicate participation on companion studies CALGB 60405 and CALGB 580603)
   - Copy of approval letter from the Alliance Imaging Core Laboratory for the FDG-PET/CT Imaging Companion
3. Fax these forms to the CTSU Patient Registrar at [redacted] between the hours of 9:00AM and 5:30PM, Mon-Fri, Eastern Time (excluding holidays); however, please be aware that registrations received after 5:00PM will be processed the next day. Registration is limited to the operating hours of the CALGB Registration Office (9:00AM – 5:00PM ET). The CTSU registrar will check the investigator and site information provided to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and follow-up with the site to resolve any discrepancies.
4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, within the confines of CALGB's registration hours. The CTSU registrar will access the CALGB's on-line registration system, to obtain assignment of treatment arm and assignment of a unique patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.

- Protocol treatment must begin within 7 days of randomization.

Registration to Companion Study (CALGB 60405)

- Sites must offer participation in the companion studies to all patients.
- Registration to the companion study CALGB 60405 for those patients who have agreed to participate, will be performed at the same time as registration to the treatment study. Although it is preferable that patients be registered to CALGB 60405 at the same time they are registered to CALGB 50303, registration to CALGB 60405 may occur up to 60 days following registration to the treatment trial.

Procedures for late enrollment on CALGB 60405 (pharmacogenomics companion):

- Submit CTSU Patient Enrollment transmittal form (with note indicating delayed registration to CALGB 60405 companion study).
- Submit revised CALGB 50303 Registration Worksheet (indicating patient consent for CALGB 60405).

Note: Although it is preferable that patients are registered to 60405 at the same time they are registered to CALGB 50303, registration to CALGB 60405 may occur up to 60 days following registration to the treatment trial.

DATA SUBMISSION AND RECONCILIATION

1. All case report forms (CRFs) and transmittals associated with this study must be downloaded from the C50303 Web page located on the CTSU registered member Web site [redacted]. Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.
2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the Alliance Statistics and Data Center (see contact table and Section 5.2 of protocol), unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.
3. The Alliance Statistics and Data Center will send (generally via e-mail but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the Alliance Statistics and Data Center (via postal mail or fax) and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP AMS account contact information current**. This will ensure timely communication between the clinical site and the Alliance Statistics and Data Center.

SPECIAL MATERIALS OR SUBSTUDIES

1. Mandatory Histologic Review (Protocol Section 5.3)
   - Fresh (frozen) tumor biopsy sample is mandatory.
   - Failure to submit a pathology block within 60 days of patient registration will be considered a major protocol violation.
   - Collect, prepare, and submit specimens as outlined in the protocol.
   - Do not send specimens, supporting clinical reports, or transmittals to the CTSU.
2. Specimen Collection for Correlatives (Protocol Section 5.4)
   - Pharmacogenomic Substudy, CALGB 60405, requires patient consent and separate registration.
   - Collect, prepare, and submit specimens as outlined in the protocol.
   - Do not send specimens, supporting clinical reports, or transmittals to the CTSU.
3. FDG-PET/CT Imaging (Protocol Section 8.0)
   - Imaging Substudy, CALGB 580603, requires separate registration
   - See instructions outlined in Protocol Section 8.0. regarding participation on this study. Sites must submit required documentation to the Imaging Core Laboratory and receive institutional approval.
   - Submit institutional approval letter for imaging substudy participation to the CTSU with each patient enrollment on CALGB 580603.
   - Do not send images to the CTSU.

SERIOUS ADVERSE EVENT (AE) REPORTING (SECTION 15.0)

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.
2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Expedited Report System (CTEP-AERS) from either the Adverse Events tab of the CTSU member home page (https://www.ctsu.org) or by selecting Adverse Event Reporting Forms from the document center drop down list on the protocol number Web page.
3. Do not send adverse event reports to the CTSU.
4. Secondary AML/MDS/ALL reporting: Report occurrence of secondary AAML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of CTEP-AERS. Submit the completed form and supporting documentation as outlined in the protocol.

DRUG PROCUREMENT (SECTION 10.0)

Commercial Agents: Doxorubicin, Etoposide, Vincristine, Cyclophosphamide, Prednisone, Rituximab, Filgrastim, Pegfilgrastim, Methotrexate

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in Section 10.0 of the protocol.
2. You may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the CALGB 50303 Web page.

REGULATORY AND MONITORING

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314, and HHS 45 CFR 46] and National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

Responsibility for assignment of the audit will be determined by the site's primary affiliation with a Cooperative Group or CTSU. For Group-aligned sites, the audit of a patient registered through CTSU will become the responsibility of the Group receiving credit for the enrollment. For CTSU Independent Clinical Research Sites (CICRS), the CTSU will coordinate the entire audit process.

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial.

Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTSU Operations Manual located on the CTSU Member Web site.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

The HIPAA Privacy Rule establishes the conditions under which protected health information may be used or disclosed by covered entities for research purposes. Research is defined in the Privacy Rule referenced in HHS 45 CFR 164.501. Templated language addressing NCI-U.S. HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU Web site.

The HIPAA Privacy Rule does not affect participants from outside the United States. Authorization to release Protected Health Information is NOT required from patients enrolled in clinical trials at non-U.S. sites.

Clinical Data System (CDS) Monitoring

This study will be monitored by the Clinical Data System (CDS-web). Cumulative CDS data will be submitted quarterly to CTEP by electronic means. The sponsoring Group fulfills this reporting obligation by electronically transmitting to CTEP the CDS data collected from the study-specific case report forms.

APPENDIX II CALGB 580603 PET/CT INSTRUMENT TECHNICAL SPECIFICATIONS FORM

(From ACRIN Credentialing Application, 2005)
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Institution: **Date:**

Institution Address:

Institution Contact Name and Telephone Number:

E-mail: **Fax:**

PET Center Name: **Date:**

PET Center Address:

PET Center Contact Name and Telephone Number:

E-mail: **Fax:**

Type of Scanner:
(e.g., GE Discovery ST, Siemens Biograph, Philips Gemini, GE Advance, Siemens/CTI ECAT, etc.)

Transmission Source:
(e.g., ^{68}GE-rods, ^{137}Cs-point, CT)

Method of Attenuation Correction:
(e.g., segmentation, subtraction of emission contribution to transmission scan, CTAC)

Routine QC Testing Performed

Daily:

Monthly:

Quarterly:

Yearly:

Other:

APPENDIX II
CALGB 580603 PET/CT INSTRUMENT TECHNICAL SPECIFICATIONS FORM
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UNIFORM PHANTOM SCAN INFORMATION

Phantom Length: **Phantom Diameter:**

Phantom Volume:

Radionuclide:

Scan Date: **Scan Time:** **Scan Duration:**

For Water-Fillable Phantoms, Provide the Following Data**:**

Assayed Activity in Syringe before injection: **mCi** **Time:**

Assayed Activity in Syringe after injection: **mCi** **Time:**

For Ge-68/Ga-68 Calibration Phantoms, Provide the Following Data**:**

Calibration Date: **Time:**

Calibration Activity: **mCi** **Time:**

Number of Pixels in ROI:	**Slice #**	**Average SUV in ROI[1]**	**Std Dev of SUV (if available)**
Report info for EVERY slice of the phantom	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____
	10	_____	_____
	11	_____	_____
	12	_____	_____
	13	_____	_____
	14	_____	_____
	15	_____	_____
	16	_____	_____
	17	_____	_____
	18	_____	_____
	19	_____	_____
	20	_____	_____

[1] A circular or elliptical region of interest (ROI) covering most of the interior of the phantom.

APPENDIX II

CALGB 580603 PET/CT INSTRUMENT TECHNICAL SPECIFICATIONS FORM
(From ACRIN Credentialing Application, 2005)

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FOR CALGB IMAGING CORE LABORTORY USE ONLY:

Phantom Images Review:

Date:
Comments:

☐ **Approved**

☐ **Disapproved**

_____ _____
Signature Date

APPENDIX II
CALGB 580603 PET/CT INSTRUMENT TECHNICAL SPECIFICATIONS FORM
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WHOLE BODY FDG-PET <u>TEST PATIENT #1</u>:

File Name: **Patient Height:** **Patient Weight:**

FDG Dose (mCi): **Dose Assay Time:**

Blood glucose prior to FDG administration (mg/dL):

Injection Time: **Start Time of Emission Scan:**

ACQUISITION No. of Bed Positions:

EMISSION TIME (MIN/BED): **TRANSMISSION TIME (MIN/BED):**

RECONSTRUCTION ALGORITHM: (e.g., 2D FBP, 2D OSEM, 3D FORE/OSEM, 3D RAMLA)	**PIXEL SIZE (mm)** X-Y: Z:

Scatter Correction Applied: ☐ **Yes** ☐ **No**

(Include algorithm type, e.g., model-based, tail-fit, Bergstrom):

Randoms Correction Applied: ☐ **Online** ☐ **Smoothed Randoms (offline)** **None** ☐

FOR CALGB IMAGING CORE LABORTORY USE ONLY:

<u>Test Patient #1</u> Review:

Date:
Comments:

☐ **Approved**

☐ **Disapproved**

_____ _____
Signature Date

APPENDIX II
CALGB 580603 PET/CT INSTRUMENT TECHNICAL SPECIFICATIONS FORM
(From ACRIN Credentialing Application, 2005)
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WHOLE BODY FDG-PET TEST PATIENT #2:

File Name: **Patient Height:** **Patient Weight:**

FDG Dose (mCi): **Dose Assay Time:**

Blood glucose prior to FDG administration (mg/dL):

Injection Time: **Start Time of Emission Scan:**

ACQUISITION No. of Bed Positions:

EMISSION TIME (MIN/BED): **TRANSMISSION TIME (MIN/BED):**

RECONSTRUCTION ALGORITHM: (e.g., 2D FBP, 2D OSEM, 3D FORE/OSEM, 3D RAMLA)	**PIXEL SIZE (mm)** X-Y: Z:

Scatter Correction Applied: ☐ **Yes** ☐ **No**

(Include algorithm type, e.g., model-based, tail-fit, Bergstrom):

Randoms Correction Applied: ☐ **Online Smoothed Randoms (offline)** ☐ **None** ☐

FOR CALGB IMAGING CORE LABORTORY USE ONLY:

Test Patient #2 Review:

Date:
Comments:

☐ **Approved**

☐ **Disapproved**

_____ _____
Signature Date

APPENDIX III CALGB 580603 IMAGING SITE PERSONNEL FORM

Responsible CRA Contact Complete Address E-mail Phone Number Fax Number	**Radiology Department Contact** Complete Address E-mail Phone Number Fax Number

Please provide the information requested above. Provide the middle initial for individuals who commonly use them. Also, please add or correct the degree/title as necessary. This information will be retained by the Alliance Imaging Core Lab at IROC Ohio.

Once completed, you may **send this form to:**

[redacted]

Call the Imaging Core Laboratory at [redacted] with any questions. Thank you for your assistance.