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MATURE B-CELL LYMPHOMA AND LEUKEMIA STUDY III

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Protocol summary

SJBC3: MATURE B-CELL LYMPHOMA AND LEUKEMIA STUDY III

Principal Investigator: Raul C. Ribeiro, M.D.

IND holder: Not applicable, non-IND study. St. Jude is the study sponsor.

Brief overview: This is a phase II/III clinical trial using risk-adapted therapy. Treatment outcomes for children with B-cell NHL are excellent. Further improvements in outcome will likely be achieved through more focused study of the biology of the tumors and prospective studies of the late effects of treatment. Toward this end, this study features a spectrum of prospective biologic and late effect studies performed in patients treated with a modified regimen derived from the very successful LMB-96 regimen.

Intervention: Chemotherapy based on assignment of group (A, B, or C). St. Jude and Rady Children's Hospital San Diego are currently the centers participating in both therapeutic and biological portion of study. International and other external sites will submit biological specimens to St. Jude for research and banking, after obtaining consent locally and enrolling participants through St. Jude Clinical Trials Office (CTO).

Brief outline of treatment plan:

Overview - the chemotherapy regimen used varies with grouping based on extent of disease (see Appendix II for definition of Group A, B, and C)

Group	Pre-Phase	Induction X 2	Consolidation X 2	Continuation
A		COPAD		
B	COP	COPAD-M_3	CYM	
C	COP	COPAD-M_8	CYVE	Seq. No 1,2,3,4

Study design: Multicenter, phase III study utilizing risk-adapted multi-agent treatment.

Sample size: Target accrual for the biological studies is 68 evaluable tumor samples from several participating international institutions and for the late effects and outcome studies 100 newly diagnosed pediatric B-cell lymphoma patients treated at St. Jude.

Data management: Data management and statistical analysis will be provided by the Comprehensive Cancer Center clinical research staff (Leukemia/Lymphoma Division) and Biostatistics Department at St. Jude Children's Research Hospital.

Human subjects: The risks to participants will be related to the toxicity of multi-agent chemotherapy. Participants will be informed of this and other potential side effects during informed consent. Adverse events will be monitored and reported and treated appropriately.

Table of Contents

APPENDICES

1.0 OBJECTIVES

1.1 Biological Primary Aims

1.1.1 To perform genomic analysis of childhood newly diagnosed mature B cell lymphomas (e.g. Burkitt lymphoma/leukemia, DLBCL, and MLBCL) obtained from different parts of the world.

1.1.2 To describe the types and frequency of mutations in the ARF-HDM2-TP53 pathway, in B-cell lymphomas in the United States and those found in selected geographic regions of the world.

1.1.3 To describe the expression of ARF-HDM2-TP53 and PUMA-associated pathways in B-cell lymphomas in the United States and that found in B-cell lymphomas of other selected geographic regions of the world.

1.1.4 To describe the pattern and frequency of XLP gene mutations presenting with B-cell lymphomas in the United States and selected geographic regions.

1.1.5 To describe the frequency of EBV-positive B-cell lymphomas in the United States and selected geographical regions of the world.

- To describe the pattern of EBV protein and gene expression (e.g., EBNA 3) in EBV-positive lymphomas.
- To compare patterns of EBV protein and gene expression with clinical, laboratory and outcome data.

1.2 Exploratory Aims

1.2.1 Neuroimaging

1.2.1.1 In participants who develop acute, clinically symptomatic neurotoxicity during treatment:

- Obtain data, especially during the early acute phase of events in order to improve our understanding of the likely sequential pathophysiological processes in acute methotrexate-induced leukoencephalopathy using diffusion-based MRI surrogate biomarkers (ADC, FA) and other advanced MR techniques (MRS, DSC-perfusion MRI)

1.2.1.2 In participants who do not develop overt, clinically symptomatic neurotoxicity during treatment:

- Determine the incidence of measurable ADC and FA changes in white matter (in particular, in normal appearing white matter) in various brain regions during the course of treatment, and
- Describe the topographical and temporal predilections of subclinical white matter lesions in order to propose a clinically feasible screening strategy for future patients

receiving such treatment with the ultimate goal to develop preventive measures to reduce the long-term burden of neurotoxicity

1.2.1.3 To describe cerebral artery perfusion rates at baseline and at various time points during therapy.

1.2.2 Neuropsychology Late Effects

To establish the prevalence and type of neuropsychological sequelae associated with a regimen derived from the LMB-96 regimen, and to compare them with neuro-imaging findings.

1.2.3 Bone Mineral Density (BMD)

1.2.3.1 Prospectively estimate BMD at diagnosis and correlate with risk factors for potential BMD deficits in pediatric patients with NHL.

1.2.3.2 Prospectively estimate BMD at end of therapy and correlate with risk factors for potential BMD deficits in pediatric patients with NHL.

1.2.4 Fertility Late Effects

To establish the prevalence of gonadal and germ cell dysfunction in children treated with this regimen.

1.2.5 Cardiac Late Effects

To establish the prevalence and degree of cardiac toxicity associated with this regimen.

1.2.6 Tumor Bank

To establish a tumor bank of mature B-cell lymphoma samples for future molecular studies aimed to clarify the role of c-*MYC* oncogene and its associated pathways, and other pathways found to harbor genetic alterations from the genomic profiling studies described in 1.1.1.

1.2.7 To determine the feasibility of administration and toxicity profile of the SJBC3 protocol, which features the use of rituximab for selected higher-risk Group B and all Group C participants.

1.2.8 To describe the relationship between treatment outcome and clinical, biological, and radiological features (including day 7 PET).

1.2.9 To estimate the complete response rate, event-free survival, and overall survival rates in patients with Burkitt lymphoma (BL), Burkitt leukemia/B-cell acute leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL) treated with a stage-adapted regimen based on the SJBCII protocol.

2.0 BACKGROUND AND RATIONALE

2.1 Background

2.1.1 Genome-Wide Profiling of Genetic Alterations in Lymphoma

Genome-wide profiling of the gene expression and genetic alterations, including DNA copy number abnormalities (CNA) and loss of heterozygosity (LOH), and genome sequencing has provided valuable insights into the pathogenesis of lymphoid neoplasms.[1,2] Gene expression profiling has been found to be an effective tool in differentiating BL from other mature B-cell lymphomas like DLBCL in adults.[3,4] These studies have shown that some cases that cannot be classified unequivocally by classical morphological or cytogenetic modalities express a clear genetic signature that allows for their classification. Three major DNA microarray studies have looked into the biological and clinical heterogeneity of adult DLBCL.[5-7] The initial DLBCL gene expression profiling study used genes that define the germinal center stage of B-cell differentiation to identify 2 prominent DLBCL subgroups. The "germinal center B-cell-like" DLBCL (GCB DLBCL) expressed genes characteristic of normal germinal center B cells (e.g. CD 10, BCL6, A-myb), whereas the "activated B cells " DLBCL subgroup (ABC DLBCL) expressed genes that are induced during mitogenic activation of peripheral blood B cells (e.g. BCL2, IRF-4, cyclin D2).[5] These two different patterns of differentiation were associated with significantly different outcomes. Another larger gene expression profiling study of DLBCL cases confirmed the existence of these two subgroups as well as a third one called "type 3" that did not resemble either and may represent an additional subgroup.[6] Genome-wide profiling of CNA in DLBCL has identified multiple recurring regions of DNA gain and loss, many of which are associated with the DLBCL subtypes identified by gene expression profiling. These include amplification of a region of chromosome 19 harboring the SPIB transcription factor, deletion of *CDKN2A/B* and trisomy 3 in ABC DLBCL; and amplification of the mir-17-92 microRNA cluster and deletion of *PTEN* and GCB DLBCL. To date, there are limited genome-wide profiling data of genetic alterations pediatric B-NHL. Our aim is to perform integrated analysis of genomic data to identify genetic pathways that are altered in this disease, to identify tumor subtypes, to examine variation in genetic features according to geographic region, and to perform a comparative analysis with existing data from adult cases.[8,9]

Techniques for studying the genomic profile of patient tumors (somatic and germline) continue to advance. These approaches to be used, depending on sample availability and quality include RNA (transcriptome) sequencing, exome sequencing, single nucleotide polymorphism array analysis and whole genome sequencing. These will be done using standard established approaches in the Mullighan laboratory and the Genomic Sequencing Facility of the Hartwell Center at St. Jude.

2.1.2 ARF-HDM2-TP53 and PUMA

Burkitt lymphoma (BL) and mature B-cell leukemia (B-ALL) are characterized by one of three reciprocal chromosomal translocations [t(8;14);t(2;8);t(8;22)]. The unifying feature of these translocations is the juxtaposition of the c-*MYC* proto-oncogene and one of the immunoglobulin genes. These translocations result in the constitutive over-expression of c-*MYC*, a transcription factor which is necessary and sufficient for promoting cell proliferation and for provoking angiogenesis. In normal B-cells, c-*MYC* expression is tightly regulated and rapidly responds to

mitogenic or growth inhibitory signals. These controls are lost in BL/B-ALL, resulting in aberrantly high levels of c-*MYC* protein. The c-*MYC* translocations that occur in BL/B-ALL have been extensively studied with respect to their breakpoint patterns and their mechanism of de-regulation of c-*MYC* expression. However, recent insights into the molecular pathogenesis of BL/B-ALL have come from a detailed analysis of Eμ-*myc* transgenic mice, a model which accurately mimics the chromosomal abnormality and pathology of BL/B-ALL.[10] In normal cells, including B lymphocytes, c-*Myc* expression activates the Arf-Mdm2-p53 pathway resulting in apoptosis.[11-13] Arf (mouse $p19^{Arf}$/human $p14^{ARF}$) is encoded by an alternative reading frame of the *Ink4a* locus and limits lymphomagenesis by antagonizing Mdm2 (murine double minute-2; HDM2 in man), a negative regulator of the tumor suppressor p53.[14,15] The c-*Myc* transgenic animal studies have shown that 80% of the lymphomas that arise in these mice carry mutations affecting apoptotic checkpoints, from either deletions of *Ink4a*/*Arf* (25%), mutation of *p53* (30%) or overexpression of *Mdm2* (50%).[13,16-20] Although data suggest that one-third of human BL/B-ALL may have mutations of TP53[21,22] many of the studies performed have been anecdotal and/or limited to the study of BL/B-ALL cell lines.[23,24] Therefore a detailed study of alterations in this pathway is currently lacking in human mature B cell malignancies.

Although these studies established that c-*Myc* activates Arf and p53, and that functional loss of this pathway impairs *Myc*-induced apoptosis, *Myc* activation can still trigger cell death in the absence of *Arf* and/or *p53*.[13] Activation of c-*Myc* in normal B cells suppresses the expression of *Bcl-2* or *Bcl-*X_L, which are anti-apoptotic proteins that protect the cells from a variety of insults, including chemotherapeutic drugs. Importantly, an analysis of lymphomas arising in Eμ-*Myc* transgenic mice has established that this pathway is also bypassed during lymphoma development and that this also occurs at an equal frequency in tumors having alterations in the *ARF-Mdm2-p53* pathway.

Recent studies have shown that about one third of BL in humans harbor c-*MYC* mutations, and two recurrent mutant c-*MYC* alleles derived from BL have been shown to be able to evade the *p53* tumor surveillance network and promote rapid lymphoma development in mice.[25] Another possible mediator in the altered apoptotic pathways of BL is *PUMA* (p53-up-regulated modulator of apoptosis). *PUMA*, a pro-apoptotic *BH3*-only Bcl-2 family protein, is an essential mediator of p53- dependent and independent cell death,[26] and recent observations have indicated that 40% of primary BLs (n=19) fail to express detectable levels of *PUMA*, and that in some cases *PUMA* is inactivated by epigenetic silencing.[27]

Understanding the exact mechanism by which the apoptotic pathways are affected in these lymphomas will provide an opportunity for the development of novel therapies. The development of more rational therapies with a selective effect in specific tumor pathways while sparing normal tissue can have a great impact in the survival of cancer especially in the developing world, where children are at high risk of dying of the toxic effects of aggressive chemotherapy due to suboptimal hospital infrastructure.

2.1.3 Epstein-Barr Virus

Epstein–Barr virus (EBV) is a B lymphotropic herpes virus linked to several human diseases including B-cell malignancies.[28] Greater than 90% of the world's population is persistently infected with EBV. It is a ubiquitous γ-herpes virus that, upon primary infection, causes a brief

and confined replication of the virus at the site of infection in the immunocompetent host.[29] In industrialized societies, infection with EBV generally occurs later in life, compared to early childhood infection common in countries with limited resources. EBV is transmitted orally from oropharyngeal secretions of asymptomatic carriers and patients with infectious mononucleosis.[28] While EBV infects B lymphocytes early in the course of primary infection, it normally does not replicate in them, but rather establishes a latent infection characterized by the limited expression of a subset of latency-associated genes.[30] The latency genes consist of six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and LP) and three membrane proteins (LMPs 1, 2A and 2B).[31] There have been three different latency-gene expression programs described after EBV infection. In BL/B-ALL only a very limited repertoire of the latency-associated genes (EBNA-1, EBV-encoded RNA (EBER)-1 and EBER-2 and the *Bam*HI-A rightwards transcripts [BARTs]) are routinely expressed (Latency I) contributing to immune evasion.[32] The Latency II program includes additional expression of LMP1 and LMP2A/B, and is commonly observed in EBV-positive Hodgkin lymphoma, some types of T and NK-cell lymphomas and some cases of B-cell lymphoma.[33] Latency III is expressed in lymphoblastoid cell lines and newly infected B cells in vivo, and includes the entire array of EBV latency-associated genes. These cells would normally be very susceptible to killing by EBV-specific T-cells, and, therefore, this pattern of expression is only seen in the EBV-associated lymphoproliferative disease (EBV-LPD) associated with primary or secondary immunodeficiency.[33] The EBER RNAs are present in all previously described programs of EBV latency, and their presence within tumor samples is evidence of a latent EBV infection.[34]

The EBNA3 proteins (3A, 3B and 3C) are not normally expressed in classic endemic BL (eBL); however, recent studies have revealed a subset of eBLs (~ 15%) that express these EBV proteins as a consequence of a deletion in the EBNA2 gene.[35,36] These tumors are particularly resistant to apoptotic stimuli. Dr. Clare Sample's laboratory has determined that, in a *SCID* mouse model of tumorigenicity, these variant EBNA-3+ eBLs are particularly aggressive relative to the eBLs that do not carry the EBNA2 deletion (C. Sample, personal communication) and additional unpublished data suggests that the EBNA-3 proteins may contribute directly to proliferation of the tumor cells. Thus, it is important to determine whether the pattern of EBV gene expression is associated with biologic and clinical and outcome features.

EBV has not been shown to be directly involved in BL pathogenesis, but has rather been suggested to increase the target pool of cells susceptible to malignant transformation. However, an interesting study by Ruf, et. al has demonstrated that the malignant phenotype of a group I BL line requires the EBV genome, and that this is associated with EBV's ability to impair the c-*MYC* apoptotic program.[37] Therefore, at least in this scenario, malignant transformation requires EBV gene expression altering apoptotic pathways. These observations suggest that EBV may influence c-*MYC*'s ability to trigger apoptosis in BL and possibly other pathways as well. Hence, we propose to evaluate and correlate EBV gene expression with alterations in the ARF-Mdm-p53 and Bcl-2/Bcl-X apoptotic pathways.

There are two main clinical presentations of BL clustering in different geographic regions. The eBL, which affects younger children and often involves the jaw, is found mainly in equatorial Africa and New Guinea.[38-40] The majority of patients with eBL have been infected with malaria. By contrast, non-endemic or sporadic BL (sBL) occurs worldwide and mainly affects young adults. Both forms of BL differ in their association with EBV: eBL is almost always associated with EBV, whereas sBL has a more irregular association, ranging from 10% to

30%.[41] However, our knowledge of other clinical and biological forms of BL, particularly in developing countries outside of Africa, is very limited. A study examining EBV association with BL in temperate countries of South America, found a 50% EBV association with predominantly abdominal presentation,[42] suggesting an intermediate form. The pattern of EBV gene expression has not been determined in these BLs. The rate of South American patients with BL infected with malaria is extremely low. Studies in Northeastern Brazil and Northern Africa have shown about 85-90% positivity for EBV (like eBL), but have a predominant abdominal presentation of the tumors (90%) similar to sBL, suggesting that other still unknown genetic or environmental factors might modify the clinical presentation, and possibly response to therapy of BL in these regions.[43,44]

2.1.4 X-Linked Lymphoproliferative Syndrome

X-linked lymphoproliferative (XLP) syndrome is a sex-linked immunodeficiency syndrome. Boys with this syndrome are at increased risk for the development of B-cell lymphomas and fatal infectious mononucleosis. Most are diagnosed by the time they are 5 years of age, although some have gone undiagnosed until much later in life (i.e. 5th or 6th decade). There may be an associated hypogammaglobulinemia; however, there is generally not a significant history of infections. As a result, patients are usually undiagnosed until they develop a B-cell lymphoma or die from infectious mononucleosis. Currently, boys with newly diagnosed high grade B-cell NHL are not screened for the XLP gene mutation. Hence the actual frequency of these mutations in boys with primary high grade B-cell lymphoma is not known.

We have identified five boys with B-cell lymphoma who have subsequently been found to have an XLP gene mutation. These cases were only identified after their clinical course suggested the possibility of XLP. Now that we have an accurate and efficient assay to screen for the XLP gene mutations, we believe that boys with BL and other B-cell lymphomas should routinely be screened for XLP mutations. It is not known whether different XLP mutations result in the same clinical phenotype or have prognostic importance.

2.1.5 Treatment

Mature B cell lymphomas (e.g. Burkitt lymphoma/ leukemia, DLBCL, and MLBCL) are aggressive B-cell neoplasms that account for about 15% of cases of childhood cancer in the developed world but make up to 50% of all childhood cancers in some parts of Africa. Dramatic improvement in the treatment outcome for children with advanced stage BL has been achieved since design of the COMP regimen.[45-50] The St. Jude Total B regimen incorporated sequential high dose methotrexate and escalating continuous infusion Ara-C.[46] This resulted in excellent outcome for patients with stage III disease; however those with stage IV disease had only a 20% long term event free survival. The POG 8617 protocol, derived from the Total B regimen which incorporated early administration of high dose pulse Ara-C resulted in improved outcome for patients with stage IV disease. During this same time period, Magrath (NCI) reported an equally improved result for patients with advanced stage disease using a CHOP-HDMTX regimen (NCI-7704).[45] Further improvement in treatment outcome has been reported by Patte (SFOP),[51] and the BFM group.[49] These regimens incorporated the above mentioned agents (COMP, high-dose MTX, and high-dose Ara-C) and VP-16 and/or ifosfamide.

Patte et al, have reported excellent results for children with mature B-cell lymphomas using the LMB 89 regimen, with approximately 87% of B-ALL patients achieving long term event-free survival.[51,52] For patients with CNS disease, a group with a poorer prognosis, they reported 70% long term event-free survival. B-ALL and the BL are rapidly growing and are often associated with tumor lysis syndrome (including hyperuricemia). Carefully attention to the control of metabolic abnormalities during induction greatly reduced early mortality associated with initial treatment. Future directions with this treatment strategy should include efforts to reduce morbidity while improving outcome for children with CNS disease.

In light of the outstanding results reported by Patte et al,[51] in the SJBCII we adopted the LMB-89 regimen as a standard of care with the following 3 modifications: 1) deletion of cranial radiation (CR RT) for those with CNS disease at diagnosis, (2) placement of an Ommaya reservoir in patients with CNS disease at diagnosis, and (3) inclusion of G-CSF after myelosuppressive therapy. Treatment was tailored based on disease stage and degree of tumor resection. Group A included only those with completely resected limited stage disease. Group C included those with either >70% replacement of marrow with lymphoma cells or those with involvement of the central nervous system at diagnosis. Group B included all those not meeting criteria for Group A or C and represents the largest and most heterogeneous patient cohort. Treatment intensity was the greatest for Group C and mildest for Group A. There were 64 evaluable patients enrolled on study. Among these, 62 (~97%) have achieved a complete remission. There have been only 6 adverse events including relapse in 4 and induction failure in 2. Fifty-nine of 64 (92%) are alive and disease-free. The international LMB 96 study (CCG, SFOP, and UKCCG) randomized patients with Group B and C to standard LMB doses versus stepwise reduced doses. Efficacy data show that in group B patients the 3-year EFS are greater than 90% in all arms.[53] Thus, in Group B patients, non-escalation of cyclophosphamide in the second course of COPADM3 and deletion of the maintenance sequence, did not compromise the excellent outcome. In contrast, dose reduction for Group C patients was associated with a poorer outcome: 3-year EFS was 90% for full dose vs. only 79% for the dose reduced arm, respectively. Finally, group C CNS positive patients had an inferior outcome with 3-year EFS of 71% (vs. 87% for CNS negative group C patients).[54] One other group which is associated with a poorer treatment outcome is those patients with mediastinal large B-cell lymphoma (MLBCL). In the LMB-96 study, the 3 year EFS for these patients was ~70%, a finding consistent with other studies. It appears therefore that they require novel or more intensive therapy.

In summary, the intensity of conventional chemotherapy regimens for mature B-cell lymphomas is reaching maximal intensity for many of the patients. To further increase the intensity of current regimens may lead to unacceptable toxicities. The introduction of compounds with diverse mechanisms of action such as rituximab into the current armamentarium of mature B-cell malignancies is being studied in large randomized clinical trials. For this study we propose to use the backbone of LMB-96 study. Specifically, the cyclophosphamide dosing is not escalated in the second COPADM block and the maintenance sequence one is deleted in Group B patients. Patients with CNS positive Group C will be considered for intra-Ommaya chemotherapy administration. Higher-risk patients (e.g., those with MLBLC, Stage III with LDH $\geq$ 2 x ULN, and/or bone marrow/CNS involvement) will be eligible to receive rituximab in addition to protocol specified chemotherapy based on disease group.

The only currently available targeted drug for B-cell lymphomas is the anti-CD20 monoclonal antibody (rituximab) which has been approved for treatment of adult B-cell lymphomas.

Rituximab has also been used in various immune-related diseases such as immune thrombocytopenic purpura, myasthenia gravis, and rheumatoid arthritis. In transplant recipients it has been used for treatment of post-transplant lymphoproliferative disease, and prevention and treatment of acute rejection. There are few data on its use in children with relapsed CD20+ lymphomas. The most recent Children's Oncology Group (COG) front-line trial for B-cell lymphomas tested the feasibility of incorporating rituximab into Groups B and C of an LMB-96 based regimen. The Group B arm was completed uneventfully; however, the Group C arm had enrollment suspended to evaluate early infectious complications – this arm of the study is now being re-opened.

2.1.6 Amendment 3.0 update

Rituximab has long been known to be active in adult CD20+ B-cell lymphomas (low grade and high grade).[55,56] Initial case reports of its activity in pediatric high grade B-cell lymphomas were followed by clinical trials which examined both its activity and safety.[57-59] The BFM are conducting a trial that features a rituximab window (prior to receiving conventional B-cell chemotherapy) that demonstrates both activity and a reasonable/acceptable toxicity profile.[57] The COG performed pilot studies of rituximab incorporating it into both Group B and Group C LMB-based therapy.[58,59] This safety data were used in part to justify the current use of rituximab for Group B primary mediastinal large B-cell lymphomas (PMLBCL) in the current SJBC3 protocol. In an effort to determine whether the incorporation of rituximab would improve outcome for children with high grade mature B-cell lymphomas, the COG is currently conducting a randomized trial (ANHL1131) in which children with Stage III disease and an LDH $\geq$2x ULN (institutional adult value), stage IV or mature-B-cell leukemia (e.g., Burkitt leukemia) are randomized to receive (or not receive) 6 doses of rituximab (Groups B and C). This features the addition of two doses of rituximab during the two courses of induction therapy (COPADM; total of 4 doses) and one dose of rituximab during the two courses of consolidation therapy (CYM and CYVE for Groups B and C, respectively; total of 2 doses) for a total of 6 doses of rituximab. The oversight committee for the COG study recently advised that the randomization be stopped because of an apparent outcome advantage for those who received rituximab (please see attached memo). We would therefore like to expand the use of rituximab in SJBC3 to include these higher risk patients studied in the COG study. By way of clarification, our institutional adult upper limit of normal for LDH is 260; therefore, stage III patients with an LDH $\geq$ 520 will receive rituximab. Additionally, any patient with Group B disease who does not meet the criteria for rituximab incorporation but is found to have high risk disease as reflected in a poor COP response (<20% shrinkage; moved to Group C), will also have rituximab added to their Group C therapy (same schedule as other Group C patients) and any patient who is not already receiving rituximab but is found to have residual disease follow CYM #1 and moved to Group C, will receive rituximab with their two courses of CYVE therapy (one dose in each course as all Group C patients). We will also permit the incorporation of rituximab for other individual patients who in the judgment of the primary physician have unique clinical features which they believe put the patient at higher risk. We will include 6 doses of rituximab to some Group B participants (e.g., those with MLBLC, LDH $\geq$ 2 x ULN, and/or bone marrow involvement) and all Group C participants, with 4 total doses to be given during induction (COPADM) and two doses given during consolidation (CYM and CYVE for Groups B and C, respectively). This dosing schedule is already in place in SJBC3 for MLBCL. A challenge for future studies includes the identification of the 10% of patients at the time of diagnosis who are at increased risk to fail therapy. We have now started an exciting era where

the interest in targeted therapy is blooming; however, without an understanding of the exact pathways involved in lymphomagenesis we will be limited in our ability to specifically target therapy. Therefore, it is critical to study the various factors and pathways that influence tumor cell proliferation and death. St. Jude is positioned to unravel these pathways because of our basic science orientation and international connections. Hence, we will provide patients with the best treatment available (cure more than 90% of patients with acceptable toxicity) and investigate tumor biology in the process.

2.1.7 Psychological Testing

Psychological testing will be performed at baseline, one, three and five years post treatment. All selected measures have age-specific norms from large, representative standardization samples. Measures also have appropriately demonstrated reliability and validity. Given the longitudinal study design, measures were chosen with appropriate test-retest reliability and negligible practice effects for the proposed interval between testing time points. An effort was made to select measures that could assess the widest age range possible given the likely age distribution at presentation and longitudinal design. Nonetheless, no single intelligence measure exists that covers this entire age spectrum, such that a separate IQ measure is needed for children less than three years of age. Children less than three years of age will be administered the Mullen Scales of Early Learning.[60] Parent questionnaires will be the same for this age group as children older than 3 years of age to allow for some longitudinal analyses including this young group. See Appendix IV for a complete listing of test measures, assessed abilities, administration age range and estimated testing time for children.

A primary assessment goal was to select a battery that could be administered in a circumscribed period of time to reduce the time burden for children, their parents and the research team. The administration time for the test battery is 1-3 hours, dependent on the age of the child and the speed with which they can complete tasks. It is anticipated that the average administration time will be 2 hours. The maximum administration time of 3 hours is only expected for older children (i.e., 7-8 years of age) or children that are notably slow (e.g., unusually slow speed of cognitive and/or physical processing). Therefore, the administration length of the current battery readily lies within the standard length of typical clinical and research batteries. It is anticipated that parents will spend on average 45 minutes completing questionnaires while their child is being assessed in a separate room.

2.1.8 Transcranial Doppler Sonography

The chronic anemia of sickle cell disease is known to result in increases in intracranial arterial blood flow velocities relative to the normal population.[61] The use of transcranial Doppler sonography (TCD) is pivotal in the management of children with sickle cell anemia who are screened for abnormally high cerebral artery velocities that place them at high risk of stroke.[61-65] In such children, a first stroke can be prevented with initiation of prophylactic red blood cell transfusion.[66] TCD has also proven to be valuable in the assessment of a wide variety of other intracranial pathologies, including: the detection and follow-up of vasoconstriction caused by subarachnoid hemorrhage; demonstration of major intracranial vessel stenoses or occlusion; determination of brain death; demonstration of the nidus of arteriovascular malformations, and; assessment of cerebral hemodynamics following trauma, stroke, or migraine.[67-70] Components of the treatment are known to be associated with neurotoxicity (methotrexate, cytarabine,

ITMHA). In addition, the anemia induced by the lymphoma itself or by chemotherapy may result in abnormally elevated cerebral blood flow velocites, such is seen in children with sickle cell anemia. The value of TCD to monitor cerebral blood flow in cancer patients has not been investigated. In this study, we will obtain preliminary TCD data to gain cursory insight into cerebral blood flow velocities before, during and after therapy for childhood B-cell lymphoma. These abnormalities will be compared with neuropsychological abnormalities and with findings on MRI studies.

2.1.9 Neuroimaging

Based on our preliminary clinical observations, we hypothesize that conventional imaging findings may be quite unremarkable in the early acute stage of methotrexate-induced neurotoxicity. Therefore, diagnosis may heavily rely on diffusion-based MRI techniques.

In a patient with clinically symptomatic acute methotrexate neurotoxicity[71] we found that ADC and FA changes in affected white matter were probably decoupled on the front end. This suggests that distinct patho-mechanisms may be responsible for the initial, very massive, restriction of water diffusion within lesion areas and the subsequent, somewhat delayed FA changes.[72] Consequently, ADC changes may be an earlier and more sensitive indicator of methotrexate-induced neurotoxicity than FA.

Based on our observations we further speculate that methotrexate-induced leukoencephalopathy may be either partly or fully reversible, or irreversible; and that the magnitude, extent and temporal profile of ADC and FA changes may allow some prediction of outcome during the early phase of toxicity. Methotrexate-induced leukoencephalopathy may be clinically asymptomatic and therefore more frequent than believed in the past.[73,74] However, even if clinically asymptomatic during the course of treatment, sub-clinical but not fully reversible changes in structural and functional integrity of white matter (and fiber tract systems) may be responsible for late adverse effects on neurocognitive functions in patients cured from the primary disease.[75] Currently available diffusion-based MRI tools (DWI and DTI) provide robust and reliable surrogate biomarkers (ADC and FA) of tissue, in particular myelin quality and integrity,[76,77] hence have the potential to allow detection and quantitative characterization of clinically asymptomatic acute methotrexate-induced toxic leukoencephalopathy, and such data could be ultimately correlated with late adverse neurocognitive sequelae in cured patients.[78]

Abnormalities in MRI neuroimaging will be compared with neuropsychological abnormalities.

2.1.10 Bone Mineral Density (BMD)

Deficits in bone mineral density have been well-documented among childhood ALL survivors and arise from the interaction of multiple factors.[79-83] Approximately 10% of pediatric patients have BMD deficits at the time of diagnosis of ALL and over 67% develop such deficits during therapy.[84] After completion of therapy, BMD deficits have been reported in up to 63% of survivors of pediatric ALL,[80,85,86] associated with a 5-year cumulative incidence of fracture of 28% +/- 3%. Risk factors include male sex, age at diagnosis ≥9 years, and treatment with corticosteroids.[82] However, bone mineral density changes in B cell NHL patients have not been extensively studied. Therefore, we will prospectively study bone mineral density changes in this patient population.

Corticosteroids and cytostatic therapy are associated with diminished BMD.[87-90] Children, like postmenopausal women, are particularly at risk for steroid-induced osteoporosis because of their more rapid bone turnover.[91,92] The mechanisms of glucocorticoid-induced osteoporosis are multiple. The greatest impact is on the vertebral trabecular bone.[93,94] Glucocorticoids decrease osteoblastic replication, differentiation, and lifespan through inhibition of genes for type 1 collagen, osteocalcin, insulin-like growth factors, bone morphogenetic proteins, RANKL, and transforming growth factor β.[95] Glucocorticoids inhibit calcium absorption and prevent 1 α-hydroxylation of vitamin D in the kidney to form the active metabolite 1,25-dihydroxy Vitamin D, further impairing calcium metabolism. Glucocorticoids directly inhibit many hormones and factors important in calcium accretion of bone.[87-90,92,94,96-98] They inhibit the expression of the vitamin D receptor in bone and the production of osteocalcin (the major bone matrix protein) and decrease local production of cytokines (which normally inhibit bone resorption).[92,96,98] Glucocorticoids inhibit osteoblastic activity and reduce the renal resorption of calcium. This effect can result in hypercalciuria and, in turn, increase parathyroid hormone which further stimulates calcium loss from bone.[94]

In summary, to further understand the pathophysiology of BMD loss in children undergoing SJBC3 therapy, we will obtain serum calcium, serum magnesium, plasma 1, 25-dihydroxyvitamin D, spot urine collection for magnesium, Creatinine, calcium, and calcium/creatinine ratio. If the calcium:creatinine ratio exceeds 0.2, then a 24-hour urine collection will be obtained for creatinine, calcium, and calcium/creatinine ratio.

2.1.11 Anthracycline Cardiotoxicity

While the anthracyclines (e.g., doxorubicin, daunorubicin) have contributed significantly to childhood cancer survival, they confer an excess risk of asymptomatic left ventricular dysfunction, cardiomyopathy, congestive heart failure and death.[99-103] Anthracycline cardiotoxicity results in loss of cardiac myocytes, which impedes myocardial development.[104] Unfortunately, myocardial effects can be harmful, asymptomatic,[101-105] and progressive.[106] Cardiotoxicity has been reported at all dose levels, but the risk increases with higher cumulative doses, younger age at first exposure, time from exposure, and female sex.[100-103] As many as 5% of at-risk survivors will develop congestive heart failure 15 years after treatment.[100] The effects may be asymptomatic in up to 57% of survivors, becoming apparent only with other physiologic stressors such as infection or pregnancy.[101,103]

Children exposed to anthracycline agents are at increased risk for cardiac toxicity that may become more clinically significant as they age. Late-onset anthracycline toxicity (occurring more than 1 year after therapy) generally manifests as left ventricular dysfunction. Although subclinical cardiac changes after anthracycline therapy are commonly reported, symptomatic disease is seen only occasionally, particularly in children treated on protocols that proactively restrict anthracycline cumulative doses.[100,101] Because most at-risk patients are asymptomatic, ongoing monitoring for late cardiac complications is critical. At a minimum, a baseline electrocardiogram and echocardiogram is recommended after the completion of therapy, with serial follow-up and cardiac referral as indicated based on age at treatment and cumulative cardiotoxic exposures.[107]

Recommended Off Therapy Follow-Up for Late Cardiac Toxicity

Age at treatment	Frequency of echocardiographic screening by doxorubicin dose		
	Group A (120 mg/m^2)	Group B (120 mg/m^2)	Group C (240 mg/m^2)
< 1 year old	Every 2 years	Every 2 years	Every year
1 to 4 years	Every 5 years	Every 5 years	Every 2 years
≥ 5 years	Every 5 years	Every 5 years	Every 5 years

2.1.12 Gonadal and Reproductive Function

Among agents used for treatment of children with non-Hodgkin lymphoma, alkylating agents confer a dose-related risk of gonadal toxicity. Because of their faster cell division, male germ cells (and their supporting Sertoli cells) are more vulnerable to toxicity from cancer therapy than the testosterone-producing Leydig cells.[108] Leydig cell failure after radiotherapy is dose-dependent and inversely related to age at treatment. Thus, infertility is more common than androgen insufficiency after treatment for cancer. Treatment with combinations of alkylating agents or cumulative doses of cyclophosphamide exceeding 7.5 grams/m^2 increases the risk of germ cell dysfunction.[109-111] Contemporary treatment protocols for non-Hodgkin lymphoma attempt to restrict these exposures in an effort to preserve reproductive function. Androgen insufficiency as a result of chemotherapy related damage to Leydig cells occurs only occasionally. In fact, most males experience normal puberty and are capable of producing normal adult levels of testosterone. Appropriate screening for signs and symptoms of androgen insufficiency should be performed annually. The Children's Oncology Group (COG) Long-Term Follow-Up Guidelines also recommend baseline serum LH, FSH, and testosterone levels at age 14 and as clinically indicated in those patients with evidence of delayed puberty or androgen insufficiency.[107] In young men at risk for infertility, semen analysis remains the most accurate noninvasive method for evaluating germ cell function.

In contrast to male physiology, injury to the female germ cells results in both infertility and endocrine dysfunction.[108] Because females have a fixed number of primordial follicles at birth that steadily decline with age, the risk for therapy-related ovarian failure and infertility is directly related to age. Thus, pre-pubertal girls, having a greater reserve of follicles, are at lowest risk for these complications.[108] Female children and adolescents treated with the cumulative doses of alkylators prescribed in regimens for non-Hodgkin lymphoma would be at relatively low risk for both gonadal or germ cell dysfunction. Evaluation for gonadal failure and infertility centers on a good history (to identify irregular or absent menses, history of pregnancy, or difficulty conceiving) and an annual physical examination with specific attention to Tanner staging. The COG currently recommends baseline serum LH, FSH, and estradiol levels at age 13 and when clinically indicated for menstrual irregularity, delayed puberty, or symptoms of estrogen insufficiency.

2.2 Rationale for this Study

Treatment outcomes for children with B-cell NHL are excellent. Further improvements in outcome will likely be achieved through more focused study of the biology of the tumors and prospective studies of the late effects of treatment. Toward this end, this study features a

spectrum of prospective biologic and late effect studies performed in patients treated with a modified regimen derived from the very successful LMB-96 regimen.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion Criteria

3.1.1 Participants from St. Jude and collaborating sites participating in therapeutic and biological objectives

- Participant must have a histologic diagnosis of a mature B cell lymphoma (e.g., Burkitt lymphoma/leukemia, atypical Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, mature B-cell lymphoma NOS) as defined in the WHO classification.

- Participant must be previously untreated, (no more than 72 hours of steroids, one intrathecal chemotherapy treatment, and/or emergency radiation).

- Participant must be < 22 years of age at the time of diagnosis.

- ***For selected higher-risk CD20$^+$ Group B and all CD20$^+$ Group C participants receiving rituximab only (e.g., those with MLBLC, Stage III with LDH ≥ 2 x ULN, and/or bone marrow/CNS involvement)***:

  All participants who will receive rituximab must have hepatitis screening prior to enrollment. Participants whose results indicate that they are carrier of hepatitis B can still be treated as per Group B or C but will NOT receive rituximab. This screening must be done for eligibility for participants who will receive rituximab, BUT the results are not needed prior to enrollment:

  - Hepatitis B immunization status (vaccination Yes or No)
  - HBsAg
  - Anti-HBs antibody
  - Anti-HBc antibody

- All participants must have screening prior to enrollment. Participants whose results indicate that they are carrier of hepatitis B can still be treated per Group B and C but will NOT receive rituximab.

- HIV test has been obtained within 42 days. Participants who test positive for HIV cannot be enrolled on therapeutic part of study but are still eligible for biology studies.

- Informed consent must be obtained according to St. Jude guidelines before enrollment into study.

3.1.2 Participants from collaborating sites participating in biological objectives only (see also Appendix VII)

- Participant must have a histologic diagnosis of a mature B cell lymphoma (e.g., Burkitt lymphoma/leukemia, atypical Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, mature B-cell lymphoma NOS) as defined in the WHO classification.

- Participant must be < 22 years of age at the time of diagnosis.

- Participant must be previously untreated, (no more than 72 hours of steroids, one intrathecal chemotherapy treatment, and/or emergency radiation) at the time of the diagnostic biopsy.

- Informed consent must be obtained by local PI or his/her designee according to ICH/Good Clinical Practice and local guidelines before enrollment into study.

3.2 Exclusion Criteria

3.2.1 Participants from collaborating sites participating in therapeutic and biological objectives

- Participants known to be HIV positive (for therapeutic part of protocol, HIV participants are eligible for biology studies).

- Participants who are pregnant or lactating.

- Inability or unwillingness of research participant or legal guardian to consent.

3.2.2 Participants from collaborating sites participating in biological objectives only

- Inability or unwillingness of research participant or legal guardian to consent.

- Histologic diagnosis other than a mature B-cell lymphoma as defined in the WHO classification.

3.3 Research Participant Recruitment and Screening

3.3.1 St. Jude Participants

Research participants at St. Jude will be recruited by study investigators through regular clinical practice.

3.3.2 Collaborating sites participating in therapeutic and biological objectives

Rady Children's Hospital San Diego will collaborate in both the therapeutic and biological objectives of this study. Study investigators will recruit research participants through regular clinical practice.

3.3.3 Collaborating sites participating in biological objectives only

When physicians consider enrolling a patient on the SJBC3 study (biology studies only), they will be asked whether frozen tumor and paraffin blocks are available. If available, investigators will recruit and consent participants in accordance with local policy. See Appendix VII for further details.

3.4 Enrollment on Study

3.4.1 St. Jude Participants

The Study Team or Clinical Trials Operations (CTO) will enter the Participant Eligibility checklist information into the central enrollment system and release the informed consent document. The signed consent form must be faxed to [redacted] or scanned and emailed the CTO at [redacted] in order to complete enrolment.

To assist with enrollments and consent release, the CTO staff is available Monday through Friday. After hours, weekends, and holidays, the study team is referred to the CTO webpage for additional resources and instructions, Link: [redacted]

3.4.2 Collaborating Sites Participants

Before the collaborating or enrolling affiliate sites screen or enroll a study participant, the site completes a RIN request form and submits it to CTO by e-mail: [redacted] or fax [redacted] (follow fax by a phone call to [redacted] to ensure receipt). A RIN is an eight-digit automated number beginning with an "R". Once the form is received, St. Jude will r*egister* the research participant and then email the RIN to the email address provided on your registration form. This will register the participant only, it will not enroll the participant on the study. After hours, holidays, and weekends the site RIN request will be answered by the Patient Registration team.

To *enroll* the study participant, after the RIN is obtained, the site study team will complete an eligibility checklist and fax it to [redacted] (follow fax by a phone call to [redacted] to ensure receipt). Clinical Trials Operations (CTO) will enter the Eligibility Checklist information into the central enrollment system to officially enroll the participant on the trial.

Collaborating sites will scan/fax a copy of the signed informed consent document to the study team at [redacted].

4.0 TREATMENT PLAN

4.1 Treatment Overview

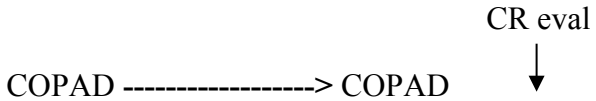
Overview - the chemotherapy regimen used varies with grouping based on extent of disease (see Appendix II for definition of Group A, B, and C).

Note about administration guidelines for all treatment groups: Minor variations in the timing, duration of chemotherapy infusions, and hydration guidelines are acceptable, as long as the treating investigator determines that there is no impact on patient safety.

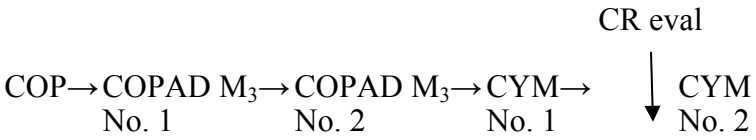
Group	Pre-Phase	Induction X 2	Consolidation X 2	Continuation
A	----------	COPAD	----------	----------
B	COP	COPAD-M_3	CYM	----------
C	COP	COPAD-M_8	CYVE	Seq. No 1,2,3,4

4.1.1 Expanded overview

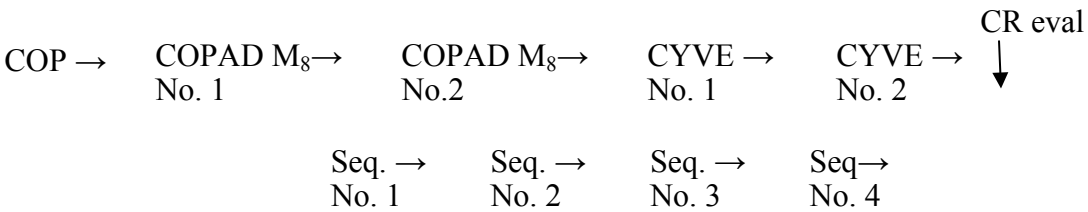
Group A (resected Stage I and resected abdominal Stage II)



Group B (participants not meeting criteria for Group A or C, see Appendix II



Group C (B-ALL with >25% BM blasts; CNS involvement, Group B COP failures i.e., <20% reduction)



4.2 Intrathecal Treatment

Intrathecal chemotherapy will be administered immediately after cerebrospinal fluid is collected for diagnosis, and the dosage is age-dependent as following:

Age	**Methotrexate**	**Hydrocortisone**	**Ara-C**	**Volume (mL)**
<1	8 mg	8 mg	16 mg	8
≥ 1 & < 2	10 mg	10 mg	20 mg	10
≥ 2 & < 3	12 mg	12 mg	24 mg	12
≥ 3	15 mg	15 mg	30 mg	15

This will apply to all treatment groups for which IT is required, except participants ≥ 3 years of age with Ommaya reservoir – see below.

4.2.1 Ommaya reservoir treatment (CNS+ only) – this is optional for CNS+ participants

For participants with an Ommaya reservoir, CNS-directed therapy will be administered as follows:

Age	Methotrexate	Hydrocortisone	Ara-C (mg/m^2)	Volume (ml)
≥3 yr	6	15	50 mg/m^2 (50 mg max)	3

Algorithm for methotrexate dose adjustment via Ommaya reservoir

Methotrexate CSF concentration (μM*)	Methotrexate boost dose
At 24 hours	
> 40	None
15-40	None
8-15	3 mg
4-8	4 mg
< 4	6 mg
At 48 hours	
>4	None
<4	2 mg

Dose adjustment targeted a CNS methotrexate exposure ≥1.0 μM for 72 hours.
**microMolar*

4.3 Chemotherapy Details

4.3.1 GROUP A Treatment Details

Participants in Group A (resected Stage I and Abdominal Stage 2) will receive two courses of COPAD. The second course can be given as soon as count recovery allows; however, it should usually be no more than 21 days after the first course.

Vincristine 2 mg/m^2 (max single dose 2 mg) IV bolus on Day 1 and 6

Prednis(ol)one* 60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive.

Cyclophosphamide 250 mg/m^2/dose every 12 hours as a 15 minute infusion on Days 1-3 inclusive (500 mg/m^2/day). The first dose should be given on day 1 prior to the start of the doxorubicin infusion. Hydration should be maintained at a rate of 3000 mL/m^2/day (125 mL/m^2/hour). Continue hydration until 12 hours after the last dose of cyclophosphamide.

Doxorubicin 60 mg/m^2 as a 6 hour infusion, after the first dose of cyclophosphamide

G-CSF *5 mcg/kg/day subcutaneously on Day 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3 even if prior to day 21.*

Group A: COPAD

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Vincristine	●					●	
Cyclophosphamide	● ●	● ●	● ●				
Doxorubicin	●						
G-CSF^							***● to D21***

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*
^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:
< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)
≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

The second course of COPAD should start at day 21 (or earlier), providing the blood count has recovered with ANC ≥ 1.0 x 10^9/L and platelet count ≥ 100 x 10^9/L. G-CSF should have been discontinued at least 24 hours previously.

4.3.2 GROUP B Treatment Details

Intravenous fluids should be given at a rate of 3000 mL/m^2/day. Use of Rasburicase may preclude the need for HCO_3

4.3.2.1 Group B Pre-Phase: COP

Cyclophosphamide 300 mg/m^2 as an infusion over 15 minutes on Day 1

Vincristine 1 mg/m^2 (max single dose 2 mg) IV bolus on Day 1

Prednis(ol)one* 60 mg/m^2/day (divided into bid doses) orally on Days 1-7

IT medications Methotrexate, hydrocortisone, and cytarabine - IT injection on Day 1 (dose varies with age – see Section 4.2)**

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Cyclophosphamide	●						
IT MTX	●						
IT HC	●						
IT Ara-C	●						

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*
***If blasts identified on this LP, participant will be moved to Group C.*

Tumor response evaluation should be performed on day 7 (see Section 7.0), before proceeding with COPAD M_3

Non-responding patients (< 20% reduction in size): Treated with Group C: start with $COPADM_8$ #1.

In some settings (e.g. critical metabolic, renal, or clinical condition) a second course of COP can be considered, see Section 4.4.2 for details of management of tumor lysis.

In cases where transaminases are > 10 x ULN, a delay in $COPADM_3$ can be considered. These cases should be discussed with the PI and PharmD.

Group B Induction: COPADM$_3$ x 2 cycles

The first COPADM$_3$ course starts on Day 8 as long as clinical condition permits. Any participant who has renal impairment during induction should be discussed with PharmD regarding need for additional renal studies.

NOTE: Group B COPAD M$_3$ cycles for 1 and 2 are identical – not to be confused with Group C. Cyclophosphamide dose is 250 mg/m^2/dose every 12 hours (500 mg/m^2/day) for Group B.

Vincristine	2 mg/m^2 (max dose 2 mg) as IV bolus on Day 1
Prednis(ol)one*	60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive
Methotrexate	3000 mg/m^2 in dextrose 5% as IV infusion over 3 hours on Day 1. See Section 4.4.1 for further details.
Leucovorin	15 mg/m^2 orally or IV every 6 hours for a total of 12 doses (or as required depending on methotrexate levels, see Section 4.4.1 for further details). This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	**250** mg/m^2/dose every 12 hours (**500** mg/m^2/day) as an infusion over 15 minutes on days 2-4 (6 doses). The first dose is given before start of the doxorubicin infusion. Continue hydration at a rate of 3000 mls/m^2/day until 12 hours after the last dose of cyclophosphamide.
Doxorubicin	60 mg/m^2 as a 6 hour infusion, after the first dose of cyclophosphamide.
IT medications	Methotrexate and hydrocortisone IT injection on Days 2 and 6 (dose varies with age – see Section 4.2). **Administer Day 2 IT 12-24 hours after HDMTX starts and before leucovorin rescue begins**
Rituximab	375 mg/m^2 Days1 and 3. *Rituximab treatment applies to higher-risk Group B participants [e.g., mediastinal large B-cell lymphoma (MLBCL), Stage III with LDH $\geq$ 2 x ULN, and/or bone marrow positive at diagnosis only].* See Appendix II for rituximab administration guidelines.
G-CSF	*5 mcg/kg/day by subcutaneous injection on Days 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21.*

Group B Induction: COPAD M_3 x 2 cycles

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Methotrexate	●						
Leucovorin		●●●●●●●●●●●●					
Cyclophosphamide		● ●	● ●	● ●			
Doxorubicin		●					
Rituximab – *higher-risk Group B participants (e.g., MLBCL, Stage III with LDH ≥ 2 x ULN, and/or bone marrow positive at diagnosis)*	●		●				
IT MTX		●				●	
IT HC		●				●	
G-CSF^							***● to D21***

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

Course #2 of COPAD M_3 should start when ANC ≥1.0 x 10^9/L and platelets ≥ 100,000 x 10^9/L. The course should not be given less than 14 days after the start of COPAD M_3 #1 and should be delayed for 24 hours from the last dose of G-CSF. If hematological recovery has not occurred by Day 25, a bone marrow examination should be considered. If there are residual blasts in the marrow COPADM_3 #2 should be given. If the marrow is hypoplastic, discuss with PI.

4.3.2.3 Group B Consolidation: CYM x 2 cycles

Each cycle of CYM should start when ANC ≥ 1.0 x 10^9/L and platelets ≥ 100,000 x 10^9/L.

Methotrexate 3000 mg/m^2 in dextrose 5% as IV infusion over 3 hours on Day 1.

Leucovorin 15 mg/m^2 orally or IV every 6 hours for a total of 12 doses (or as required depending on methotrexate levels, see Section 4.4.1.3 for further details). This begins at 24 hours from the start of the methotrexate infusion.

Cytarabine 100 mg/m^2 in either dextrose or saline as continuous infusion over 24 hours. Repeat daily from Day 2-6 inclusive (total of 5 days).

IT medications Methotrexate and hydrocortisone IT injection on Day 2. Cytarabine and hydrocortisone IT injection on Day 7 (dose varies with age – see Section 4.2). **Administer Day 2 IT methotrexate and hydrocortisone 12-24 hours after HDMTX starts and before leucovorin begins.**

Rituximab* 375 mg/m^2 day 1**.** *Rituximab treatment applies to higher-risk Group B participants (e.g., mediastinal large B-cell lymphoma (MLBCL) and Stage III with LDH ≥ 2 x ULN, and/or bone marrow positive at diagnosis*. See Appendix I for rituximab administration guidelines.

G-CSF *5 mcg/kg/day by subcutaneous injection on Days 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21.*

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Methotrexate	●							
Leucovorin		●●●●●●●●●●●						
Cytarabine		●	●	●	●	●		
Rituximab (e.g., *MLBCL, Stage III with LDH ≥ 2 x ULN, and/or bone marrow positive at diagnosis)*	●							
IT MTX		●						
IT Ara-C							●	
IT HC		●					●	
G-CSF^								***● to D21***

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:
< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)
≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

Following recovery from CYM 1, a full assessment of response should be carried out. Any residual masses should be surgically excised, or biopsied if excision is not possible (see Section 7.0).	
If histology negative	Continue with CYM 2
If histology positive (even if completely resected)	Change to Arm C1 starting with CYVE 1

4.3.3 Group C Treatment Details

Intravenous fluids should be given at a rate of 3000 mL/m^2/day. Use of rasburicase may preclude the need for HCO_3

4.3.3.1 Group C Pre-Phase: COP

Cyclophosphamide 300 mg/m^2 as an infusion over 15 minutes on Day 1

Vincristine 1 mg/m^2 (max single dose 2 mg) IV bolus on Day 1

Prednis(ol)one* 60 mg/m^2/day (divided into bid doses) orally on Days 1-7

IT medications Methotrexate, cytarabine, and hydrocortisone IT injection on Days 1, 3, and 5 (dose varies with age – see Section 4.2)

Leucovorin 5 mg/m^2/dose (max 5 mg) po given at 24 and 30 hours after IT on Days 2 and 4

Days	1	2	3	4	5	6	7
Cyclophosphamide	●						
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
IT MTX	●		●		●		
IT HC	●		●		●		
IT Ara-C	●		●		●		
Leucovorin		● ●		● ●			

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*

Tumor response evaluation should be performed on day 7.

In some settings (e.g. critical metabolic, renal, or clinical condition) a second course of COP can be considered. See Section 4.4.2 for details of management of tumor lysis.

In cases where transaminases are > 10 x ULN, a delay in $COPADM_3$ can be considered. These cases should be discussed with the PI or PharmD.

4.3.3.2 Group C Induction: COPADM$_8$ cycle 1

COPAD M_8 should start on day 8 of COP pre-phase therapy, as long as clinical condition permits. Any participant who has renal impairment should be discussed with PharmD regarding need for additional renal studies.

Vincristine	2 mg/m^2 (max dose 2 mg) as IV bolus on Day 1
Prednis(ol)one*	60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive
Methotrexate	8000 mg/m^2 in dextrose 5% as IV infusion over 4 hours on Day 1. **NOTE: Higher dose than for Group B.** See Section 4.4.1 for details.
Leucovorin	15 mg/m^2 orally or IV every 6 hours for a total of 12 doses (or as required depending on methotrexate levels, see Section 4.4.1.3 for further details). This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	**250** mg/m^2/dose every 12 hours **(500** mg/m^2/day) as an infusion over 15 minutes on days 2-4 (6 doses). The first dose is given before start of the doxorubicin infusion. Continue hydration until 12 hours after the last dose of cyclophosphamide.
Doxorubicin	60 mg/m^2 as a 6 hour infusion, after the first dose of cyclophosphamide
Rituximab	375 mg/m^2 Days 1 and 3**.** All Group C participants will receive rituximab with COPADM$_8$ cycles. See Appendix I for rituximab administration guidelines.
IT medications**	Methotrexate, cytarabine, and hydrocortisone IT injection on Days 2, 4, and 6 (dose varies with age – see Section 4.2). **Administer Day 2 IT 12-24 hours after HDMTX starts and before leucovorin rescue begins**.
G-CSF	*5 mcg/kg/day by subcutaneous injection on Days 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21.*

Group C Induction: COPADM$_8$ cycle 1

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Methotrexate	●						
Leucovorin		●●●●●●●●●●●					
Cyclophosphamide		● ●	● ●	● ●			
Doxorubicin		●					
Rituximab	●		●				
IT MTX		●		●		●	
IT HC		●		●		●	
IT Ara-C		●		●		●	
G-CSF^							***● to D21***

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*

***CNS+ participants with Ommaya reservoirs, receive Intra-Ommaya treatment on Day 2 only [plus MTX boost doses as needed (section 4.2.1)]*

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

4.3.3.3 Group C Induction: COPAD M_8 Cycle 2

Course #2 of COPAD M_3 should start when ANC $\geq$1.0 x 10^9/L and platelets $\geq$ 100,000 x 10^9/L. The course should not be given less than 14 days after the start of COPAD M_3 #1 and should be delayed for 24 hours from the last dose of G-CSF. If hematological recovery has not occurred by Day 25, a bone marrow examination should be considered. If there are residual blasts in the marrow COPADM_3 #2 should be given. If the marrow is hypoplastic, discuss with PI. **Note: Group C COPAD M_8, cycle 2 cyclophosphamide dose is higher than Group B dose.**

Vincristine	2 mg/m^2 (max dose 2 mg) as IV bolus on Day 1
Prednis(ol)one*	60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive.
Methotrexate	8000 mg/m^2 in dextrose 5% as IV infusion over 4 hours on Day 1. **NOTE: Higher dose than for Group B.** See Section 4.4.1 for further details.
Leucovorin	15 mg/m^2 orally or IV every 6 hours for a total of 12 doses (or as required depending on methotrexate levels, see Section 4.4.1.3 for further details). This begins at 24 hours from the start of the methotrexate infusion.
Cyclophosphamide	**500** mg/m^2/dose every 12 hours (**1000 mg/m^2/day**) as an infusion over 15 minutes on days 2-4 (6 doses). The first dose is given before start of the doxorubicin infusion. Continue hydration until 12 hours after the last dose of cyclophosphamide.
Doxorubicin	60 mg/m^2 as a 6 hour infusion, after the first dose of cyclophosphamide
Rituximab	375 mg/m^2 Days 1 and 3**.** All Group C participants will receive rituximab with COPADM$_8$ cycles. See Appendix I for rituximab administration guidelines.
IT medications**	Methotrexate, cytarabine, and hydrocortisone IT injection on Days 2, 4, and 6 (dose varies with age – see Section 4.2). **Administer Day 2 IT 12-24 hours after HDMTX starts and before leucovorin rescue begins**.
G-CSF	*5 mcg/kg/day by subcutaneous injection on Days 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21.*

Group C Induction: COPADM$_8$ Cycle 2

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Methotrexate	●						
Leucovorin		●●●●●●●●●●●●					
Cyclophosphamide		● ●	● ●	● ●			
Doxorubicin		●					
Rituximab	●		●				
IT MTX		●		●		●	
IT HC		●		●		●	
IT Ara-C		●		●		●	
G-CSF^							***● to D21***

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*

*****CNS+ participants with Ommaya reservoirs, receive Intra-Ommaya treatment on Day 2 only [plus MTX boost doses as needed (section 4.2.1)]***

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

4.3.3.4 Group C Consolidation CYVE x 2 cycles

The first of these two courses should start after COPAD M8 when ANC $\geq 1.0 \times 10^9$/L and platelets $\geq 100{,}000 \times 10^9$/L. G-CSF should have been stopped for 24 hours before the start of this course.

Variations in the start/stop times of the agents below are acceptable, as long as the sequence of administration of the agents and duration of infusions are as close as possible.

Cytarabine 50 mg/m^2 by continuous infusion over 12 hours. This should start at 2000 hours and run until 0800 the following day. Repeat daily x 5.

High-Dose Ara-C 3000 mg/m^2 as IV infusion over 3 hours, to start at the end of the 12 hour infusion of cytarabine for 4 doses (from 0800 to 1100 hours).

Etoposide 200 mg/m^2 in saline (concentration $\leq$ 0.4 mg/mL) as IV infusion over 2 hours daily x 4 doses. Etoposide starts at 1400 hours, 3 hours after end of high dose cytarabine.

Rituximab 375 mg/m^2 Day 1**.** All Group C participants will receive rituximab with CYVE cycles. See Appendix I for rituximab administration guidelines.

G-CSF *5 mcg/kg/day by subcutaneous injection on Days 7-21 inclusive. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21.*

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Cytarabine (continuous)	●	●	●	●	●	Runs from 2000-0800	
High dose Ara-C		●	●	●	●	Runs from 0800-1100	
Etoposide		●	●	●	●	Runs from 1400-1600	
Rituximab	●					See Appendix I	
G-CSF^							***● to D21***

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:
< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)
≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

CYVE Course #2: This course is the same as CYVE #1 and starts once ANC $\geq$ 1.0 x 10^9/L and platelets $\geq$ 100,000 x 10^9/L (usually by days 25 to 28).

Following recovery from CYVE2, a complete response evaluation should be performed. Residual masses should be surgically excised or biopsied if excision is not possible (see Section 4.6.5)	
If histology negative	Continue on protocol
If histology positive	Remove from protocol treatment; additional treatment to be determined by primary physician.

4.3.3.5 Group C Maintenance

Maintenance therapy includes 4 sequences/blocks of chemotherapy. Each sequence will begin when ANC $\geq 1.0 \times 10^9$/L and platelets $\geq 100{,}000 \times 10^9$/L.

GROUP C MAINTENANCE SEQUENCE No. 1

Vincristine 2 mg/m^2 (max dose 2 mg) as IV bolus on Day 1

Prednis(ol)one* 60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive

Cyclophosphamide 500 mg/m^2/day given daily as IV infusion over 30 minutes on Days 2 and 3. First dose is given before doxorubicin.

Methotrexate 8000 mg/m^2 in dextrose 5% IV infusion over 4 hours on Day 1. **NOTE: Higher dose than for Group B. See Section 4.4.1**

Leucovorin 15 mg/m^2 orally or IV every 6 hours for a total of 12 doses (or as modified depending on methotrexate levels, see Section 4.4.1.3 for details). This begins 24 hours from the start of the methotrexate infusion.

Doxorubicin 60 mg/m^2 as a 6 hour infusion, after first dose of cyclophosphamide

IT medications** Methotrexate, cytarabine, and hydrocortisone IT injection on Day 2 (dose varies with age – see Section 4.2). **Administer Day 2 IT 12-24 hours after HDMTX starts and before leucovorin rescue begins**.

G-CSF *5 mcg/kg/day by subcutaneous injection starting 24 hours after completion of chemotherapy. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3*

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Cyclophosphamide		●	●				
Methotrexate	●						
Leucovorin		●●●●●●●●●●●					
Doxorubicin		●					
IT MTX		●					
IT HC		●					
IT Ara-C		●					
G-CSF^							●

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*
***CNS+ participants with Ommaya reservoirs, receive Intra-Ommaya treatment on Day 2 only [plus MTX boost doses as needed (section 4.2.1)]*

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

GROUP C MAINTENANCE SEQUENCE No. 2

Cytarabine 50 mg/m^2/dose as subcutaneous injection every 12 hours (100 mg/m^2/day) Days 1-5

Etoposide 150 mg/m^2 IV infusion over 90 minutes Days 1-3

G-CSF *5 mcg/kg/day by subcutaneous injection starting 24 hours after completion of chemotherapy. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3*

IT Medications **For CNS positive participants only (see chart)**

Days	1	2	3	4	5	6
Cytarabine	● ●	● ●	● ●	● ●	● ●	
Etoposide	●	●	●			
G-CSF^						●
IT MTX*	●					
IT HC*	●					
IT Ara-C*	●					

**CNS positive participants only, (either ITMHA or Intra-Ommaya MHA).*
See Section 4.2 for boost.

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:
< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)
≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

GROUP C MAINTENANCE SEQUENCE No. 3

Vincristine 2 mg/m^2 (max dose 2 mg) as IV bolus on Day 1

Prednis(ol)one 60 mg/m^2/day (divided into bid doses) orally on Days 1-7 inclusive

Cyclophosphamide 500 mg/m^2/day given as IV infusion over 30 minutes on Days 1 & 2. First dose is given before doxorubicin. Maintain hydration at 3000 mL/m^2/day until 12 hours after 2nd dose.

Doxorubicin 60 mg/m^2 as a 6 hour infusion, after the first dose of cyclophosphamide.

G-CSF *5 mcg/kg/day by subcutaneous injection starting 24 hours after completion of chemotherapy. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3, even if prior to Day 21*

IT Medications **For CNS positive participants only (see chart)**

Days	1	2	3	4	5	6	7
Vincristine	●						
Prednis(ol)one*	● ●	● ●	● ●	● ●	● ●	● ●	● ●
Cyclophosphamide	●	●					
Doxorubicin	●						
G-CSF^						●	
IT MTX**	●						
IT HC**	●						
IT Ara-C**	●						

**Oral prednisone can be substituted with methylprednisolone at 30 mg/m^2/day IV (divided into bid doses) for patients who cannot tolerate the oral medication.*

***CNS positive patients only (either ITMHA or Intra-Ommaya MHA). See Section 4.2 for boost*

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

GROUP C MAINTENANCE SEQUENCE No. 4

Cytarabine 50 mg/m^2/dose as subcutaneous injection every 12 hours (100 mg/m^2/day) Days 1-5

Etoposide 150 mg/m^2 IV infusion over 90 minutes Days 1-3

G-CSF *5 mcg/kg/day by subcutaneous injection starting 24 hours after completion of chemotherapy. G-CSF should be discontinued when the post nadir ANC reaches 2000/mm^3*

IT Medications **For CNS positive participants only (see chart)**

Days	1	2	3	4	5	6
Cytarabine	● ●	● ●	● ●	● ●	● ●	
Etoposide	●	●	●			
G-CSF^						●
IT MTX*	●					
IT HC*	●					
IT Ara-C*	●					

**CNS positive patients only (either ITMHA or Intra-Ommaya MHA). See Section 4.2 for boost*

^In the event the patient cannot receive G-CSF, Peg-Filgrastim can be substituted:

< 45 kg: 0.1 mg/kg/dose subQ Once (to be given the same day GCSF were to start)

≥ 45 kg: 6 mg subQ Once (to be given the same day GCSF were to start)

4.4 Supportive Care Guidelines

These guidelines are provided to help physicians caring for participants treated on this protocol. They are guidelines and not protocol requirements. Nothing in these guidelines is intended to supplant the judgment of the treating physician regarding patient management. Current institutional practice may dictate other approaches to the management of the areas discussed in this section.

4.4.1 High-Dose Methotrexate Administration

Hydration

Pre-hydrate with 250 ml/m^2/hr with alkalinization for a minimum of 2 hours in order to achieve a pH of $\geq$ 6.5.

Methotrexate is administered in dextrose (piggyback into IVF with bicarb) at a dose of 3000 mg/m^2 over 3 hours in Group B and a dose of 8000 mg/m^2 over 4 hours in Group C participants.

After methotrexate infusion, continue hydration at a rate of 3000 mLs/m^2/day with dextrose 5% with added NaHCO3 (40 mEq/l) and KCL (20 mEq/l) until MTX level is < 0.5µM. When MTX level < 0.5µM added NaHC03 may be removed and urine pH's do not need to be checked. Increased rate of hydration must continue until 12 hours after the last dose of cyclophosphamide.

Note: doxorubicin is unstable in alkaline solution, so must give via a separate lumen from alkaline hydration fluids. If no alternate lumen is available, then oral alkalinization is an alternative. Give 1 mEq/kg $NaHCO_3$ every 6 hours and increase as necessary to maintain the urine pH at $\geq$ 7 as an alternative. It is important to check that the urine pH is maintained $\geq$ 7.

Drug interactions

Drugs which compromise renal function (e.g. aminoglycosides) can decrease clearance of methotrexate and lead to systemic toxicity. Avoid concurrent use of NSAIDs, omeprazole, azole antifungals, salicylates and sulphonamides. Large doses of penicillin may interfere with the active renal tubular section of methotrexate.

Leucovorin

Leucovorin (folinic acid) 15 mg/m^2 should be given orally or IV every 6 hours. The dose should be rounded up to the nearest 5 mg. The rescue begins 24 hours from the start of the methotrexate infusion. Intrathecal drugs should be given before rescue starts. If vomiting occurs within 30 minutes, repeat the dose. If persistent vomiting or diarrhea occurs, give leucovorin by IV injection. Methotrexate levels, urea and electrolytes should be measured daily for 3 days after methotrexate infusion. Strict attention should be paid to fluid balance. The leucovorin dose should be modified as required based in the methotrexate level.

Leucovorin dosage adjustments: Patients will have their leucovorin rescue increased if they meet any of the following criteria:

	3 g/m^2	8 g/m^2
MTX level		
24 hour	>5 µM	>10 µM
48 hour*	>1 µM	>1 µM
72 hour	>0.1 µM	>0.1 µM

If the patient meets any of these criteria, the leucovorin dosage will be individualized and plasma concentrations will be monitored until the plasma level is <0.1 µM. Concomitant administration of vincristine and HD MTX may delay the MTX clearance.

***If the 48 hour MTX level is < 0.15 µM/l, discontinue leucovorin rescue.**

Group C: Patients who experience Grade IV GI toxicity after COPAD M_8 #1 will receive COPAD M_3 for their subsequent courses. Patients who experience Grade IV GI toxicity after COPAM M_3 #1 will begin leucovorin at 18 instead of 24 hours with subsequent HDMTXs.

A further check on the MTX level should be done 24 hours after the day 6 IT in Group C COPADM_8 courses to ensure that the level does not increase again after IT MTX and to alter leucovorin rescue as required. Hydration should continue beyond 72 hours in the following situations:

- If there is still evidence of tumor lysis
- If cyclophosphamide infusion is still in process
- If the MTX level is still > 0.15 µmol/L (1.5 x 10^{-7}M)

4.4.2 Management of Tumor Lysis Syndrome (TLS)

Patients with mature B cell lymphomas are at risk for Tumor Lysis Syndrome (hyperkalemia, hyperuricemia, hyperphosphatemia, hypocalcemia, and secondary renal dysfunction).

It is suggested that baseline serum chemistries (urate, urea, creatinine, electrolytes, Ca, Mg, and PO_4) be obtained pre-treatment and frequently after therapy is started, and a central venous line (Double Lumen Hickman Catheter or DLHC) be inserted for fluid administration and monitoring.

Preventative measures

1. Give allopurinol 300 mg/m^2 daily divided TID. Alternatively, rasburicase may be given daily until the lysis period is over.
2. Intravenous fluids should be started at least 12 hours before chemotherapy, 3000 mL/m^2/day. **NO ADDED POTASSIUM.**

Suggested monitoring during Induction chemotherapy

1. Strict monitoring of fluid balance is essential.
2. Check blood pressure frequently.
3. Daily weights.
4. Measurement of serum chemistries at least every 6 hours.

4.4.4 Prophylactic antibiotics

Co-trimoxazole prophylaxis is strongly recommended for all participants, starting with COPADM #1 and continuing for 6 months after completion of treatment.

4.4.5 Fever and neutropenia

Fever with neutropenia should be evaluated immediately. After blood cultures are obtained, broad spectrum antibiotics should be started as soon as possible.

4.4.6 Blood product support

Blood product support should be provided as indicated clinically. It is suggested to keep platelet count >20,000 x 10^9/L.

4.4.7 Growth factors (G-CSF)

G-CSF should be stopped for at least 24 hours prior to next course of chemotherapy.

4.5 Dosage Modifications for Toxicity

4.5.1 Seizures

Discussion with PharmD and PI regarding anticonvulsants and potential need for modification of chemotherapy should be considered in the event of seizures.

4.5.2 Etoposide reactions

Cardiovascular effects

Transient hypotension has occurred in about 1 to 2 % of patients following rapid IV administration of etoposide during clinical trials. However, hypotension has not been associated with cardiac toxicity or electrocardiogram changes. Blood pressure usually normalizes within a few hours after discontinuation of the infusion. To avoid this complication, etoposide should be infused over 30 – 60 minutes. If hypotension should occur, stop the infusion, and if necessary, give 10 mL/kg NS bolus over 15 minutes. Repeat as necessary. Once symptoms resolve, resume infusion at ½ previous infusion rate until full dose administered. If hypotension recurs, stop infusion and administer 10 mL/kg NS bolus as indicated. Once hypotension resolves, resume infusion at ½ previous infusion rate until complete. Consider infusing NS at 1-1.5 x maintenance during remainder of infusion. For all subsequent doses, further dilute and infuse over 2 hours.

Sensitivity reactions

Anaphylactoid reactions consisting principally of chills, rigors, diaphoresis, pruritis, loss of consciousness, nausea, vomiting, fever, bronchospasm, dyspnea, tachycardia, hypertension, and/or hypotension have occurred in 0.7 – 3% of patients receiving etoposide. Other manifestations include flushing, rash, substernal chest pain, lacrimations, sneezing, coryza, throat pain, back pain, abdominal cramps, and auditory impairment. Facial/lingual swelling, coughing, diaphoresis, cyanosis, tightness in the throat, and laryngospasm have also occurred.

If an anaphylactoid reaction should occur:

1. Stop the infusion immediately and notify H/O Fellow or Attending MD
2. Administer the following as indicated:
    a) diphenhydramine 1mg/kg IV (max dose 50 mg)
    b) hydrocortisone 50 – 100 mg/m^2 IV
    c) epinephrine 0.01 mg/kg of a 1:1000 concentration for SQ administration
    d) fluid bolus 10 mL/kg NS infused over 15 minutes
3. Once symptoms have resolved, resume infusion at ½ previous rate until infusion complete. Consider infusing NS at 1-1.5 x maintenance during remainder of infusion.
4. If anaphylaxis recurs, stop the infusion and re-treat as above. Do not administer remainder of dose. Consider substituting etoposide with etoposide phosphate (Etopophos®) for all subsequent doses.
5. If anaphylaxis does not recur, pre-medicate all subsequent doses with diphenhydramine 1mg/kg (max 50 mg) and hydrocortisone 50 – 100 mg/m^2. Consider slowing the loading dose to be administered over 1 hour.
6. Have at bedside all of the following for all subsequent infusions:
    a) Diphenhydramine 1mg/kg IV (max 50 mg)
    b) Hydrocortisone 50 – 100 mg/m^2 IV
    c) Epinephrine 0.01 mg/kg of a 1:1000 concentration for SQ administration

    Note: anaphylactoid reactions are still possible with etoposide phosphate. If the patient cannot tolerate the substitution, drug is contraindicated and must be discontinued.

4.5.3 Nephrotoxicity

Consultation with PharmD and PI is suggested for severe renal dysfunction. In the event of toxicity secondary to high dose methotrexate, consider carboxypeptidase (glucarpidase).

4.5.4 Cardiotoxicity

Hold doxorubicin until fractional shortening is > 28%. Consultation with cardiologist suggested if clinically indicated.

4.5.5 Hepatic toxicity

Prior to starting IV methotrexate

If SGOT or SGPT is > 20 x upper limit of normal, consult with PI for further recommendation. If transaminases are between 10 and 20 x upper limit of normal, wait 48 hours and re-check to ensure that they are decreasing.

Anthracyclines and vincristine

Anthracyclines and vincristine dosages should be modified in patients with elevated direct bilirubin concentrations or other evidence of biliary obstruction.

- Direct bilirubin 2-4 mg/dl: 50% dosage decrease
- Direct bilirubin 4-6 mg/dl: 75% dosage decrease
- Direct bilirubin >6 mg/dl: withhold dose

4.5.7 Modifications for age

For participants < 1 year of age, a 1/3 dosage reduction should be made; if this course is well tolerated, an increase to full dosage may be considered. Intrathecal doses are age specific, see Section 4.2.

4.6 Surgical Guidelines

4.6.1 Surgical intervention at diagnosis

Obtaining tissue for diagnosis should be done with the least invasive procedure possible. Surgical resection of Stage I or localized abdominal disease (i.e., ileo-caecal area with involvement of regional mesenteric lymph nodes only) should be considered in consultation with surgery.

4.6.2 Abdominal disease

Debulking procedures are not indicated (see Section 4.6.1).

4.6.3 Mediastinal mass

Consultation with Anesthesia, Surgery, and Interventional Radiology is suggested to determine the safest procedure for obtaining tissue for diagnosis.

4.6.4 Bone primary

Consultation with Surgery is suggested for those presenting with primary involvement of bone.

4.6.5 Secondary surgery

For residual masses prior to CYM #2 in Group B patients, after CYVE #2 in Group C patients

Histological evaluation of residual masses should be done in consultation with Surgery. Complete resection is advisable; however, biopsy (with interventional radiology) may be considered depending on site of disease and potential risk.

Group B patients

If there is histologic confirmation of residual tumor (even if this is resected with the surgery) the patient should be transferred to Group C (starting with CYVE #1).

Group C patients

If there is histologic confirmation of residual tumor (even if this is resected with the surgery) the participant is considered a treatment failure and should be taken off treatment.

Residual bone lesions

Residual diagnostic imaging abnormalities are common. Consultation with Orthopedics and Radiology is suggested as clinically indicated.

4.7 Radiation Therapy Guidelines

There are no specific indications for RT in this study.

5.0 DRUG INFORMATION

5.1 Cytarabine (Ara-C) (Cytosar-U®)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50mg/ml. he 1000 and 2000mg vials are reconstituted with 20ml and 40 ml respectively resulting in a final concentration of 50mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia, and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

5.2 Cytarabine (High Dose Ara-C)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50mg/ml. The 1000 and 2000mg vials are reconstituted with 20ml and 40 ml respectively resulting in a final concentration of 50mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available.

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia, and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

5.3 Cyclophosphamide

Source and pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell-cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of > 75%. Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of < 50 ml/min.

Formulation and stability: Cyclophosphamide is available in 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.

Supplier: Commercially available

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decrease the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

5.4 Doxorubicin (Adriamycin®)

Source and pharmacology: Doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius*. Doxorubicin exerts its anti-tumor effects in several different ways. Doxorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, doxorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Lastly, doxorubicin undergoes enzymatic electron reduction to generate highly reactive species, including the hydroxyl free radical, which is thought to be responsible for the drug's cardiac toxicity, but may play a role in its anti-tumor activity as well.

Doxorubicin is cell-cycle, phase non-specific. Doxorubicin is widely distributed in the tissues and plasma, but does not cross the blood brain barrier to an appreciable extent. It is metabolized to doxorubicinol, which is thought to be the major active metabolite, and aglycones. Doxorubicin and its metabolites are excreted mainly in the bile and feces (≈80%). The remainder is excreted in the urine. Dosage should be reduced in patients with liver dysfunction (bilirubin > 1.2 mg/dl) or renal dysfunction (creatinine > 3 mg/dl).

Formulation and stability: Doxorubicin is available in vials containing 10 mg, 20 mg, 50 mg and 200 mg as a 2mg/ml red-orange solution. It is also available in vials containing 10 mg, 20 mg, 50 mg, 100 mg and 150 mg of doxorubicin as a red-orange lyophilized powder. Intact vials of doxorubicin solution should be stored under refrigeration while the lyophilized product should be stored at room temperature. Both products should be protected from light. Lyophilized doxorubicin can be reconstituted by adding 5, 10, 25, 50 or 75 ml of 0.9% NaCl respectively to the 10, 20, 50, 100 and 150 mg vials to produce a final concentration of 2 mg/ml. Bacteriostatic diluents are not recommended. After reconstitution, the resultant solution should be protected from light and is stable for 7 days at room temperature and 15 days if refrigerated.

Supplier: Commercially available

Toxicity: Dose-limiting toxicities include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred. Typhlitis can occur when combined with cytarabine.

5.5 Etoposide (VP16) (Vepesid®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllumpelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide is available in multi-dose vials containing 100mg, 150mg, 500mg and 1000mg of etoposide as a 20mg/ml solution and 30% alcohol. Etoposide is also available as a 50 mg capsule. The intact vials of etoposide solution should be stored at room temperature. The capsules should be stored under refrigeration. Etoposide solution should be

diluted in D5W or 0.9% NaCl prior to administration. Solutions with a final concentration of 0.2 and 0.4 mg/ml are stable at room temperature for 96 hours and 24 hours respectively.

Supplier: Commercially available

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

5.6 Etoposide phosphate (Etopophos®)

To be used in case of etoposide reactions (see Section 4.4.3).

Source and pharmacology: Etoposide is an epipodophylotoxin derived from *Podophyllumpelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide phosphate is a water-soluble ester of etoposide. The higher water solubility of etoposide phosphate than that of etoposide lessens the potential for precipitation following dilution and during administration. Etoposide phosphate is available in single-dose vials containing etoposide phosphate equivalent to 100mg etoposide. The intact vials of etoposide solution should be stored at 2 to 8 degrees Celsius. Etoposide phosphate solution should be diluted in D5W or 0.9% NaCl prior to administration. Solution is stable at room temperature for 24 hours.

Supplier: Commercially available.

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

5.7 Filgrastim (G-CSF, Neupogen®)

Source and pharmacology: G-CSF (granulocytic colony stimulating factor), is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of *Escherichia coli.* G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.

Formulation and stability: G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300 mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for IV infusion in 5% dextrose. Do not dilute in saline---precipitate may form. If the final concentration of this product is < 15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.

Supplier: Commercially available

Toxicity: G-CSF causes marked leukocytosis. Adverse reactions reported commonly include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in patients with allergy to E. coli derived products.

5.8 Peg-filgrastim (pegylated filgrastim, PEG-filgrastim, Neulasta®)

Source and pharmacology: Pegfilgrastim is the pegylated form of recombinant methionyl human G-CSF (filgrastim). Pegfilgrastim is produced by covalently binding a 20-kilodalton (kD) monomethoxypolyethylene glycol molecule to the N-terminal methionyl residue of filgrastim. The molecular weight of pegfilgrastim is 39 kD. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens).

After subcutaneous injection the elimination half-life of pegfilgrastim ranges from 15 to 80 hours and the time to peak concentration ranges from 24 to 72 hours. Serum levels are sustained in most patients during the neutropenic period post-chemotherapy and begin to decline after the start of neutrophil recovery, consistent with neutrophil-dependent elimination.

Toxicity: mild to moderate medullary bone pain, local irritation at the injection site, headache, elevations in alkaline phosphatase, LDH, uric acid, thrombocytopenia, low grade fever, allergic reactions, splenomegaly, splenic rupture, exacerbation of pre-existing skin rashes, sickle cell crisis in patients with SCD, excessive leukocytosis, ARDS. Fetal toxicities and teratogenic effects in humans are unknown. There is conflicting data in animals. It is unknown whether the drug is excreted in breast milk.

Formulation and stability: Supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single dose syringe with 27 g, ½ inch needle with an UltraSafe® Needle Guard. Store refrigerated at 2º-8ºC (36º-46ºF) and in the carton to protect from light. Prior to injection, pegfilgrastim may be allowed to reach room temperature protected from light for a maximum of 48 hours. Avoid freezing.

Guidelines for administration: Pegfilgrastim should not be administered in the period between 2 weeks before and 24 hours after chemotherapy. Do not shake. Dose and administration as clinically indicated, per institutional guidelines. See Treatment and Dose Modifications sections of the protocol.

Supplier: Commercially available.

5.9 Hydrocortisone, Intrathecal (Cortef, Solu-Cortef)

Source and pharmacology: Hydrocortisone is a synthetic steroid akin to the natural adrenal hormone cortisol. Hydrocortisone has phase-specific cytotoxicity, killing lymphoblasts primarily during S phase. It has catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

Formulation and stability: Solu-Cortef sterile powder is supplied in the following package: 100 mg plain, and 100 mg, 250 mg, 500 mg, and 1000 mg ACT-O-VIAL (MIX-O-VIAL). Store unreconstituted product at controlled room temperature 15-30°C (59-86°F). Store reconstituted solution in the refrigerator and protect from light. Unused solution should be discarded after 3 days. Use Solu-Cortef (plain vial) for intrathecal use, and reconstitute with 0.9% sodium chloride, USP for injection.

Supplier: Commercially available

Toxicity: If given intrathecally, sterile arachnoiditis may occur. Headache, seizures, unusual feelings or sensations, loss of feeling or ability to move arms or legs, and difficulty with urination or bowel movements may also occur.

5.10 Leucovorin (folinic acid)

Source and pharmacology: Leucovorin is a racemic mixture of tetrahydrofolic acid, which is involved as a cofactor for 1-carbon transfer reactions in the synthesis of purine and pyrimidines. Leucovorin is a potent antidote for both the hematopoietic and reticuloendothelial toxic effects of folic acid antagonists by replenishing reduced folate pools. It is postulated that in some cancers, leucovorin enters and “rescues” normal cells from the toxic effects of folic acid antagonists, in preference to tumor cells, because of differences in membrane transport and affinity for polyglutamylation. Leucovorin is converted in the intestinal mucosa and the liver to 5-methyl-tetrahydrofolate, which is also active as a reduced folate. It is excreted primarily in the urine with minor excretion occurring in the feces.

Formulation and stability: Leucovorin is supplied in 5, 15 and 25 mg tablets and vials containing 50, 100 or 350 mg of leucovorin as a lyophilized powder. The tablets and the lyophilized powder can be stored at room temperature. The 50 mg and 100 vials can be reconstituted by adding 5 or 10 ml of sterile water or bacteriostatic water for injection respectively to yield a final concentration of 10 mg/ml. The 350 mg vials can be reconstituted with 17 ml of sterile water or bacteriostatic water for injection to yield a final concentration of 20 mg/ml. The reconstituted solution is stable for at least 7 days at room temperature. Leucovorin may be further diluted in 5% dextrose or 0.9% NaCl containing solutions.

Supplier: Commercially available

Toxicity: Leucovorin is generally well tolerated. Toxicities that have been reported uncommonly include rash, mild nausea, headache, and wheezing (possible allergic reaction). Intrathecal leucovorin is contraindicated and has caused neurotoxic deaths. There have been rare reports of leucovorin promoting seizures.

5.11 Methotrexate

Source and pharmacology: Methotrexate is a folate analogue that acts by inhibiting dihydrofolatereductase. Dihydrofolatereductase is an enzyme important in the conversion of folic acid to tetrahydrofolic acid, which is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the production of tetrahydrofolic acid, methotrexate interferes with DNA, RNA and protein synthesis. Methotrexate is poorly and variably absorbed orally, with an average of ≈ 40% for doses of ≤ 30 mg/m^2. At higher dosages, the extent of absorption decreases. Methotrexate is approximately 50% protein bound. It distributes widely into body tissues and fluids with sustained concentrations in the kidney and the liver. Methotrexate undergoes metabolism by cytosolic aldehyde oxidase to hydroxy methotrexate. It is excreted mainly in the urine as unchanged drug with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination with an initial half-life of ≈ 2-3 hours and a terminal half-life of 10-12 hours. Methotrexate may be "sequestered" in body fluid collections and eliminated slowly from these areas. Patients with effusions or GI obstruction should have plasma levels monitored closely for delayed excretion following high-dose methotrexate.

Formulation and stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250 mg of methotrexate as a 25 mg/ml preservative-free solution and in vials containing 20mg, 50 mg, 100mg, 250 mg and 1000mg of lyophilized drug. It is also available in 2.5 mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets can also be stored at room temperature. The vials containing 20, 50, 100 and 250 mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25 mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50 mg/ml.

Supplier: Commercially available.
Toxicity: The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, alopecia, hepatic toxicity and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and mucositis. Leucovorin may also be necessary after IT administration, especially if IT methotrexate therapy is given to patients with

renal dysfunction. Patients with Down syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.

5.12 Prednisone (prednisolone)

Source and pharmacology: Prednisone is a synthetic congener of hydrocortisone, the natural adrenal hormone. Prednisone is a white or yellowish crystalline powder. It binds with steroid receptors on nuclear membranes, impairs cellular mitosis and inhibits protein synthesis. Prednisone also has potent anti-inflammatory effects and suppresses the immune system. Prednisone is well absorbed orally. It is converted to prednisolone, the pharmacologically active metabolite, in the liver. Prednisolone is further metabolized to inactive compounds in the liver. The metabolites are excreted mainly in the urine.

Formulation and stability: Prednisone is available as various strength tablets and oral solution from multiple manufacturers. All dosage forms can be stored at room temperature. At St. Jude Children's Research Hospital, prednisolone oral solution may be substituted for prednisone liquid at equal doses due to its superior palatability.

Supplier: commercially available.

Toxicity: Side effects of prednisone vary depending on the duration of its use. Side effects that can occur with short term use include sodium and water retention with associated hypertension, peptic ulcer with possible perforation and hemorrhage, increased susceptibility to infections, emotional instability, insomnia, increased appetite, weight gain, acne and hyperglycemia. Side effects more commonly associated with prolonged use include cataracts, increased intraocular pressure and associated glaucoma, development of a "cushingoid" state, compression fractures, menstrual irregularities, suppression of growth in children, secondary adrenocortical and pituitary unresponsiveness particularly in times of stress as in trauma, surgery or illness, osteoporosis and muscle wasting.

5.13 Rituximab (Rituxan®)

Source and pharmacology: Rituximab is a murine /human chimeric monoclonal antibody. It is specific for the CD20 antigen located on B-cells. Rituximab has been shown to mediate complement-dependent tumor cell lysis and antibody-dependent cellular cytotoxicity. Direct binding to the CD20 antigen is thought to play a role in inhibition of cell growth. Rituximab is administered intravenously. The mean serum half-life after a single IV dose of 375 mg/m^2 is 59.8 hours (range 11.1-104.6 hours).

Formulation and stability: Rituximab is available as 100 mg/10 ml single-use and 500 mg/50 ml single-use vials. Each vial also contains sodium chloride 9mg/ml, sodium citrate 7.35 mg/ml, polysorbate 80 0.7 mg/ml and water for injection. Rituximab for injection concentration must be diluted with 5% Dextrose or 0.9% NaCl prior to administration. After dilution, unused drug is stable for 24 hours when refrigerated (2-8 degrees Celsius) and 12 hours at room temperature. Vials should be protected from direct sunlight.

Supplier: Commercially available.

Toxicity: Hypersensitivity reactions may occur; therefore, premedication with acetaminophen and diphenhydramine should be considered before each infusion. The most common toxicities are infusion related and may include chills, fever, headache, nausea, vomiting, angioedema (13%), hypotension (10%), bronchospasm (8%), and arrhythmia. Other possible adverse reactions include thrombocytopenia, myalgias, arthralgias, asthenia, and throat irritation.

Dosage and route of administration: Do not administer as an intravenous push or bolus. Infusions should be initiated at 50 mg/hour for 30 minutes. If hypersensitivity or infusion-related events do not occur, subsequent infusions can be administered at an initial rate of 100 mg/hour and increased by 100 mg/hour every 30 minutes as tolerated. Infuse at a maximum rate of 400 mg/hour.

5.14 Vincristine

Source and pharmacology: Vincristine is an alkaloid obtained from the periwinkle *(Vinca rosea)* plant. It reversibly binds to microtubule and spindle proteins causing metaphase arrest. Vincristine has poor penetration into the CSF. It is approximately 75% protein bound. Extensive metabolism occurs in the liver. Excretion is primarily in the bile. A dosage decrease is recommended in patients with a bilirubin > 3 mg/dl.

Formulation and stability: Vincristine is supplied in multiple-dose 1 mg/ml vials containing 1 ml, 2 ml and 5 ml. The intact vials should be stored under refrigeration and protected from light.

Supplier: Commercially available.

Toxicity: Dose limiting toxicity is neurotoxicity. This can be characterized by constipation and/or paralytic ileus, ptosis, vocal cord paralysis, weakness, jaw pain, abdominal pain, peripheral neuropathies, loss of deep tendon reflexes and “foot drop”. Peripheral neuropathy is often the first sign of neurotoxicity and is initially reversible. Other toxicities reported include alopecia, mild nausea and vomiting, SIADH, myelosuppression, orthostatic hypotension, optic atrophy, transient cortical blindness, and auditory damage. Acute shortness of breath and severe bronchospasm have been reported following the administration of vinca alkaloids. Myelosuppression is rare at usual doses. Vincristine is a vesicant and may cause severe tissue damage if extravasation occurs. NOTE: dose reduction may be necessary in patients < 1 year of age. Dosing on a per kg (rather than per m^2) basis has been advocated for infants in order to decrease toxicity.

6.0 EVALUATIONS, TESTS, AND OBSERVATIONS

6.1 Suggested Evaluations Before and During Therapy – for Good Medical Care**

All entry/eligibility studies should be performed within 2 weeks prior to entry onto the trial (unless otherwise specified). Imaging studies are required within 4 weeks prior to study entry.

	Pre-Treatment	COP	Induction	Consolidation	Continuation	Off Therapy	Follow-Up
H & P	X		Weekly	Weekly	Prior to each Rx	X	See Section 6.4
CBC & Diff	X		Weekly	Weekly or as indicated	Weekly or as indicated	X	See Section 6.4
CMP, Mg++, PO_4, LDH, uric acid	X	Daily	Weekly	Weekly	Weekly or as indicated	X	
Hepatitis Screening (Section 3.1.1) – required for patients receiving rituximab	X						
PT/PTT, INR, fibrinogen, ESR	X						
U/A	X	Daily	Prior to Rx	Prior to Rx	Prior to Cyclophosphamide	X	
Bone marrow aspiration & biopsy (bilateral)	X		See below CR Evaluation	See below CR Evaluation		X	1 year from enrollment
CSF evaluation*	X					X	1 year from enrollment
CT – Neck, chest, abdomen, pelvis	X	CT of tumor on day 7 COP	See below CR Evaluation Group A	See below CR Evaluation Groups B and C		As clinically indicated	As clinically indicated
FDG-PET	X	Day 7 (research – See Table 6.2) (***to be done only in patients who do not require sedation***)	See below CR Evaluation Group A	See below CR Evaluation Groups B and C		As clinically indicated	As clinically indicated
EKG, 2D-echocardiogram	X				Prior to each dose of Adriamycin, if clinically indicated	X	2 and 5 years from enrollment

CMP-comprehensive metabolic panel (Na+, K+, Cl, CO_2, Ca++, BUN, Cr, Blood sugar, total protein, albumin, Alk Phos),
Obtain other studies as needed for good patient care.
** Also with all IT Treatments; first CSF examination done with first intrathecal treatment after enrollment*
***Participants of childbearing potential require a negative pregnancy test prior to starting treatment.*
CR Evaluation: Includes CSF exam (not required if no CNS involvement at diagnosis), bone marrow exam (not required if bone marrow negative at diagnosis) and imaging studies of primary tumor sites or as clinically indicated. The time of evaluation varies with group: Group A - after completion of COPAD #2, Group B - after completion of CYM #1, Group C - after completion of CYVE #2

6.2 Suggested Evaluations for Research

The following research tests are optional, but strongly encouraged

	Pre-Treatment***	COP	Induction	Consolidation	Continuation	Off Therapy	Follow-Up
Plasma 1,25-dihydroxyvitamin D	X					X	
25-hydroxyvitamin D, osteocalcin	X					X	
Spot urine for Mg, creatinine, calcium, and calcium:creatinine ratio (obtain 24 hour if calcium:creatinine exceeds 0.2).	X					X	
Fertility Studies (See Section 6.4.2.1)	X					X	2 and 5 years from enrollment
Serum Troponin T, Natriuretic hormone levels (NT-PRO BNP) (see Section 6.4.2.2)	X					X	2 and 5 years from enrollment
Serum for XLP studies**	X						
Transcranial Doppler studies (TCD) ¥	X		X (Day 15 COPADM)			X	1 year from enrollment
QCT- BMD¥	X					X	1 and 5 years from enrollment
PET-CT¥ (***to be done only in patients who do not require sedation***)		Day 7 - research				X	
MRI Brain*¥ (***to be done only in patients who do not require sedation***)	X		X (Day 15 COPADM #1)	See below CR Evaluation		X	1 year from enrollment
Psychological Testing¥	X						1, 3 and 5 years from enrollment

*PET-CT on day 7 is research and s**hould only be done in patients who do not require sedation**. Other PET-CT scans performed as part of good medical care per table 6.1*
CR Evaluation: Includes CSF exam, bone marrow exam and imaging studies of primary tumor sites or as clinically indicated. The time of evaluation varies with group:
Group A - after completion of COPAD #2; Group B - after completion of CYM #1; Group C - after completion of CYVE #2
**For Group B and C only*
***XLP studies may be obtained after chemotherapy has started. The results of XLP studies will be reported to the treating physician for those patients enrolled outside of St. Jude. For those treated at St. Jude, the primary MD will discuss the results with the family and the patient will be referred for genetic counseling, if clinically indicated.*
****If research imaging and psych testing cannot be scheduled pre-treatment, can obtain as soon as possible after therapy starts.*
¥Day 7 PET, psychological testing, Brain MRIs, QCT-BMD, transcranial Doppler studies, Troponin T levels, natriuretic hormone levels will not be done at Rady Children's Hospital/UCSD.

6.3 Advanced MR Imaging Methods

6.3.1 MR diffusion and perfusion imaging

In addition to the conventional MR neuroimaging prescribed for clinical evaluation, diffusion tensor imaging (DTI) will be acquired as a set of diffusion images in six or more directions. DTI will be acquired using bipolar diffusion-encoding gradients to reduce gradient-induced eddy currents that cause image distortion and degradation.[112,113] All images will be acquired at either 1.5 or 3 T using a double spin echo EPI pulse sequence (TR/TE = 10/100 ms, b=1000 or 700 ms). Imaging sets will be acquired as forty 3 mm thick contiguous sections with whole-head coverage and a 128 square matrix. These data sets will be used to fully define a diffusion tensor for each point in the image[114,115] Once the tensors have been calculated, Eigen values can be derived and used to calculate apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps for the whole brain. A commercial workstation and software will be used for post processing of this data as well as other custom analysis software developed at St. Jude. These measures are related to the integrity of the myelin sheath which restricts the diffusion of water perpendicular to the axons within the white matter fiber tracts.

Perfusion imaging will be evaluated with a dynamic-susceptibility contrast imaging. Imaging is acquired as fifteen 4 mm thick contiguous sections centered to ensure coverage across the entire corpus callosum and with a 256 square matrix to match the resolution of the conventional imaging. T2*-weighted images will be acquired with an axial echo-planer gradient-echo sequence (TR/TE = 2000/50 ms, 1 average). Fifty imaging sets with a temporal sampling of every 2 seconds will be acquired after the contrast injection. Standard bolus tracking of 0.1 mmol/kg gadolinium followed by a 20 ml saline flush, both delivered at 1 ml/s will be performed. A commercial workstation and software will be used for post processing of this data as well as other custom analysis software developed at St. Jude.

6.3.2 Proton MR Spectroscopy

Proton MR spectroscopy will be performed using FDA approved commercially available data acquisition technology, provided by vendor (Siemens Medical Systems). Typically, 2D CSI (chemical shift imaging) technique will be used, depending on lesion extension within cerebral white matter, either single slice, or multislice. Occasionally, single voxel technique may be employed. Post processing of raw data will be done off line, using a multimodality workstation, provided by the same vendor using proprietary software. Individual, voxel-based MR spectra as well as metabolic maps of the entire region of interest for NAA (N-acetylaspartate), Cho (choline compounds), Cr (creatine) will be generated. Metabolic ratios (typically Cho/Naa) will be calculated based on relative metabolic concentrations.

6.4 Suggested Long-Term Follow-up Evaluations

6.4.1 Investigations at completion of therapy

At completion of therapy all initially positive sites should be investigated to confirm remission (radiological, bone marrow, and cerebrospinal fluid examinations).

6.4.2 Suggested Follow-up and Late Effects Monitoring

Upon completion of therapy, it is *suggested* that participants be seen on a monthly basis (PE, CBC, chem panel including LDH) until the one year anniversary date from diagnosis. After one year from diagnosis, it is suggested that participants be seen at 3 monthly intervals (PE, CBC, chem panel including LDH) for the next year, and then annually (PE, CBC, chem panel including LDH). The late effects that may arise following this treatment include cardiotoxicity and infertility.

Suggested Fertility Studies

The following tests/evaluations are recommended to monitor for late effects on fertility at the following time points: Pre-treatment, off therapy, two years from enrollment, five years from enrollment, at Tanner III and V (if not already studied):

Males

- Pubertal development evaluation (annually)
- FSH, LH, and testosterone (annually)
- Semen analysis (through Fertility Associates of Memphis)
- Inhibin B level

Females

- Pubertal development evaluation (annually)
- FSH, LH, and Estradiol (annually)
- Anti-mullerian hormone (AMH)

Suggested Cardiac Studies

The following tests/evaluations are recommended to monitor for cardiac late effects at the following time points: pre-treatment, off therapy, **two** year from enrollment, and five years from enrollment:

- 2D-Echocardiogram
- Troponin T levels (will not be done at UCSD)
- Natriuretic hormone levels (NT-PRO BNP) (will not be done at UCSD).

6.5 Modifications for Collaborating Sites Participating in Therapeutic & Biological Objectives

The evaluations listed in Table 6.2 as indicated by "¥" will not be performed at Rady Children's Hospital, San Diego. This includes the PET scan on Day 7, Psychological testing (all evaluations), Brain MRI (all evaluations), pre-treatment bone density/BMD-QCT, transcranial Doppler studies, Troponin T levels, natriuretic hormone levels.

7.0 EVALUATION CRITERIA

7.1 Definition of Evaluable Disease

All abnormal sites at diagnosis should be evaluated using whatever modality is most appropriate. If bone marrow or CNS is involved, marrow and CSF should be examined. The timing of investigations is described in Section 6.0.

7.2 Definition of Response

Complete response: Complete disappearance of all measurable or evaluable lesions (except bone), no L3 blasts in the bone marrow or in the CSF.

Incomplete response: 20-99% reduction in the product of the two largest diameters (perpendicular) of measurable lesions and/or in the case of leukemia 20-99% reduction in the number of L3 blasts in the bone marrow and/or in the CSF.

Non-response: < 20% tumor reduction of the product of the two largest diameters (perpendicular) of measurable lesions, or tumor progression, or tumor re-growth after initial shrinkage.

Relapse: Defined as recurrence of disease at any site after achieving a CR.

Failure: defined as relapses, deaths and failures to achieve a CR within the time frame described in the protocol.

7.3 Response Evaluations According to Treatment Group

7.3.1 Group A: participants who are not in remission after two courses of COPAD (confirmed histologically) will advance to induction in Group C ($COPADM_8$ Course 2).

7.3.2 Group B: Participants with < 20% tumor reduction after COP will move to Group C, starting at $COPADM_8$ Course #1. Those remaining in Group B should be in complete remission after completing CYM Course #1. Residual masses are to be biopsied (surgical consultation is suggested). If there is histologic confirmation of viable tumor, the participant will be moved to Group C, starting with CYVE #1 as per Section 4.6.6. If the mass contains no histological evidence of viable tumor, the participant is considered to be in complete remission and will stay on treatment.

7.3.3 Group C: Participants should be in complete remission after completing CYVE Course #2. Residual masses are to be biopsied (surgical consultation is suggested). If the mass contains histologic evidence of viable tumor, the participant will be removed from protocol treatment. If the mass contains no viable tumor, the participant is considered to be in complete remission and stays on treatment.

7.4 Toxicity Evaluation Criteria

Common Terminology Criteria for Adverse Events v4.0 (CTCAE): This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page (http://ctep.info.nih.gov). Additionally, toxicities are to be reported on the appropriate data collection screens/forms.

7.5 Acceptable Percentage of Missed Doses for Commercially Available Drugs

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB. However, it is expected that participants will occasionally miss some doses or receive the wrong dose of oral chemotherapy. Compliance with oral medication will be captured in the CRIS database and appropriately documented in the participants' medical records. Appropriately documented doses of missed or wrong doses of chemotherapy will not constitute a deviation unless the amount in question is over *10%* of the expected total dose due in the respective protocol cycles (these are specified in the CRIS SJBC3 database). Missed doses do not include doses held or reduced for medical reasons (toxicity, illness) and will not be considered protocol deviations or violations.

8.0 OFF PROTOCOL TREATMENT AND OFF-STUDY CRITERIA

8.1 Off Treatment Criteria

A patient will be taken off SJBC3 treatment if any of the following occurs:

- Failure to achieve a complete response to induction therapy:
  - Group A: failure to achieve CR after CYVE #2
  - Group C: failure to achieve CR after CYVE #2
- Bone marrow or extramedullary relapse
- Second malignancy (e.g., therapy-induced AML or brain tumor)
- Development of unacceptable toxicity during treatment (with concurrence of the PI or co-PI)
- Patients with severe congenital immunodeficiency (e.g. ataxia telangiectasia) or HIV infection will be taken off SJBC3 therapy and will receive alternate therapy (to prevent excessive toxicities) with curative intent.
- Completion of all protocol-prescribed treatment
- Participant/family decision to withdraw from protocol treatment at any time for any reason
- Investigator decides continued protocol treatment is no longer in patient's best interest

8.2 Off Study Criteria

A patient is taken off SJBC3 study if any of the following occurs:

- Death
- Lost to follow-up
- Withdrawal of consent for continued follow-up
- Found to be ineligible (e.g., incorrect diagnosis)

Follow up will stop at the time the patient is off study. The follow up time of an off-study patient is censored at this point, and no outcome data beyond the off-study time will be used in analyses.

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Reporting Adverse Experiences (AEs) and Deaths to the St. Jude IRB

Only "unanticipated problems involving risks to participants or others" referred to hereafter as "unanticipated problems" are required to be reported to the St. Jude IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only adverse events that constitute unanticipated problems are reportable to the St. Jude IRB.

As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Deaths that occur > 30 days after protocol treatment that expected and unrelated to protocol therapy do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death; this includes any

death that occurs while the participant is on treatment or that occurs ≤ 30 days of last protocol treatment, regardless of cause.

The following definitions apply with respect to reporting adverse experiences:

Serious Adverse Event: Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

Unexpected Adverse Event:

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Internal Events: Events experienced by a research participant enrolled at a site under the jurisdiction of St. Jude IRB for either multicenter or single-center research projects.

External Events: Events experienced by participants enrolled at a site external to the jurisdiction of the St. Jude Institutional Review Board (IRB) or in a study for which St. Jude is not the coordinating center or the IRB of record.

Unanticipated Problem Involving Risks to Subjects or Others: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur, and which increases the degree of risk posed to research participants.

Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Consistent with FDA and OHRP guidance on reporting unanticipated problems and adverse events to IRBs, the St. Jude IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an "unanticipated problem involving risks to subjects or others" it must be reported in accordance with this policy. In general, to be reportable external events need to have implications for the conduct of the study (for example, requiring a significant and usually safety-related change in the protocol and/or informed consent form).

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events. Examples of unanticipated problems involving risks to subjects or others include:

- Improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

9.2 Recording AEs and SAEs

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into CRIS protocol-specific database. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing), 4) action taken for event, 5) patient outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "SAEs".

These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above.

Cumulative summary of Grade 3-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

The study team will meet regularly to discuss AEs (and other study progress as required by institutional DSMP). The PI will review Adverse Event reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his/her review and approval of the AE as entered in the research database.

9.3 Reporting AEs to and from St. Jude and Collaborating Sites/Affiliates

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE report from collaborating sites for AEs that are serious, unexpected, and at least possibly related to protocol treatment or interventions will be reported to site IRB and the St. Jude IRB within the reporting requirements described above. The PI will determine if this is an event that will need to be reported expeditiously to all participating sites, considering the following criteria:

- Is the AE serious, unexpected, and related or possibly related to participation in the research?
- Is the AE expected, but occurring at a significantly higher frequency or severity than expected?
- Is this an AE that is unexpected (regardless of severity that may alter the IRB's analysis of the risk versus potential benefit of the research *and*, as a result, warrant consideration of substantive changes in the research protocol or informed consent process/document?

With the submission of the "Reportable Event" in St. Jude TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects). Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI.

A cumulative summary of Grade 3-5 AEs and expected/unrelated deaths that occur more than 30 days off last protocol treatment will be reported to all sites with study progress report at the time of continuing review.

10.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

10.1 Data Collection

Electronic case report forms (e-CRFs) will be completed by the St. Jude Leukemia/Lymphoma CRAs. Data will be entered from record directly into a secure CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Leukemia/Lymphoma Division, working with Dr. Ribeiro or his designee. All protocol-specific data and all grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary St. Jude medical chart.

Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review.

10.2 Data Collection Instructions for Collaborating Sites

Collaborating sites will collect abstract data from medical records and submit by using e-CRFs via remote electronic data entry. All protocol-specific data and all grade 3-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of study phase.

10.3 Study Monitoring

Monitoring of this protocol is considered to be in the **moderate risk** category, as per the St. Jude Data Safety and Monitoring Plan (DSMP).

The study team will hold monthly meetings and review case histories or quality summaries on participants.

Source document verification of eligibility and informed consent for 50% of St. Jude participants will be performed by the Eligibility Coordinators within 5 working days of completion of enrollment.

The Clinical Research Monitor will perform monitoring of applicable essential regulatory documentation. Also, reviewing for the timeliness of serious adverse event reporting (type, grade, attribution, duration, timeliness and appropriateness) for selected study participants *semi-annually* and track accrual continuously. The monitor will verify those data points relating to the primary study objective for a certain number of study enrollees as specified in the Moderate Risk monitoring plan checklist for this study. Protocol compliance monitoring will include participant status, safety assessments, eligibility, the informed consent process,

participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Monitoring may be conducted more frequently if deemed necessary by the CTO or IMC. Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB/OHSP.

This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in TRACKS.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the DSMP.

10.4 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and St. Jude clinical research monitors.

11.0 STATISTICAL CONSIDERATIONS

11.1 Biological Aims

The SJBC3 trial has several exploratory biological studies as its primary objectives. Statistical considerations on accrual, sample size, and levels of statistical errors for the first aim are given below.

To perform transcriptional profiling and genome-wide analysis of DNA copy number abnormalities and loss-of-heterozygosity using DNA microarrays in children with newly diagnosed mature B cell lymphomas (e.g. Burkitt lymphoma/leukemia, DLBCL, and MLBCL) from different parts of the world.

Accrual. It is difficult to get a precise projection of accrual rate at this point although we have some expectation based on prior experience. Below we give a conservative projection for the biological study in terms of number of evaluable tumor samples after 5 year accrual although we anticipate the trial may run beyond 5 years. Experience from the SJBCII trial indicates that the annually 6 patients can be accrued at St. Jude, giving approximately 30 patients after 5 years of accrual at this institution. Because there is not another childhood B-cell lymphoma trial currently in US, we anticipate the accrual may increase to 8 to 12 patients per year. The genomic analysis requires tumor biopsy samples from participating international institutions along with St. Jude. The international institutions will participate in the biological studies and provide biopsy samples but will not participate in the SJBC3 therapy. We also note that it is

rather difficult to obtain tumor biopsy samples from patients enrolled here for various logistical reasons. We expect that there will be about 10, 1 to 2, and 5 per year from Latin America, North America (St. Jude), and other geographic regions respectively. Five year accrual can provide about 50, 5 to 10, and 25 tumor samples respectively. Approximately 80% of the tumors are expected to be Burkitt's lymphoma (BL). Moreover, we conservatively assume that 80% of the samples from each region are eventually evaluable for genomic analyses, resulting in 40, 8 and 20 evaluable tumor samples from Latin America, North America, and other regions respectively.

Five year accrual of evaluable tumor samples from each region for this aim is summarized below.

Number of evaluable tumor samples after 5-year accrual

	BL	Non-BL	Total
Latin America	32	8	40
North America	6	2	8
Other	16	4	20
Total	54	14	68

At this point we set the accrual target for the biological aims as 68 evaluable tumor samples and anticipate completing accrual in 5 to 6 years for the biological aims. We set the accrual target for other aims to 60 patients treated at St. Jude and anticipate completion in 5 to 6 years. We will amend the protocol to extend the enrollment time if the targets cannot be met in 5 years.

Amendment 3.0 update:

We recognize that we need an extension in duration of study to complete the biology (genomics) objectives. We anticipate that the study will need to remain open for an additional 5 years. We estimate that we will accrue an additional 40 participants on the therapeutic and biology part of the study during that time, for a total enrollment of 168 patients (100 therapeutic and biology, and 68 biology only).

Increasing treatment enrollment/accrual for the "Therapeutic and Biology" component is justified by (1) providing potentially more tumor samples from North America, and (2) strengthening the analyses of the exploratory objectives, especially the very promising preliminary results of the neuroimaging studies (abstract included in earlier continuing review report).

Analyses. There will be three major components in the analyses for this objective. Each is discussed below, with very limited preliminary genomic data in pediatric lymphomas.

Gene differential profiling of BL vs. non-BL. Identification of gene differential expression signatures requires inference (comparison) of gene expression levels in BL vs. non-BL. It is important to realize that the tumor samples come from dramatically different geographical regions where ethnic and environmental factors (e.g., diet) can greatly affect gene expression. Additionally, the extent of differential expression between BL and non-BL may also be

affected by environmental, endemic and related biological factors (e.g., involvement of EBV in BL). Thus, it is important to adjust the gene expression comparison by geographic region, which serves as an aggregate of all the possible region-related confounding and stratification factors, in the absence of the data on such factors. Each participating institute defines a geographic region; from example, St. Jude defines the "North America" region. At this point we expect in addition to St. Jude, there will be one institute in Brazil (Latin America) and one in Singapore (Asia) participating the biological studies. Modeling, testing, and interpretation for a single gene (probeset) is given below, followed by methods of handling massive multiple tests. For samples with suitable RNA quality, gene expression profiling will be performed using transcriptome sequencing in preference to microarray analysis, in view of the more complete data provided by this platform.

The following two-factor Poisson regression model will be used for each gene, where E[Y(bgi)] denotes the mean expression of a gene in the i-th tumor sample from region g (g=Latin America, North America, etc.) and disease b (b=BL, non-BL):

$$\text{Log}\{E[Y(bgi)]\}=\mu+\alpha(b)+\beta(g)+\alpha\beta(g,b).$$

From a completely biological stand point the primary interest here is the main effect α(b). For each mRNA expression we will test the null hypothesis α(BL)= α(non-BL)=0 against the two-sided alternative at least one of them is non-zero. Statistical significance of this effect indicates that it is likely the gene is differentially expressed between BL and non-BL. From a molecular epidemiology (and to a lesser extent biological) stand point the disease-region interaction effect αβ(g,b) is important, as it signifies that the extent of differential expression between BL and non-BL may be affected by geographical region. Statistical significance of this interaction effect indicates that it is likely the differential expression between BL and non-BL is different from one region to another; this would be an epidemiologically and biologically interesting finding as it helps to define the RNA-level differences between endemic and non-endemic Burkitt lymphoma.

Hence, we will be testing the main effect α(b) and the interaction effect for each RNA expression measurement. Because EBV positivity in BL may have an important effect on gene differential expression, in the final analysis we will also consider defining the disease main effect α(b) as a three-level factor: b = EBV+ BL, EBV- BL, non-BL.

It is imperative to assess the levels of statistical errors, especially false positives, made in these massive multiple tests. An assessment of the level of false negative errors with the available sample size will be given in the next paragraph. Following the published microarray studies many of which from St. Jude, we will first assess the ensemble distribution of the P values and use the profile information criterion[116] to obtain a data-suggested level of statistical significance and the corresponding estimated false discovery rate (FDR),[116,117] and then estimate the false discovery rate corresponding to a set of statistical significance levels specified around the data-suggested level. Estimates of FDR will be computed using methods in the literature.[116,118,119] We will analyze further the "significant genes" so identified by modeling there expressions with disease subtype as well as potential confounding factors as explanatory variables.

Recognizing the fast changes in biotechnology, we'll use the most effective technology and appropriate statistical methods at the time of final analyses.

Levels of statistical errors – an assessment by published data. All of the published gene expression profiling studies of mature B-cell lymphomas focused on adults, but Hummel et al. (2006)[120] included a number of pediatric samples (age≤18 years). None of the published studies considered geographic region as a factor and thus there is no preliminary data available. Therefore the preliminary microarray gene expression data on pediatric mature B-cell lymphomas are very limited. Nonetheless we provide a rough assessment of the levels of false positive and false negative errors that we may encounter in the analysis based on the pediatric data[120] followed by a discussion of caveats and limitations of this assessment.

Here we consider the tests of the main effect α(b) for the m (≈ 22000) Affymetrix GeneChip probesets. Adapting the concept of anticipated FDR (aFDR)[121] to the fixed sample size situation and the planned analysis outlined above, we define the aFDR at a given significance level α as

$$aFDR(\alpha) = \frac{\pi_0 \alpha}{\pi_0 \alpha + (1-\pi_0) AP}.$$

Here π_0 is the proportion of true null hypotheses (non-differentially expressed genes), and AP is the *average power* which depends on the effect size, sample size, and significance level. The quantity $(1-\pi_0)(1-AP)$ is the expected proportion of true alternative hypotheses the multiple tests fail to capture, hence a measure of the level of false negative errors; we call this the *false negative proportion.*[116]

Hummel et al. (2006) included 32 pediatric samples run on Affymetrix U133A GeneChip.[120] In these pediatric lymphoma samples 6 are traditional BL, 10 are atypical BL, 12 are DLBCL, and 4 are aggressive B-NHL. The 32 samples are put into two groups: BL (traditional and atypical BL) and non-BL (DLBCL and aggressive B-NHL). To get some preliminary information about π_0, per-probeset comparisons of BL vs. non-BL by the two-sample t test yield 22,215 P values, one for each probeset. The profile information criterion (Ip)[116] produces a significance threshold (P value cut-off) to declare the significant tests in a way to balance the levels of false positive and false negative errors, and a conservative (biased up) estimate of the corresponding FDR; it also provides an estimate of π_0 as part of the procedure. Applying this procedure to the 22,215 P values yields an estimate of π_0 0.6525 (65.25%), the significance threshold 0.004547, and the corresponding FDR 0.1377 (13.77%) among the 478 probesets declared differentially expressed at the 0.004547 significance level. For each probeset, the mean in the BL and non-BL group along with the within-group variance are computed to provide an estimate of the non-centrality parameter of the t test, and then is shrunk toward zero by the q-value weighting as described.[121] The probesets are then sorted by the magnitude of the shrunken non-centrality parameter in descending order. To be on the conservative side, for each postulated null proportion ($\boldsymbol{\pi_0}$) in the table below, the non-centrality parameters of the top $m(1-\boldsymbol{\pi_0})$ probesets are all set to the same as the $[m(1-\boldsymbol{\pi_0})]$th largest one, and the remaining non-centrality parameters are set to zero. Next, average power (AP) and $\boldsymbol{\pi_0}$ is estimated after this additional shrinkage, giving enlarged $\boldsymbol{\pi_0}$.

Based on the π_0 estimate and the significance threshold suggested by Ip, we provide the final non-centrality after shrinkage, estimates of aFDR, the false negative proportion FNP, and average power (AP) for a range of postulated and enlarged π_0 values and significance levels (P value cutoffs) in the following table.

Anticipated False Discovery Rate and False Negative Proportion

		π_0					
	Postulated	**0.60**	**0.65**	**0.80**	**0.90**	**0.95**	**0.99**
	Enlarged	**0.7969**	**0.7994**	**0.8469**	**0.9117**	**0.9532**	**0.9902**
	Non-centrality	**3.39**	**4.17**	**7.57**	**11.82**	**15.81**	**24.38**
α	**0.0010 aFDR**	0.1107	0.0823	0.0388	0.0306	0.0372	0.1116
	FNP	0.3874	0.3344	0.1726	0.0673	0.0236	0.0019
	AP	0.0315	0.0443	0.1371	0.3268	0.5274	0.8079
	0.0025	0.1471	0.1141	0.0621	0.0599	0.0737	0.2241
		0.3773	0.3229	0.1583	0.0564	0.0180	0.0012
		0.0568	0.0773	0.2086	0.4358	0.6405	0.8783
	0.0045	0.1775	0.1417	0.0850	0.0830	0.1142	0.3330
		0.3673	0.3120	0.1464	0.0487	0.0144	0.0009
		0.0818	0.1086	0.2680	0.5134	0.7114	0.9144
	0.0050	0.1836	0.1473	0.0899	0.0891	0.1235	0.3554
		0.3651	0.3096	0.1440	0.0472	0.0138	0.0008
		0.0872	0.1153	0.2798	0.5278	0.7237	0.9201
	0.0075	0.2093	0.1715	0.1120	0.1171	0.1658	0.4474
		0.3555	0.2994	0.1342	0.0416	0.0115	0.0006
		0.1111	0.1444	0.3287	0.5838	0.7696	0.9396
	0.0100	0.2298	0.1911	0.1311	0.1419	0.2030	0.5160
		0.3474	0.2909	0.1267	0.0376	0.0100	0.0005
		0.1315	0.1687	0.3667	0.6239	0.8003	0.9514

For example, if the proportion of non-differentially expressed probesets π_0=0.65 (65%) and all probesets with P value ≤ α=0.0045 are declared as significantly differentially expressed between BL and non-BL, we anticipate that the false discovery rate aDFR=0.1417, i.e., 14.17% of these "significant" probesets are false positives; and that the false negative proportion FNP=0.3120, i.e., we expect to miss 31.2% of the truly differentially expressed probesets.

It should be noted that the non-centrality parameters estimated above in fact correspond to one-factor ANOVA. Unfortunately however, there is no preliminary data on the second factor (geographic region). The numerator of the non-centrality parameter determined from the one-factor model could be too optimistic for the two-factor mode, but the denominator (the within-group/residual standard deviation) determined by the one-factor model may be larger than that from the two-factor model.

The total number of tests performed for this analysis will be over 44,000 although the above error assessment was done for 22,000+ tests. Note however, aFDR and FNR do not depend directly on the total number of tests; rather they depend directly on the proportion of true null hypothesis π_0.

It should be noted that there will be very limited statistical power for gene expression profiling for BL vs. non-BL in a single geographic region, except perhaps in Latin America where the sample size is the largest among all regions.

Additionally, unsupervised analyses such as hierarchical clustering will be performed to explore novel subtypes in childhood lymphomas. Also, identified gene differential signatures will be compared with published results (in adult lymphomas) using methods such as gene set enrichment analysis.

Catalog and estimate frequencies of copy number variations in childhood lymphomas. There is no preliminary data on copy number variations (CNVs) in childhood lymphomas. A study of DLBCL in adults[10] reported the prevalence of CNVs in certain subsets ranged roughly from 4% to 40%. So, conservatively, with 14 samples in the non-BL group, if a CNV is observed in 1 sample (sample proportion 0.07), then the exact 95% confidence interval is [0.0037, 0.3122]; if a CNV is observed in 6 samples (sample proportion 0.43), the exact 95% confidence interval is [0.2061, 0.6878]. Additionally, we will compare the prevalence of CNVs between different subtypes of childhood lymphomas and geographic regions with exact chi-square or Fisher's test, taking population structures into account if necessary. DNA extracted from peripheral blood will be used as matched control tissue. Loss of heterozygosity (LOH) will be analyzed by comparison of SNP genotype data of the tumor sample to matched germline samples, or, in the case of samples lacking a matched germline sample, pools of reference samples already accrued at St Jude. LOH data will be analyzed using publicly available algorithms (e.g. dChip using the Hidden Markov Model) and alternative algorithms that will be tested and developed at St Jude during the course of the study. These analyses will complement analyses identifying copy number alteration, and will permit identification of both deletional and copy-neutral loss-of-heterozygosity.

Integrated analysis of CNVs and gene expressions. For CNVs with a reasonable prevalence, we will explore the association between the identified CNVs and gene expressions in the study cohort. We will identify individual gene expression correlated with a CNV, and a gene expression profile (signature) associated with a CNV. General linear models will be used to perform statistical tests, and multiple tests will be addressed using FDR, as described before.

To describe the types and frequency of mutations in the ARF-HDM2-TP53 pathway, in "non-endemic" B-cell lymphomas (United States) and those found in selected geographic regions of the world (Brazil, Chile, Guatemala, Honduras, El-Salvador, Singapore, and others)

This exploratory aim will be analyzed by enumerating the types of mutations and estimating the frequency of each type of mutation in each geographical region as well as in all regions pooled. Point estimates and 95% confidence intervals (CI) will be provided.

To describe the expression of ARF-HDM2-TP53 and PUMA-associated pathways in non-endemic B-cell lymphomas (United States) and that found in B-cell lymphomas of other selected geographic regions of the world (see above)

Protein as well as mRNA expression levels of important genes on these pathways from various bioassays will be summarized using descriptive statistics including mean, standard deviation, and the five-number summary in each geographical region as well as in all regions pooled.

To describe the pattern and frequency of XLP gene mutations presenting with B-cell lymphomas in the United States and selected geographic regions.

Point estimates and 95% CIs of the frequency of XLP mutation among boys will be calculated in each geographical region as well as in all regions pooled. Relationships of XLP mutations with clinical phenotypes will be explored by appropriate statistical models including contingency table analysis and/or logistic regression modeling (for categorical/binary phenotypes), general linear models for continuous phenotypes, and hazard regression models for right-censored failure times.

To describe the frequency of EBV-positive Burkitt lymphomas between "non-endemic" and endemic B-cell lymphomas;

a. *To describe the pattern of EBV protein and gene expression (e.g., EBNA 3) in EBV-positive lymphomas.*
b. *To relate EBV protein and gene expression with clinical, laboratory and outcome data.*

It is expected that among BL, about 80% are EBV positive in endemic BL (Latin America, Africa) and 45% are EBV positive in non-endemic BL (US, Mideast), and about 80% of the available tumor samples are BL. Point estimates and 95% CIs of the frequency of EBV-positive BL will be calculated for each geographical region. EBV protein expression will be described by summary statistics including mean, standard deviation, and the five-number summary in each geographical region as well as in all regions pooled. Relationship of EBV protein expression with clinical, laboratory, and outcome features will be explored using appropriate statistical methods such as general linear models.

11.2 Exploratory Aims

Acute and Late Effects Objectives

These objectives are completely exploratory. Only patients treated at St. Jude will be asked to participate in these studies. As stated earlier, we anticipate the accrual rate of 8-12 patients per year and expect to accrual approximately 100 patients in 12 years. We also note that although we planned 6 year accrual at St. Jude for this and the outcome aim, similar to SJBCII, the SJBC3 trial will run beyond five years in this institution. SJBCII opened in September 1994 with the first and last patient enrolled on 12/6/1994 and 9/23/2005 respectively. Descriptive statistics and appropriate statistical models (regression, logistic regression, etc.) will be applied to analyze the data obtained for these aims.

To estimate the complete response, event-free survival, and overall survival rates in patients with Burkitt lymphoma (BL), Burkitt leukemia/B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL) treated with a stage-adapted regimen based on the SJBCII protocol.
All eligible patients treated at St. Jude will be included in the analysis. SJBCII experience shows the accrual rate at St. Jude is about 6 per year; thus approximately 30 patients after 5 years. It should be noted that although we plan 6 year accrual at St. Jude, similar to SJBCII, the

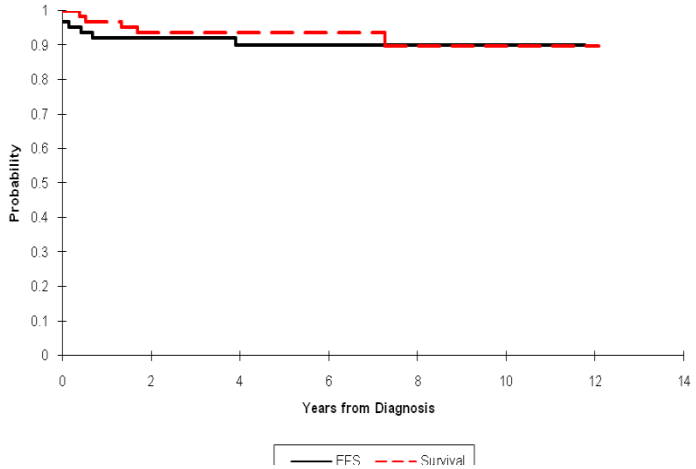
SJBC3 trial may run beyond 6 years in this institution. SJBCII opened in September 1994 with the first and last patient enrolled on 12/6/1994 and 9/23/2005 respectively.

Complete response (CR), event-free survival (EFS), and overall survival (OS) will be analyzed only for eligible patients treated at St. Jude. Point estimate and exact 95% CI based on the binomial distribution will be calculated for the rate (probability) of CR. For EFS, failures will include (1) death while in continuous CR, (2) relapse, (3) secondary malignancy, and (4) failure to achieve CR. Failure time is defined to be the time between on-study and the time of failure or the last follow-up time for patients who had not failed at the last follow-up time. The failure time of patients failing to achieve CR is set to zero. The survival distribution of EFS and OS will be estimated by the Kaplan-Meier estimator, and the standard deviation will be calculated by Peto's method. CR rate, EFS and OS of patients treated on SJBC3 at St. Jude will be compared historically with those treated on SJBCII. This comparison defines the continued improvement in the treatment of childhood mature B-cell lymphomas and leukemia in this institution.

Of the 64 patients on the SJBCII trial there are 3 patients in Group A, 37 in Group B, and 24 in Group C. Two (2/64=3.1%) patients failed to achieve CR. As of May 2007 the median follow-up time was 6.07 years (range 1.05 to 12.07 years). Ninety five percent (95%) of the patients were contacted within a year.

The overall five-year EFS and OS were 90.1% ± 4.9% and 93.6% ± 3.7% respectively (see also figure below).

Figure 1. Event Free Survival (EFS) and Survival of Patients Enrolled on Total SJBCII



Five-year EFS and OS in each treatment group in the SJBCII trial are summarized below.

Five-year EFS and OS by Group on SJBCII

Group	5-Y EFS	5-Y OS
A	100%	100%
B	96.4%±3.7%	100%
C	79.2%±1.2%	82.9%±9.9%

Figure 3. EFS by ARM

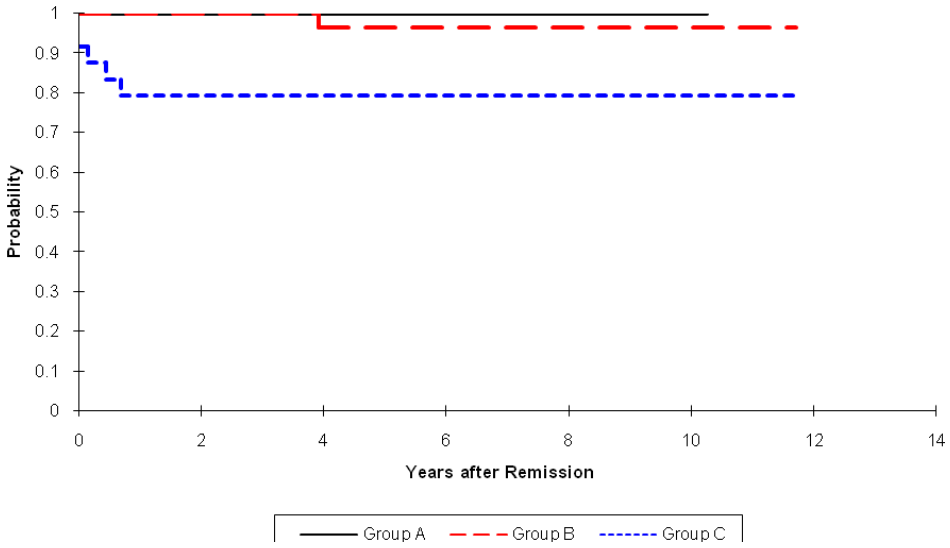
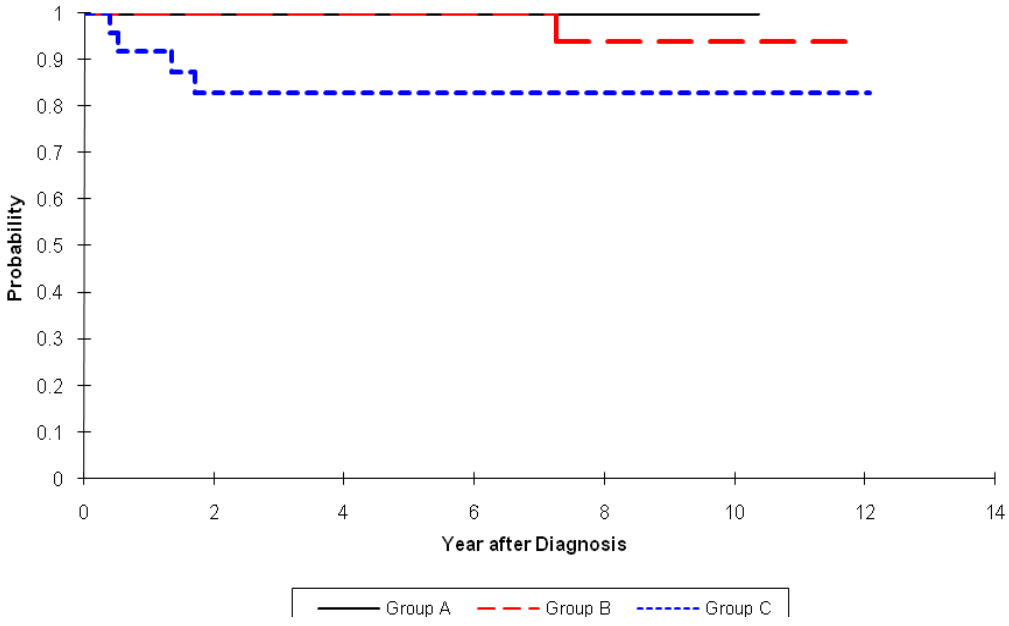


Figure 4. OS by ARM



To determine the feasibility of administration and toxicity profile of the SJBC3 protocol, which features the use of rituximab for selected higher-risk Group B and all Group C participants.

Frequencies of possible rituximab-related toxicities will be described in terms of the estimates of the number of episodes and the cumulative incidence throughout the treatment period for each type of toxicity.

12.0 OBTAINING INFORMED CONSENT

12.1 Consent/Assent at Enrollment

The process of informed consent for SJBC3 will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), blood transfusion and other procedures as necessary. After the diagnosis of mature B-cell NHL or leukemia is established, we will invite the patient to participate in the SJBC3 protocol.

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at St. Jude and collaborating sites, and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all participants ≥14 years of age. Participants who reach the age of majority while on study will be re-consented for continued participation on SJBC3, according to Cancer Center and institutional policy.

12.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday. If an affiliate or collaborating site is located in a country or state where a different age of majority applies, that location must consent the participants according to their local laws.

12.3 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CTO websites.

Collaborating Sites will follow institutional policy for consenting non-English speaking participants (and will provide institutional policy to St. Jude).

12.4 Collection of Collaborating Institution Consent Forms

Signed collaborating institution's consent forms will be faxed/scanned to [redacted]

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APPENDIX I: PEDIATRIC RITUXIMAB INFUSION GUIDELINES

Dose = 375 mg/m^2

- Dilute in NS to final concentration 1 mg/ml for ease of administration.
- Administer intravenously through a dedicated line

Pre-medicate with:

- Acetaminophen 15 mg/kg PO (max 650 mg)
- Benadryl 1 mg/kg IV or PO (max 50 mg)

FIRST INFUSION:

See current St. Jude formulary from Intranet.

SUBSEQUENT INFUSIONS:

See current St. Jude formulary from Intranet.

GUIDELINES:

1. Monitor blood pressure, pulse, respiration and temperature every 15 minutes for the first hour or until the participant is stable, then hourly until the infusion is complete.
2. Have epinephrine and diphenhydramine available along with resuscitation equipment for the emergency management of anaphylactic reactions.
3. If infusion related events occur, slow the infusion or stop the infusion until resolution, treating the participant if necessary. If it is determined that the participant can be safely re-challenged, begin the infusion at 50% of the rate at which the infusion was running when stopped.
4. Verify that the participant has not taken antihypertensive medications within 12 hours of beginning the rituximab infusion. Hypotension may occur during the infusion.
5. Participants with a high number of circulating cancer cells (> 25,000/mm^3) may be at a higher risk for tumor lysis syndrome. These patients should receive allopurinol and intravenous hydration and should be monitored.
6. Participants who experience severe infusional symptoms may need to be hospitalized for observation. Participants and families should be counseled to be seen in the emergency room if infusion-related symptoms occur again after the infusion is complete (i.e. at home at a later time).
7. Monitor participants during and for a few weeks after receiving rituximab for the development of mucositis with a sore throat or mouth ulcers followed by a diffuse skin rash which can worsen rapidly and result in total body skin sloughing and can become life threatening.
8. Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis throughout their study participation.

APPENDIX II: GROUP CLASSIFICATION/MURPHY STAGE

Group Classification

Group A - Completely resected stage I or completely resected abdominal stage II lesions.

Group B - All cases not eligible for Group A or Group C. (Murphy Stage III and non-CNS Stage IV)

Group C - Any CNS involvement and/or bone marrow involvement ≥ 25% blasts. For CNS involvement one or more of the following applies:

(1) Any L3 blasts in CSF
(2) Cranial nerve palsy (if not explained by extracranial tumor)
(3) Clinical spinal cord compression
(4) Isolated intracerebral mass
(5) Parameningeal extension: cranial and/or spinal

Murphy's Staging

Stage 1: **A** single tumor (extranodal) or a single anatomical site (nodal) with exclusion of the mediastinum or abdomen.

Stage 2: A single tumor (extranodal) with regional involvement:

- Two or more nodal areas on the same side of the diaphragm
- Two single (extranodal) tumors with or without regional node involvement on the same side of the diaphragm. A primary gastrointestinal tract tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only, grossly completely resected

Stage 3: Two single tumors (extranodal) on opposite sides of the diaphragm

- Two or more nodal areas above and below the diaphragm
- All primary intrathoracic tumors (mediastinal, pleural, thymic)
- All extensive primary intra-abdominal disease, unresectable
- All paraspinal or epidural tumors, regardless of other tumor sites

Stage 4: Any of the above with initial CNS and/or bone marrow involvement

APPENDIX III: BIOLOGICAL SPECIMENS

To ensure high quality data from the genomic profiling studies, it is important to immediately process tissue obtained at the time of tumor sampling to maximize DNA and RNA integrity. Tissue samples should be placed in liquid nitrogen as soon as possible (or after an intermediate freezing step, such as in a dry ice and ethanol slurry). If access to very low temperature freezing is not available, a portion of the tissue sample should be placed immediately in a solution that prevent RNA degradation, and permit subsequent RNA extraction, e.g. RNA*later* (Ambion).

DNA studies:

DNA will be isolated from peripheral blood and tumors using commercially available reagents, such as the DNA blood and tissue mini kit (Qiagen, Valencia, CA) or by phenol-chloroform extraction. DNA purity and integrity will be assessed by spectrophotometry and by agarose gel electrophoresis. Analysis of DNA copy number alterations and LOH will be performed using Affymetrix SNP 6.0 arrays (Affymetrix, Santa Clara, CA) using a bioinformatic analysis pipeline established and developed at St. Jude. Validation of CNA will be performed using genomic quantitative PCR and/or fluorescence in situ hybridization on paraffin embedded tissue. Mutational analysis of genes including *TP53*, c-*MYC* and *ARF,* and other genes found to be targeted by recurring genetic alterations on SNP array analysis, will be performed by PCR and direct sequencing on ABI 3730 analyzers at St. Jude[25,122,123]. Methylation analysis of individual genes will be performed using mass spectrometry of bisulfate treated DNA using the MassARRAY platform (Sequenom, San Diego, CA).

RNA studies:

RNA will be extracted from frozen tissue samples or samples placed in RNA*later* using TriZOL reagent, or alternative commercially available reagents, such as the QiagenRNeasy RNA Midi-Prep kit (Valencia, CA) according to the Manufacturer's recommendations[122]. The RNA will be resuspended in diethylpyrocarbonate-treated water, quantified by UV absorbance at 260/280 nm and stored at -80^{o}C.

a) Quantitative Real-Time PCR: cDNA will be generated from 1 μg total RNA using commercially available kits (e.g., iScriptcDNA amplification kit, Bio-Rad Laboratories, Hercules, CA). Specific gene transcripts will be identified and quantified by amplifying cDNA using DNA oligonucleotide primers and intercalating fluorescent dyes (e.g., SybrGreen) or custom specific fluorescent probes (e.g. 6FAM and MGB labeled probes designed using Primer Express, Applied Biosystems, Foster City, CA).[122,124] GAPDH, RNAseP or other housekeeping genes will also be analyzed as an internal loading control. Expression levels will be determined by the comparison of standard curves or$\Delta\Delta$Ct methods[125].
b) Affymetrix Gene Expression: Intact total cellular RNA (~5-10 μg) will be processed according to the Affymetrix one-cycle labeling protocol (http:www.affymetrix.com/support/technical/manual/expression_manual.affx) and analyzed using the Affymetrix U133 Plus 2.0GeneChip through the assistance of the Hartwell Center. Data will be analyzed using the AffymetrixMAS 5.0, Bioconductor (e.g. *limma*module) software or other appropriate programs.

Protein studies:

Protein will be isolated from whole cell extracts in NuPAGE (Invitrogen), T-PER (Pierce Chemical, Rockford, IL) or other suitable lysis buffers containing a protease and or phosphatase inhibitor cocktail (Roche Diagnostics Corporation, Indianapolis, IN) or by other appropriate methods. Total protein will be separated by SDS-PAGE and transferred to nylon or nitrocellulose membranes. The filters will be blocked in buffer containing milk or bovine serum albumin and probed with primary antibodies that are specific for p53, HDM2, $p14^{ARF}$, c-MYC and other constituents of the TP53 signaling pathway, and other genes found to be targets of recurring genetic alterations. Horseradish peroxidase-linked secondary antibodies and chemiluminescent-based reagents (e.g., Supersignal West Femto kit, Pierce Chemical) will be used to detect the level of expression of each protein.

Return of results:

As in other tumor profiling projects, germline material is being used to serve as a comparator to tumor material to facilitate the identification of tumor-specific (i.e. somatic) mutations. We will not perform interrogative germline analyses. These studies are being done in a non-CLIA certified research laboratory, and therefore the results will not be returned to the patient/parent. This has been made clear in the consent form. In the unlikely event that a genetic abnormality was discovered that is thought to have clinical significance (after consultation with experts such as members in the cancer predisposition team), we would approach the IRB for a decision as to how to best handle the situation. Patients will all be enrolled on the TBANK protocol, which permits contact with participant/family if something is discovered that could potentially affect the health and wellbeing of a child under the age of 18.

The non-CLIA certified laboratory results for the participants from international sites will not be returned to the primary physician or family, as indicated in the consent form.

EBV METHODS

Initial identification of EBV-positive BLs will be determined by in situ hybridization with EBV EBER RNA. Tumors will be analyzed for the presence of a variant latency program where EBNA-3 proteins are expressed or for the latency I program found in the majority of BL.

Classification of EBV genomes: To identify the latency program expressed in these tumors, the EBV genome will be examined by PCR. In African tumors, this pattern of latency is associated with a deletion in the region of the genome encoding the EBNA-2 latency protein; the known deletions fall within a defined region and can be identified by long range PCR analysis using a common set of primers. These tumors also carry wild-type EBV genomes that are silenced by an as yet undefined mechanism. Therefore, tumors will be analyzed for the presence of both wild-type and deleted genomes, allowing the detection of wild-type genomes. Detection of wild-type genomes will verify the presence of EBV in the tumor and serve as a positive control for the PCR reaction. DNA will be amplified using the CG Rich PCR System (Roche) according to the manufacturer's instructions. Each 50µl reaction will contain 1 x GC Rich PCR buffer, 200 µM each dNTP, 400 nM forward and reverse primer, 100ng DNA and 2units of GC Rich enzyme mix. Primers to amplify the deleted EBV genome will be 5'-TCCTCTCCAACCTTCGCTCC-3' (forward primer) and 5'-

GCCTTCGCTGGCTTCTAACATC-3' (reverse primer); primers for amplification of wild type EBV will be 5'-CTCTGCCACCTGCAACACTA-3' (forward primer) and 5'-GAGGGTGCATTGATTGGTCT-3' (reverse primer). Conditions for PCR have been previously established in the laboratory and these primers have been found to amplify EBV DNA from 4 distinct tumors. Products will be examined by agarose gel electrophoresis.

EBV gene expression: The presence or absence of the EBNA-3 proteins will be determined using RT-PCR analysis of RNA isolated from the tumors. If sufficient sample is available (10^6 or fewer cells are required), these results will be confirmed by immunoblot analysis using monospecific antibodies to each individual EBNA-3 protein and the EBNA-1 protein. Ongoing studies in the Sample laboratory are directed at identifying cellular changes in the subset of BL associated with the expression of the EBNA-3 proteins, and these will be incorporated into the analysis as appropriate.

APPENDIX IV: PSYCHOLOGICAL TESTING

Cognitive Domain	Measure	Broad Skill	Task Characteristics	Age Range	Admin. Time
IQ Estimate	SB-V Routing Subtests	Global Cognitive Function	Object Series/Matrices and Descriptive Vocabulary subtests	3+	20 min.
Attention & Executive Function	Digit Span- WISCIV Integrated	Forward- Brief Auditory Attention Backward- Auditory Working Memory	Forward- repeat random digit strands of increasing length Backward- repeat random digit strands in reverse order	*6-17 (WAIS-17+)	5 min.
	Conners' CPT	Sustained Visual Attention	Selectively respond to target stimuli (all letters except X) and not a distractor (X); computerized	4+	20 min.
	Auditory Working Memory- WJIII	Auditory Working Memory	Repeat words and numbers after organizing based on rule	4+	5 min.
	Retrieval Fluency- WJIII	Verbal Fluency	Name foods, people names & animals, each in 1 min. trial	3+	5 min.
(Parent Measure)	BRIEF Questionnaire	Executive Function	Questionnaire assessing executive dysfunction in community	2-5/6-18	15 min.
Memory	CVLT-C/CVLT-II	Verbal List Learning	Learn 15 item list with multiple presentations, interference, delay & recognition	5-17 (CVLTII-17+)	15 min.
	Bead Memory- SBIV	Visual Learning & Memory	Recreate bead patterns (color & shape) of increasing length	3+	5 min.
	Rey-O	Complex Visual Memory Task	Copy a complex figure followed by drawing from memory after short and long delays; recognition trial included	6+	20 min.

Processing Speed	Visual Matching-WJIII	Processing Speed	Find matching pairs; timed	3+	3 min.
	Decision Speed-WJIII	Processing Speed	Pick two conceptually similar pairs; timed	3+	3 min.
Visual Spatial/ Visual Motor	Beery VMI	Visual Perception & Visual-Motor Integration	Blue- Match shapes of decreasing size Green- Draw shapes of increasing complexity	3-19	15 min.
Achievement**	Letter- Spelling, Letter Word Identification & Applied Problems-WJIII	Academic Achievement	Single word reading, spelling to dictations and solving math word problems	3+	20 min.
Motor	Purdue Pegboard	Fine Motor Speed & Dexterity	Place pegs using dominant, nondominant & both hands; timed	3+	3 min.
Adaptive	ABAS-II	Adaptive Functioning	Inquires about adaptive skills across functional domains	birth-89	15 min.
Social-Emotional	BASC-II	Psycho-social Adjustment	Inquires about 9 behavioral domains- aggression, anxiety, etc.	2.0- 21.11	15 min.

** Tests that will be tried at ages below normative population for longitudinal assessment of individual change*
Table Abbreviations: - SB-V- Stanford-Binet, Fifth Edition; WISC-IV- Wechsler Intelligence Scale for Children, Fourth Edition; WJIII- Woodcock Johnson, Third Edition; TOVA- Test of Variables of Attention; BRIEF- Brief Rating Inventory of Executive Function; Beery VMI- Beery-Buktenica Test of Visual-Motor Integration; CVLT-C- California Verbal Learning Test- Children's Version; CVLT-II- California Verbal Learning Test, Second Edition Rey-O- Rey-Osterrieth Complex Figure; ABAS-II- Adaptive Behavior Assessment System, Second Edition; BASC-II- Behavior Assessment System for Children, Second Edition;
***Only administered at end of therapy in lieu of the Rey-O*

APPENDIX V: COLLECTION AND SUBMISSION OF SPECIMENS FOR BIOLOGY STUDIES

Collection of Tissues: Tumor tissue obtained at diagnosis must be immediately processed to ensure high quality, non-degraded nucleic acid material (DNA and RNA) for genomic analysis.

One of two methods can be used:

1. Snap freezing in liquid nitrogen

   Small tissue pieces (less than 0.5 cm in any one direction) should be wrapped in foil and snap frozen either in liquid nitrogen, or in dry ice/ethanol slurry, and then subsequently stored in liquid nitrogen. This material can then be shipped to St Jude on dry ice, and used for subsequent DNA and RNA extraction.

   OR

2. Storage in RNA later

   If liquid nitrogen storage is not available, small tissue pieces (less than 0.5 cm in any one direction) should be placed in approximately 5 volumes of RNA*later* (Ambion). e.g. 0.5g sample requires approximately 2.5ml of RNA later. This solution permeates the cells, stabilizing the RNA for subsequent extraction. The solution may then be stored for 1 day at 37°C, 1 week at 25°C, 1 month at 4°C or indefinitely at -20°C. This tissue will then be processed for RNA and DNA.

Germline DNA will be obtained from peripheral blood samples, and may be drawn after chemotherapy has started (primary tumor for genomics still must be obtained pre chemo apart from listed exceptions as specified in the protocol eligibility). 5ml of EDTA-anti-coagulated blood should be collected and buffy coat leukocyte preparations made. These cell preparations may then be used for DNA extraction using either phenol-chloroform or column based methods. For sample from non-St Jude sites, the blood sample may be centrifuged, the buffy coat aspirated, washed in phosphate buffered saline, and then the leukocytes pelleted, and the cell pellet frozen on dry ice. The pellet is then stored on liquid nitrogen. **If liquid nitrogen is not available, ship the EDTA tube to St. Jude within 24 hours of collection at room temperature**.

Mail all specimens to:

Charles Mullighan / Audrey Phillips
St Jude Children's Research Hospital
262 Danny Thomas Place
Pathology, Mail Stop 342, Room D4036
Memphis, TN, 38105
[redacted]

For questions, e-mail or call Dr. Mullighan, or call Assistant: Carolyn Reed: T: [redacted] or Lab Manager: Audrey Phillips: T: [redacted]

APPENDIX VI: CLINICAL DATA FOR PARTICIPANTS AT COLLABORATING SITES PARTICIPATING IN BIOLOGIC OBJECTIVES ONLY

Age
Race
Gender
Diagnosis Date
LDH
CBC with diff
Diagnosis
Sites of Disease
Stage
Treatment Regimen(s)
Treatment Start Date
Treatment Best Response
Date of Response
Date Completed Therapy
Significant Events (e.g. relapse, induction failure, death, cause of death, second malignancy)
Date Event
Last Contact Date

APPENDIX VII: SUB-STUDY FOR COLLABORATING INSTITUTIONS PARTICIPATING IN BIOLOGICAL OBJECTIVES ONLY

1.0 OBJECTIVES

1.1 Biological Primary Objectives

1.1.1 To perform genomic analysis of childhood newly diagnosed mature B cell lymphomas (e.g. Burkitt lymphoma/leukemia, DLBCL, and MLBCL) obtained from different parts of the world.

1.1.2 To describe the types and frequency of mutations in the ARF-HDM2-TP53 pathway, in B-cell lymphomas in the United States and those found in selected geographic regions of the world.

1.1.3 To describe the expression of ARF-HDM2-TP53 and PUMA-associated pathways in B-cell lymphomas in the United States and that found in B-cell lymphomas of other selected geographic regions of the world.

1.1.4 To describe the pattern and frequency of XLP gene mutations presenting with B-cell lymphomas in the United States and selected geographic regions.

1.1.5 To describe the frequency of EBV-positive B-cell lymphomas in the United States and selected geographical regions of the world.

- To describe the pattern of EBV protein and gene expression (e.g., EBNA 3) in EBV-positive lymphomas.
- To compare patterns of EBV protein and gene expression with clinical, laboratory and outcome data.

1.2 Exploratory Aim

1.2.1 Tumor Bank

To establish a tumor bank of mature B-cell lymphoma samples for future molecular studies aimed to clarify the role of c-*MYC* oncogene and its associated pathways, and other pathways found to harbor genetic alterations from the genomic profiling studies described in 1.1.1.

2.0 BACKGROUND AND RATIONALE

2.1 Background

2.1.1 Genome-Wide Profiling of Genetic Alterations in Lymphoma

Genome-wide profiling of the gene expression and genetic alterations, including DNA copy number abnormalities (CNA) and loss of heterozygosity (LOH), and genome sequencing has provided valuable insights into the pathogenesis of lymphoid neoplasms.[1,2] Gene expression profiling has been found to be an effective tool in differentiating BL from other mature B-cell lymphomas like DLBCL in adults.[3,4] These

studies have shown that some cases that cannot be classified unequivocally by classical morphological or cytogenetic modalities express a clear genetic signature that allows for their classification. Three major DNA microarray studies have looked into the biological and clinical heterogeneity of adult DLBCL.[5-7] The initial DLBCL gene expression profiling study used genes that define the germinal center stage of B-cell differentiation to identify 2 prominent DLBCL subgroups. The "germinal center B-cell-like" DLBCL (GCB DLBCL) expressed genes characteristic of normal germinal center B cells (e.g. CD 10, BCL6, A-myb), whereas the "activated B cells " DLBCL subgroup (ABC DLBCL) expressed genes that are induced during mitogenic activation of peripheral blood B cells (e.g. BCL2, IRF-4, cyclin D2)[5]. These two different patterns of differentiation were associated with significantly different outcomes. Another larger gene expression profiling study of DLBCL cases confirmed the existence of these two subgroups as well as a third one called "type 3" that did not resemble either and may represent an additional subgroup.[6] Genome-wide profiling of CNA in DLBCL has identified multiple recurring regions of DNA gain and loss, many of which are associated with the DLBCL subtypes identified by gene expression profiling. These include amplification of a region of chromosome 19 harboring the SPIB transcription factor, deletion of *CDKN2A/B* and trisomy 3 in ABC DLBCL; and amplification of the mir-17-92 microRNA cluster and deletion of *PTEN* and GCB DLBCL. To date, there are limited genome-wide profiling data of genetic alterations pediatric B-NHL. Our aim is to perform integrated analysis of genomic data to identify genetic pathways that are altered in this disease, to identify tumor subtypes, to examine variation in genetic features according to geographic region, and to perform a comparative analysis with existing data from adult cases.[5,9]

Techniques for studying the genomic profile of patient tumors (somatic and germline) continue to advance. These approaches to be used, depending on sample availability and quality include RNA (transcriptome) sequencing, exome sequencing, single nucleotide polymorphism array analysis and whole genome sequencing. These will be done using standard established approaches in the Mullighan laboratory and the Genomic Sequencing Facility of the Hartwell Center at St. Jude.

2.1.2 ARF-HDM2-TP53 and PUMA

Burkitt lymphoma (BL) and mature B-cell leukemia (B-ALL) are characterized by one of three reciprocal chromosomal translocations [t(8;14);t(2;8);t(8;22)]. The unifying feature of these translocations is the juxtaposition of the c-*MYC* proto-oncogene and one of the immunoglobulin genes. These translocations result in the constitutive over-expression of c-*MYC*, a transcription factor which is necessary and sufficient for promoting cell proliferation and for provoking angiogenesis. In normal B-cells, c-*MYC* expression is tightly regulated and rapidly responds to mitogenic or growth inhibitory signals. These controls are lost in BL/B-ALL, resulting in aberrantly high levels of c-*MYC* protein. The c-*MYC* translocations that occur in BL/B-ALL have been extensively studied with respect to their breakpoint patterns and their mechanism of de-regulation of c-*MYC* expression. However, recent insights into the molecular pathogenesis of BL/B-ALL have come from a detailed analysis of Eμ-*myc* transgenic mice, a model which accurately mimics the chromosomal abnormality and pathology of BL/B-ALL[12]. In normal cells, including B

lymphocytes, c-*Myc* expression activates the Arf-Mdm2-p53 pathway resulting in apoptosis.[13-15] Arf (mouse $p19^{Arf}$/human $p14^{ARF}$) is encoded by an alternative reading frame of the *Ink4a* locus and limits lymphomagenesis by antagonizing Mdm2 (murine double minute-2; HDM2 in man), a negative regulator of the tumor suppressor p53.[16,17] The c-*Myc* transgenic animal studies have shown that 80% of the lymphomas that arise in these mice carry mutations affecting apoptotic checkpoints, from either deletions of *Ink4a*/*Arf* (25%), mutation of *p53* (30%) or overexpression of *Mdm2* (50%).[15,18-22] Although data suggest that one-third of human BL/B-ALL may have mutations of *TP53*[23,24] many of the studies performed have been anecdotal and/or limited to the study of BL/B-ALL cell lines[25,26]. Therefore, a detailed study of alterations in this pathway is currently lacking in human mature B cell malignancies.

Although these studies established that c-*Myc* activates Arf and p53, and that functional loss of this pathway impairs *Myc*-induced apoptosis, *Myc* activation can still trigger cell death in the absence of *Arf* and/or *p53*.[15] Activation of c-*Myc* in normal B cells suppresses the expression of *Bcl-2* or *Bcl-*X_L, which are anti-apoptotic proteins that protect the cells from a variety of insults, including chemotherapeutic drugs. Importantly, an analysis of lymphomas arising in Eμ-*Myc* transgenic mice has established that this pathway is also bypassed during lymphoma development and that this also occurs at an equal frequency in tumors having alterations in the *ARF-Mdm2-p53* pathway.

Recent studies have shown that about one third of BL in humans harbor c-*MYC* mutations, and two recurrent mutant c-*MYC* alleles derived from BL have been shown to be able to evade the *p53* tumor surveillance network and promote rapid lymphoma development in mice.[25] Another possible mediator in the altered apoptotic pathways of BL is *PUMA* (p53-up-regulated modulator of apoptosis). *PUMA*, a pro-apoptotic *BH3*-only Bcl-2 family protein, is an essential mediator of p53- dependent and independent cell death,[26] and recent observations have indicated that 40% of primary BLs (n=19) fail to express detectable levels of *PUMA*, and that in some cases *PUMA* is inactivated by epigenetic silencing.[27]

Understanding the exact mechanism by which the apoptotic pathways are affected in these lymphomas will provide an opportunity for the development of novel therapies. The development of more rational therapies with a selective effect in specific tumor pathways while sparing normal tissue can have a great impact in the survival of cancer especially in the developing world, where children are at high risk of dying of the toxic effects of aggressive chemotherapy due to suboptimal hospital infrastructure.

2.1.3 Epstein-Barr Virus

Epstein–Barr virus (EBV) is a B lymphotropic herpes virus linked to several human diseases including B-cell malignancies.[126] Greater than 90% of the world's population is persistently infected with EBV. It is a ubiquitous γ-herpes virus that, upon primary infection, causes a brief and confined replication of the virus at the site of infection in the immunocompetent host.[29] In industrialized societies, infection with EBV generally occurs later in life, compared to early childhood infection common in countries with limited

resources. EBV is transmitted orally from oropharyngeal secretions of asymptomatic carriers and patients with infectious mononucleosis.[28] While EBV infects B lymphocytes early in the course of primary infection, it normally does not replicate in them, but rather establishes a latent infection characterized by the limited expression of a subset of latency-associated genes.[30] The latency genes consist of six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and LP) and three membrane proteins (LMPs 1, 2A and 2B).[31] There have been three different latency-gene expression programs described after EBV infection. In BL/B-ALL only a very limited repertoire of the latency-associated genes (EBNA-1, EBV-encoded RNA (EBER)-1 and EBER-2 and the *Bam*HI-A rightwards transcripts [BARTs]) are routinely expressed (Latency I) contributing to immune evasion.[32] The Latency II program includes additional expression of LMP1 and LMP2A/B, and is commonly observed in EBV-positive Hodgkin lymphoma, some types of T and NK-cell lymphomas and some cases of B-cell lymphoma.[33] Latency III is expressed in lymphoblastoid cell lines and newly infected B cells in vivo, and includes the entire array of EBV latency-associated genes. These cells would normally be very susceptible to killing by EBV-specific T-cells, and, therefore, this pattern of expression is only seen in the EBV-associated lymphoproliferative disease (EBV-LPD) associated with primary or secondary immunodeficiency.[33] The EBER RNAs are present in all previously described programs of EBV latency, and their presence within tumor samples is evidence of a latent EBV infection.[34]

The EBNA3 proteins (3A, 3B and 3C) are not normally expressed in classic endemic BL (eBL); however, recent studies have revealed a subset of eBLs (~ 15%) that express these EBV proteins as a consequence of a deletion in the EBNA2 gene.[35,36] These tumors are particularly resistant to apoptotic stimuli. Dr. Clare Sample's laboratory has determined that, in a *SCID* mouse model of tumorigenicity, these variant EBNA-3+ eBLs are particularly aggressive relative to the eBLs that do not carry the EBNA2 deletion (C. Sample, personal communication) and additional unpublished data suggests that the EBNA-3 proteins may contribute directly to proliferation of the tumor cells. Thus, it is important to determine whether the pattern of EBV gene expression is associated with biologic and clinical and outcome features.

EBV has not been shown to be directly involved in BL pathogenesis, but has rather been suggested to increase the target pool of cells susceptible to malignant transformation. However, an interesting study by Ruf, et. al has demonstrated that the malignant phenotype of a group I BL line requires the EBV genome, and that this is associated with EBV's ability to impair the c-*MYC* apoptotic program.[37] Therefore, at least in this scenario, malignant transformation requires EBV gene expression altering apoptotic pathways. These observations suggest that EBV may influence c-*MYC*'s ability to trigger apoptosis in BL and possibly other pathways as well. Hence, we propose to evaluate and correlate EBV gene expression with alterations in the ARF-Mdm-p53 and Bcl-2/Bcl-X apoptotic pathways.

There are two main clinical presentations of BL clustering in different geographic regions. The eBL, which affects younger children and often involves the jaw, is found mainly in equatorial Africa and New Guinea.[38-40] The majority of patients with eBL have

been infected with malaria. By contrast, non-endemic or sporadic BL (sBL) occurs worldwide and mainly affects young adults. Both forms of BL differ in their association with EBV: eBL is almost always associated with EBV, whereas sBL has a more irregular association, ranging from 10% to 30%.[41] However, our knowledge of other clinical and biological forms of BL, particularly in developing countries outside of Africa, is very limited. A study examining EBV association with BL in temperate countries of South America, found a 50% EBV association with predominantly abdominal presentation,[42] suggesting an intermediate form. The pattern of EBV gene expression has not been determined in these BLs. The rate of South American patients with BL infected with malaria is extremely low. Studies in Northeastern Brazil and Northern Africa have shown about 85-90% positivity for EBV (like eBL), but have a predominant abdominal presentation of the tumors (90%) similar to sBL, suggesting that other still unknown genetic or environmental factors might modify the clinical presentation, and possibly response to therapy of BL in these regions.[43,44]

2.1.4 X-Linked Lymphoproliferative syndrome

X-linked lymphoproliferative (XLP) syndrome is a sex-linked immunodeficiency syndrome. Boys with this syndrome are at increased risk for the development of B-cell lymphomas and fatal infectious mononucleosis. Most are diagnosed by the time they are 5 years of age, although some have gone undiagnosed until much later in life (i.e. 5th or 6th decade). There may be an associated hypogammaglobulinemia; however, there is generally not a significant history of infections. As a result, patients are usually undiagnosed until they develop a B-cell lymphoma or die from infectious mononucleosis. Currently, boys with newly diagnosed high grade B-cell NHL are not screened for the XLP gene mutation. Hence, the actual frequency of these mutations in boys with primary high grade B-cell lymphoma is not known.

We have identified five boys with B-cell lymphoma who have subsequently been found to have an XLP gene mutation. These cases were only identified after their clinical course suggested the possibility of XLP. Now that we have an accurate and efficient assay to screen for the XLP gene mutations, we believe that boys with BL and other B-cell lymphomas should routinely be screened for XLP mutations. It is not known whether different XLP mutations result in the same clinical phenotype or have prognostic importance.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 Inclusion criteria for subjects participating in biological objectives only

- Participant must have a histologic diagnosis of a mature B cell lymphoma (e.g., Burkitt lymphoma/leukemia, atypical Burkitt lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, mature B-cell lymphoma NOS) as defined in the WHO classification.
- Participant must be < 22 years of age at the time of diagnosis.

- Participant must be previously untreated, (no more than 72 hours of steroids, one intrathecal chemotherapy treatment, and/or emergency radiation) at the time of the diagnostic biopsy.
- Informed consent must be obtained by local PI or his/her designee according to ICH/Good Clinical Practice and local guidelines before enrollment into study.

3.2 Exclusion criteria for subjects participating in biological objectives only

- Inability or unwillingness of research participant or legal guardian to consent.
- Histologic diagnosis other than a mature B-cell lymphoma as defined in the WHO classification.

4.0 DESIGN AND METHODS

4.1 Biological Specimens

To ensure high quality data from the genomic profiling studies, it is important to immediately process tissue obtained at the time of tumor sampling to maximize DNA and RNA integrity. Tissue samples should be placed in liquid nitrogen as soon as possible (or after an intermediate freezing step, such as in a dry ice and ethanol slurry). If access to very low temperature freezing is not available, a portion of the tissue sample should be placed immediately in a solution that prevent RNA degradation, and permit subsequent RNA extraction, e.g. RNA*later* (Ambion). See Section 6.2 of main protocol for further details.

4.1.1 DNA studies:

DNA will be isolated from peripheral blood and tumors using commercially available reagents, such as the DNA blood and tissue mini kit (Qiagen, Valencia, CA) or by phenol-chloroform extraction. DNA purity and integrity will be assessed by spectrophotometry and by agarose gel electrophoresis. Analysis of DNA copy number alterations and LOH will be performed using Affymetrix SNP 6.0 arrays (Affymetrix, Santa Clara, CA) using a bioinformatic analysis pipeline established and developed at St. Jude. Validation of CNA will be performed using genomic quantitative PCR and/or fluorescence in situ hybridization on paraffin embedded tissue. Mutational analysis of genes including *TP53*, c-*MYC* and *ARF,* and other genes found to be targeted by recurring genetic alterations on SNP array analysis, will be performed by PCR and direct sequencing on ABI 3730 analyzers at St. Jude[24,122,123]. Methylation analysis of individual genes will be performed using mass spectrometry of bisulfate treated DNA using the MassARRAY platform (Sequenom, San Diego, CA).

4.1.2 RNA studies:

RNA will be extracted from frozen tissue samples or samples placed in RNA *later* using TriZOL reagent, or alternative commercially available reagents, such as the QiagenRNeasy RNA Midi-Prep kit (Valencia, CA) according to the Manufacturer's

recommendations.[122] The RNA will be resuspended in diethylpyrocarbonate-treated water, quantified by UV absorbance at 260/280 nm and stored at -80^{o}C.

a) Quantitative Real-Time PCR: cDNA will be generated from 1 μg total RNA using commercially available kits (e.g., iScriptcDNA amplification kit, Bio-Rad Laboratories, Hercules, CA). Specific gene transcripts will be identified and quantified by amplifying cDNA using DNA oligonucleotide primers and intercalating fluorescent dyes (e.g., SybrGreen) or custom specific fluorescent probes (e.g. 6FAM and MGB labeled probes designed using Primer Express, Applied Biosystems, Foster City, CA).[122,124] GAPDH, RNAseP or other housekeeping genes will also be analyzed as an internal loading control. Expression levels will be determined by the comparison of standard curves orΔΔCt methods.[125]

b) Affymetrix Gene Expression: Intact total cellular RNA (~5-10 μg) will be processed according to the Affymetrix one-cycle labeling protocol (http:www.affymetrix.com/support/technical/manual/expression_manual.affx) and analyzed using the Affymetrix U133 Plus 2.0 GeneChip through the assistance of the Hartwell Center. Data will be analyzed using the AffymetrixMAS 5.0, Bioconductor (e.g. *limma*module) software or other appropriate programs.

The non-CLIA certified laboratory results for the participants from international sites will not be returned to the primary physician or family, as indicated in the consent form

4.1.3 Protein studies:

Protein will be isolated from whole cell extracts in NuPAGE (Invitrogen), T-PER (Pierce Chemical, Rockford, IL) or other suitable lysis buffers containing a protease and or phosphatase inhibitor cocktail (Roche Diagnostics Corporation, Indianapolis, IN) or by other appropriate methods. Total protein will be separated by SDS-PAGE and transferred to nylon or nitrocellulose membranes. The filters will be blocked in buffer containing milk or bovine serum albumin and probed with primary antibodies that are specific for p53, HDM2, p14^{ARF}, c-MYC and other constituents of the TP53 signaling pathway, and other genes found to be targets of recurring genetic alterations. Horseradish peroxidase-linked secondary antibodies and chemiluminescent-based reagents (e.g., Supersignal West Femto kit, Pierce Chemical) will be used to detect the level of expression of each protein.

4.1.4 EBV METHODS

Initial identification of EBV-positive BLs will be determined by in situ hybridization with EBV EBER RNA. Tumors will be analyzed for the presence of a variant latency program where EBNA-3 proteins are expressed or for the latency I program found in the majority of BL.

Classification of EBV genomes: To identify the latency program expressed in these tumors, the EBV genome will be examined by PCR. In African tumors, this pattern of latency is associated with a deletion in the region of the genome encoding the EBNA-2 latency protein; the known deletions fall within a defined region and can be identified by long range PCR analysis using a common set of primers. These tumors also carry wild-type EBV genomes that are silenced by an as yet undefined mechanism. Therefore, tumors will be analyzed for the presence of both wild-type and deleted genomes, allowing the detection of wild-type genomes. Detection of wild-type genomes will verify the presence of EBV in the tumor and serve as a positive control for the PCR reaction. DNA will be amplified using the CG Rich PCR System (Roche) according to the manufacturer's instructions. Each 50µl reaction will contain 1 x GC Rich PCR buffer, 200 µM each dNTP, 400 nM forward and reverse primer, 100ng DNA and 2units of GC Rich enzyme mix. Primers to amplify the deleted EBV genome will be 5'-TCCTCTCCAACCTTCGCTCC-3' (forward primer) and 5'-GCCTTCGCTGGCTTCTAACATC-3' (reverse primer); primers for amplification of wildtype EBV will be 5'-CTCTGCCACCTGCAACACTA-3' (forward primer) and 5'-GAGGGTGCATTGATTGGTCT-3' (reverse primer). Conditions for PCR have been previously established in the laboratory and these primers have been found to amplify EBV DNA from 4 distinct tumors. Products will be examined by agarose gel electrophoresis.

EBV gene expression: The presence or absence of the EBNA-3 proteins will be determined using RT-PCR analysis of RNA isolated from the tumors. If sufficient sample is available (10^6 or fewer cells are required), these results will be confirmed by immunoblot analysis using monospecific antibodies to each individual EBNA-3 protein and the EBNA-1 protein. Ongoing studies in the Sample laboratory are directed at identifying cellular changes in the subset of BL associated with the expression of the EBNA-3 proteins, and these will be incorporated into the analysis as appropriate.

4.2 Collection and Submission of Specimens for Biology Studies

4.2.1 Collection of tissues:

Tumor tissue obtained at diagnosis must be immediately processed to ensure high quality, non-degraded nucleic acid material (DNA and RNA) for genomic analysis.

One of two methods:

1. Snap freezing in liquid nitrogen

   Small tissue pieces (less than 0.5 cm in any one direction) should be wrapped in foil and snap frozen either in liquid nitrogen, or in dry ice/ethanol slurry, and then subsequently stored in liquid nitrogen. This material can then be shipped to St Jude on dry ice and used for subsequent DNA and RNA extraction.

OR

2. Storage in RNA later

   If liquid nitrogen storage is not available, small tissue pieces (less than 0.5 cm in any one direction) should be placed in approximately 5 volumes of RNAlater (Ambion), e.g. 0.5g sample requires approximately 2.5ml of RNA later. This solution permeates the cells, stabilizing the RNA for subsequent extraction. The solution may then be stored for 1 day at 37°C, 1 week at 25°C, 1 month at 4°C or indefinitely at -20°C. This tissue will be processed for RNA and DNA.

4.2.2 Collection of Blood for Germline DNA

5ml of EDTA-anti-coagulated blood should be collected and buffy coat leukocyte preparations made. These cell preparations may then be used for DNA extraction using either phenol-chloroform or column based methods. For sample from non-St Jude sites, the blood sample may be centrifuged, the buffy coat aspirated, washed in phosphate buffered saline, and then the leukocytes pelleted, and the cell pellet frozen on dry ice. The pellet is then stored on liquid nitrogen. If liquid nitrogen is not available, ship the EDTA tube to St. Jude within 24 hours of collection at room temperature.

This sample may be collected any time before or after chemotherapy.

4.2.3 Mail all specimens to:

Charles Mullighan / Audrey Phillips
St Jude Children's Research Hospital
262 Danny Thomas Place
Pathology, Mail Stop 342, Room D4036
Memphis, TN, 38105
[redacted]

Email: [redacted]. For questions, e-mail or call Dr. Mullighan, or call his assistant: Carolyn Reed: T: [redacted], or Lab Manager: Audrey Phillips: T: [redacted].

5.0 DATA COLLECTION AND PROCEDURES FOR SITES PARTICIPATING IN BIOLOGICAL OBJECTIVES ONLY

5.1 Pre-enrollment:

Before the collaborating or enrolling affiliate sites screen or enroll a study participant, the site completes a RIN request form and submits it to CTO by e-mail: [redacted] or fax [redacted] (follow fax by a phone call to [redacted] to ensure receipt). A RIN is an eight-digit automated number beginning with an

"R". Once the form is received, St. Jude will r*egister* the research participant and then email the RIN to the email address provided on your registration form. This will register the participant only, it will not enroll the participant on the study. After hours, holidays, and weekends the site RIN request will be answered by the Patient Registration team.

To *enroll* the study participant, after the RIN is obtained, the site study team will complete an eligibility checklist and fax it to [redacted] (follow fax by a phone call to [redacted] to ensure receipt). Clinical Trials Operations (CTO) will enter the Eligibility Checklist information into the central enrollment system to officially enroll the participant on the trial.

Collaborating sites will scan/fax a copy of the signed informed consent document to the study team at [redacted].

5.2 Post-enrollment:

1) The St. Jude PI and the study coordinator receive a notification of the enrollment completion from the St. Jude Data Warehouse Service
2) The PI completes the Registration Form and the study coordinator enters the information into a secured study database.
3) The research subject and the referring physician receive a copy of the signed consent form.
4) The referring physician sends St. Jude paraffin blocks, slides, frozen tumor, and blood samples if they are available and patient/parents/guardians have signed the consent form (Specimen Guidelines – Appendix III).
5) If the diagnosis is not confirmed, the biologic studies will not be performed, and the samples will be returned or discarded.

5.3 Yearly follow-up:

The St. Jude PI or designee contacts the treating physician for yearly follow up information.

5.4 Clinical Data

Age	Treatment regimen(s)
Race	Treatment start date
Gender	Treatment best response
Diagnosis date	Date of response
LDH	Date completed therapy
CBC with diff	Significant events (e.g., relapse, induction failure,
Diagnosis	death, cause of death, second malignancy)
Sites of disease	Date of event
Stage	Last contact date and survival status

6.0 STATISTICAL CONSIDERATIONS

6.1 Biological Aims

The SJBC3 trial has several exploratory biological studies as its primary objectives. Statistical considerations on accrual, sample size, and levels of statistical errors for the first aim are given below.

To perform transcriptional profiling and genome-wide analysis of DNA copy number abnormalities and loss-of-heterozygosity using DNA microarrays in children with newly diagnosed mature B cell lymphomas (e.g. Burkitt lymphoma/leukemia, DLBCL, and MLBCL) from different parts of the world.

Accrual. It is difficult to get a precise projection of accrual rate at this point although we have some expectation based on prior experience. Below we give a conservative projection for the biological study in terms of number of evaluable tumor samples after 5 year accrual although we anticipate the trial may run beyond 5 years. Experience from the SJBCII trial indicates that the annually 6 patients can be accrued at St. Jude, giving approximately 30 patients after 5 years of accrual at this institution. Because there is not another childhood B-cell lymphoma trial currently in US, we anticipate the accrual may increase to 8 to 12 patients per year. The genomic analysis requires tumor biopsy samples from participating international institutions along with St. Jude. The international institutions will participate in the biological studies and provide biopsy samples but will not participate in the SJBC3 therapy. We also note that it is rather difficult to obtain tumor biopsy samples from patients enrolled here for various logistical reasons. We expect that there will be about 10, 1 to 2, and 5 per year from Latin America, North America (St. Jude), and other geographic regions respectively. Five-year accrual can provide about 50, 5 to 10, and 25 tumor samples respectively. Approximately 80% of the tumors are expected to be Burkitts Lymphoma (BL). Moreover, we conservatively assume that 80% of the samples from each region are eventually evaluable for genomic analyses, resulting in 40, 8 and 20 evaluable tumor samples from Latin America, North America, and other regions respectively. Five-year accrual of evaluable tumor samples from each region for this aim is summarized below.

Number of evaluable tumor samples after 5-year accrual

	BL	Non-BL	Total
Latin America	32	8	40
North America (St. Jude)	6	2	8
Other	16	4	20
Total	54	14	68

At this point we set the accrual target for the biological aims as 68 evaluable tumor samples and anticipate completing accrual in 12 years for the biological aims. We set the accrual target for other aims to 100 patients treated at St. Jude and anticipate completing in 12 years.

Analyses. There will be three major components in the analyses for this objective. Each is discussed below, with very limited preliminary genomic data in pediatric lymphomas.

Gene differential profiling of BL vs. non-BL. Identification of gene differential expression signatures requires inference (comparison) of gene expression levels in BL vs. non-BL. It is important to realize that the tumor samples come from dramatically different geographical regions where ethnic and environmental factors (e.g., diet) can greatly affect gene expression. Additionally, the extent of differential expression between BL and non-BL may also be affected by environmental, endemic and related biological factors (e.g., involvement of EBV in BL). Thus, it is important to adjust the gene expression comparison by geographic region, which serves as an aggregate of all the possible region-related confounding and stratification factors, in the absence of the data on such factors. Each participating institute defines a geographic region; from example, St. Jude defines the "North America" region. At this point we expect in addition to St. Jude, there will be one institute in Brazil (Latin America) and one in Singapore (Asia) participating the biological studies. Modeling, testing, and interpretation for a single gene (probeset) is given below, followed by methods of handling massive multiple tests. For samples with suitable RNA quality, gene expression profiling will be performed using transcriptome sequencing in preference to microarray analysis, in view of the more complete data provided by this platform.

The following two-factor Poisson regression model will be used for each gene, where E[Y(bgi)] denotes the mean expression of a gene in the i-th tumor sample from region g (g=Latin America, North America, etc.) and disease b (b=BL, non-BL):

$$\text{Log}\{E[Y(bgi)]\}=\mu+\alpha(b)+\beta(g)+\alpha\beta(g,b).$$

From a completely biological stand point the primary interest here is the main effect α(b). For each mRNA expression we will test the null hypothesis α(BL)= α(non-BL)=0 against the two-sided alternative at least one of them is non-zero. Statistical significance of this effect indicates that it is likely the gene is differentially expressed between BL and non-BL. From a molecular epidemiology (and to a lesser extent biological) stand point the disease-region interaction effect αβ(g,b) is important, as it signifies that the extent of differential expression between BL and non-BL may be affected by geographical region. Statistical significance of this interaction effect indicates that it is likely the differential expression between BL and non-BL is different from one region to another; this would be an epidemiologically and biologically interesting finding as it helps to define the RNA-level differences between endemic and non-endemic Burkitt lymphoma.

Hence, we will be testing the main effect α(b) and the interaction effect for each RNA expression measurement. Because EBV positivity in BL may have an important effect on gene differential expression, in the final analysis we will also consider defining the disease main effect α(b) as a three-level factor: b = EBV+ BL, EBV- BL, non-BL.

It is imperative to assess the levels of statistical errors, especially false positives, made in these massive multiple tests. An assessment of the level of false negative errors with the

available sample size will be given in the next paragraph. Following the published microarray studies many of which from St. Jude, we will first assess the ensemble distribution of the P values and use the profile information criterion[116] to obtain a data-suggested level of statistical significance and the corresponding estimated false discovery rate (FDR),[116,117] and then estimate the false discovery rate corresponding to a set of statistical significance levels specified around the data-suggested level. Estimates of FDR will be computed using methods in the literature.[116,118,119] We will analyze further the "significant genes" so identified by modeling there expressions with disease subtype as well as potential confounding factors as explanatory variables.

Recognizing the fast changes in biotechnology, we'll use the most effective technology and appropriate statistical methods at the time of final analyses.

Levels of statistical errors – an assessment by published data. All of the published gene expression profiling studies of mature B-cell lymphomas focused on adults, but Hummel et al.[120] included a number of pediatric samples (age≤18 years). None of the published studies considered geographic region as a factor and thus there is no preliminary data available. Therefore, the preliminary microarray gene expression data on pediatric mature B-cell lymphomas are very limited. Nonetheless we provide a rough assessment of the levels of false positive and false negative errors that we may encounter in the analysis based on the pediatric data[120] followed by a discussion of caveats and limitations of this assessment.

Here we consider the tests of the main effect α(b) for the *m* (≈ 22000) Affymetrix GeneChip probesets. Adapting the concept of anticipated FDR (aFDR)[121] to the fixed sample size situation and the planned analysis outlined above, we define the aFDR at a given significance level α as

$$aFDR(\alpha) = \frac{\pi_0 \alpha}{\pi_0 \alpha + (1-\pi_0) AP}$$

Here π_0 is the proportion of true null hypotheses (non-differentially expressed genes), and AP is the *average power* which depends on the effect size, sample size, and significance level. The quantity $(1-\pi_0)(1-AP)$ is the expected proportion of true alternative hypotheses the multiple tests fail to capture, hence a measure of the level of false negative errors; we call this the *false negative proportion*.[116]

Hummel et al. (2006) included 32 pediatric samples run on Affymetrix U133A GeneChip[120]. In these pediatric lymphoma samples 6 are traditional BL, 10 are atypical BL, 12 are DLBCL, and 4 are aggressive B-NHL. The 32 samples are put into two groups: BL (traditional and atypical BL) and non-BL (DLBCL and aggressive B-NHL). To get some preliminary information about π_0, per-probeset comparisons of BL vs. non-BL by the two-sample t test yield 22,215 P values, one for each probeset. The profile information criterion (Ip)[116] produces a significance threshold (P value cut-off) to declare the significant tests in a way to balance the levels of false positive and false negative

errors, and a conservative (biased up) estimate of the corresponding FDR; it also provides an estimate of π_0 as part of the procedure. Applying this procedure to the 22,215 P values yields an estimate of π_0 0.6525 (65.25%), the significance threshold 0.004547, and the corresponding FDR 0.1377 (13.77%) among the 478 probesets declared differentially expressed at the 0.004547 significance level. For each probeset, the mean in the BL and non-BL group along with the within-group variance are computed to provide an estimate of the non-centrality parameter of the t test, and then is shrunk toward zero by the q-value weighting as described.[121] The probesets are then sorted by the magnitude of the shrunken non-centrality parameter in descending order. To be on the conservative side, for each postulated null proportion ($\boldsymbol{\pi_0}$) in the table below, the non-centrality parameters of the top $m(1\text{-}\boldsymbol{\pi_0})$ probesets are all set to the same as the $[m(1\text{-}\boldsymbol{\pi_0})]$th largest one, and the remaining non-centrality parameters are set to zero. Next, average power (AP) and $\boldsymbol{\pi_0}$ is estimated after this additional shrinkage, giving enlarged $\boldsymbol{\pi_0}$.

Based on the π_0 estimate and the significance threshold suggested by Ip, we provide the final non-centrality after shrinkage, estimates of aFDR, the false negative proportion FNP, and average power (AP) for a range of postulated and enlarged π_0 values and significance levels (P value cutoffs) in the following table.

Anticipated False Discovery Rate and False Negative Proportion

		$\boldsymbol{\pi_0}$					
	Postulated	**0.60**	**0.65**	**0.80**	**0.90**	**0.95**	**0.99**
	Enlarged	**0.7969**	**0.7994**	**0.8469**	**0.9117**	**0.9532**	**0.9902**
	Non-centrality	**3.39**	**4.17**	**7.57**	**11.82**	**15.81**	**24.38**
α	**0.0010 aFDR**	0.1107	0.0823	0.0388	0.0306	0.0372	0.1116
	FNP	0.3874	0.3344	0.1726	0.0673	0.0236	0.0019
	AP	0.0315	0.0443	0.1371	0.3268	0.5274	0.8079
	0.0025	0.1471	0.1141	0.0621	0.0599	0.0737	0.2241
		0.3773	0.3229	0.1583	0.0564	0.0180	0.0012
		0.0568	0.0773	0.2086	0.4358	0.6405	0.8783
	0.0045	0.1775	0.1417	0.0850	0.0830	0.1142	0.3330
		0.3673	0.3120	0.1464	0.0487	0.0144	0.0009
		0.0818	0.1086	0.2680	0.5134	0.7114	0.9144
	0.0050	0.1836	0.1473	0.0899	0.0891	0.1235	0.3554
		0.3651	0.3096	0.1440	0.0472	0.0138	0.0008
		0.0872	0.1153	0.2798	0.5278	0.7237	0.9201
	0.0075	0.2093	0.1715	0.1120	0.1171	0.1658	0.4474
		0.3555	0.2994	0.1342	0.0416	0.0115	0.0006
		0.1111	0.1444	0.3287	0.5838	0.7696	0.9396
	0.0100	0.2298	0.1911	0.1311	0.1419	0.2030	0.5160
		0.3474	0.2909	0.1267	0.0376	0.0100	0.0005
		0.1315	0.1687	0.3667	0.6239	0.8003	0.9514

For example, if the proportion of non-differentially expressed probesets π_0=0.65 (65%) and all probesets with P value ≤ α=0.0045 are declared as significantly differentially expressed between BL and non-BL, we anticipate that the false discovery rate aDFR=0.1417, i.e., 14.17% of these "significant" probesets are false positives; and that

the false negative proportion FNP=0.3120, i.e., we expect to miss 31.2% of the truly differentially expressed probesets.

It should be noted that the non-centrality parameters estimated above in fact correspond to one-factor ANOVA. Unfortunately, however, there is no preliminary data on the second factor (geographic region). The numerator of the non-centrality parameter determined from the one-factor model could be too optimistic for the two-factor mode, but the denominator (the within-group/residual standard deviation) determined by the one-factor model may be larger than that from the two-factor model.

The total number of tests performed for this analysis will be over 44,000 although the above error assessment was done for 22,000+ tests. Note however, aFDR and FNR do not depend directly on the total number of tests; rather they depend directly on the proportion of true null hypothesis π_0.

It should be noted that there will be very limited statistical power for gene expression profiling for BL vs. non-BL in a single geographic region, except perhaps in Latin America where the sample size is the largest among all regions.

Additionally, unsupervised analyses such as hierarchical clustering will be performed to explore novel subtypes in childhood lymphomas. Also, identified gene differential signatures will be compared with published results (in adult lymphomas) using methods such as gene set enrichment analysis.

Catalog and estimate frequencies of copy number variations in childhood lymphomas. There is no preliminary data on copy number variations (CNVs) in childhood lymphomas. A study of DLBCL in adults[8] reported the prevalence of CNVs in certain subsets ranged roughly from 4% to 40%. So, conservatively, with 14 samples in the non-BL group, if a CNV is observed in 1 sample (sample proportion 0.07), then the exact 95% confidence interval is [0.0037, 0.3122]; if a CNV is observed in 6 samples (sample proportion 0.43), the exact 95% confidence interval is [0.2061, 0.6878]. Additionally, we will compare the prevalence of CNVs between different subtypes of childhood lymphomas and geographic regions with exact chi-square or Fisher's test, taking population structures into account if necessary. DNA extracted from peripheral blood will be used as matched control tissue. Loss of heterozygosity (LOH) will be analyzed by comparison of SNP genotype data of the tumor sample to matched germline samples, or, in the case of samples lacking a matched germline sample, pools of reference samples already accrued at St Jude. LOH data will be analyzed using publicly available algorithms (e.g. dChip using the Hidden Markov Model) and alternative algorithms that will be tested and developed at St Jude during the course of the study. These analyses will complement analyses identifying copy number alteration and will permit identification of both deletional and copy-neutral loss-of-heterozygosity.

Integrated analysis of CNVs and gene expressions. For CNVs with a reasonable prevalence, we will explore the association between the identified CNVs and gene expressions in the study cohort. We will identify individual gene expression correlated

with a CNV, and a gene expression profile (signature) associated with a CNV. General linear models will be used to perform statistical tests, and multiple tests will be addressed using FDR, as described before.

To describe the types and frequency of mutations in the ARF-HDM2-TP53 pathway, in "non-endemic" B-cell lymphomas (United States) and those found in selected geographic regions of the world (Brazil, Chile, Guatemala, Honduras, El-Salvador, Singapore, and others).

This exploratory aim will be analyzed by enumerating the types of mutations and estimating the frequency of each type of mutation in each geographical region as well as in all regions pooled. Point estimates and 95% confidence intervals (CI) will be provided.

To describe the expression of ARF-HDM2-TP53 and PUMA-associated pathways in non-endemic B-cell lymphomas (United States) and that found in B-cell lymphomas of other selected geographic regions of the world (see above)

Protein as well as mRNA expression levels of important genes on these pathways from various bioassays will be summarized using descriptive statistics including mean, standard deviation, and the five-number summary in each geographical region as well as in all regions pooled.

To describe the pattern and frequency of XLP gene mutations presenting with B-cell lymphomas in the United States and selected geographic regions.

Point estimates and 95% CIs of the frequency of XLP mutation among boys will be calculated in each geographical region as well as in all regions pooled. Relationships of XLP mutations with clinical phenotypes will be explored by appropriate statistical models including contingency table analysis and/or logistic regression modeling (for categorical/binary phenotypes), general linear models for continuous phenotypes, and hazard regression models for right-censored failure times.

To describe the frequency of EBV-positive Burkitt lymphomas between "non-endemic" and endemic B-cell lymphomas;

a. *To describe the pattern of EBV protein and gene expression (e.g., EBNA 3) in EBV-positive lymphomas.*
b. *To relate EBV protein and gene expression with clinical, laboratory and outcome data.*

It is expected that among BL, about 80% are EBV positive in endemic BL (Latin America, Africa) and 45% are EBV positive in non-endemic BL (US, Mideast), and about 80% of the available tumor samples are BL. Point estimates and 95% CIs of the frequency of EBV-positive BL will be calculated for each geographical region. EBV protein expression will be described by summary statistics including mean, standard deviation, and the five-number summary in each geographical region as well as in all

regions pooled. Relationship of EBV protein expression with clinical, laboratory, and outcome features will be explored using appropriate statistical methods such as general linear models.