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TACL
Therapeutic Advances in Childhood Leukemia & Lymphoma

T2014-001 A Phase I Trial of Temsirolimus (CCI-779, Pfizer, Inc.) in Combination with Etoposide and Cyclophosphamide in Children with Relapsed Acute Lymphoblastic Leukemia and Non-Hodgkins Lymphoma

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STUDY COMMITTEE

ABSTRACT

One in five children with acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) will develop recurrent disease despite strategies of risk stratification and modern intensified chemotherapy. The combination of etoposide and cyclophosphamide is used widely in an attempt to salvage the refractory and relapsed lymphoblastic leukemia and lymphoma population. Therefore we propose a Phase 1 study to determine the feasibility and safety of adding two weekly doses of intravenous temsirolimus to standard ALL salvage therapy with 5 days of cyclophosphamide and etoposide.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase involved in signal transduction pathways which regulate cell growth and proliferation.[1-3] While mutations in mTOR do not frequently occur in human cancer[46], constitutive activation of signal transduction proteins related to the mTOR pathway (AKT kinase, S6K1, PI3K) or loss of proteins linked to mTOR (PTEN) are found in numerous malignancies.[1, 3, 4] Studies have shown that mTOR inhibitors (MTI) inhibit growth of pre-B and T-cell ALL cell lines *in vitro* and in ALL xenograft models.[5] In preclinical studies, ALL cell lines treated with temsirolimus showed synergism with many chemotherapeutic agents, including etoposide and dexamethasone.[6] The PPTP found *in vivo* combinations of sirolimus and cyclophosphamide were more effective than either agent used alone.[7]

The MTI temsirolimus was chosen for use in this study due to its weekly intravenous dosing, its more predictable blood levels, and availability of a single-agent pediatric MTD and its sustained biologic effect due to conversion to sirolimus.[8]

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Objectives

1.1.1 To determine the maximum tolerated dose (MTD) of temsirolimus when combined with etoposide and cyclophosphamide in pediatric and young adult patients with relapsed ALL or NHL.

1.1.2 To define the dose-limiting toxicity (DLT) and describe other serious toxicities of temsirolimus in combination with etoposide and cyclophosphamide in pediatric and young adult patients with relapsed ALL or NHL.

1.2 Secondary Objectives

1.2.1 To determine the response rate in refractory ALL and NHL patients within the confines of a Phase 1 study.

1.2.2 To evaluate responsiveness of patient lymphoblasts to mTOR inhibition using *in vitro* and *in vivo* pharmacodynamic (PD) assessments.

1.2.3 To describe minimal residual disease (MRD) levels present at end of cycle 1 therapy in patients with bone marrow involvement of disease.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase involved in signal transduction pathways which regulate cell growth and proliferation [1-3]. Its activation triggers resting cells to increase the translation of a subset of messenger RNAs whose proteins are required for cell-cycle progression from the G1 to S phase of the cell cycle (reviewed in [1-3]). While mTOR itself is not frequently mutated in human cancer, constitutive activation of signal transduction proteins related to the mTOR pathway (AKT kinase, S6K1, PI3K) or loss of proteins linked to mTOR (PTEN) are found in numerous malignancies.[1-4]

One in five children with ALL will develop recurrent disease despite strategies of risk stratification and modern intensified chemotherapy. The prognosis for these patients is poor even with aggressive retrieval strategies with high dose chemotherapy and stem cell rescue.,[9,10] Newer agents are needed to treat these patients. Biologically targeted cancer agents, including signal transduction inhibitors like mammalian target of rapamycin inhibitors (MTIs), have shown great promise in treating hematologic malignancies.[2, 5, 11] While signal transduction inhibitors may be efficacious as single agents, it is more likely that these targeted agents will demonstrate greater efficacy in combination with cytotoxic agents.

2.1.1 Rationale for Chemotherapy Backbone

The combination of etoposide and cyclophosphamide is used widely in an attempt to salvage the refractory and relapsed lymphoblastic leukemia and lymphoma population. Using the 3-[4,5-dimethyl-thiazole-2,5-diphenyl] tetrazolium bromide (MTT) cytotoxicity assay, both case/control and matched set comparisons of initial and relapse samples showed that relapse samples retain sensitivity to etoposide and cyclophosphamide, which are used minimally in front-line protocols.[12] Combining newer, targeted agents with known successful salvage therapies may potentiate response.

2.2 Preclinical Studies

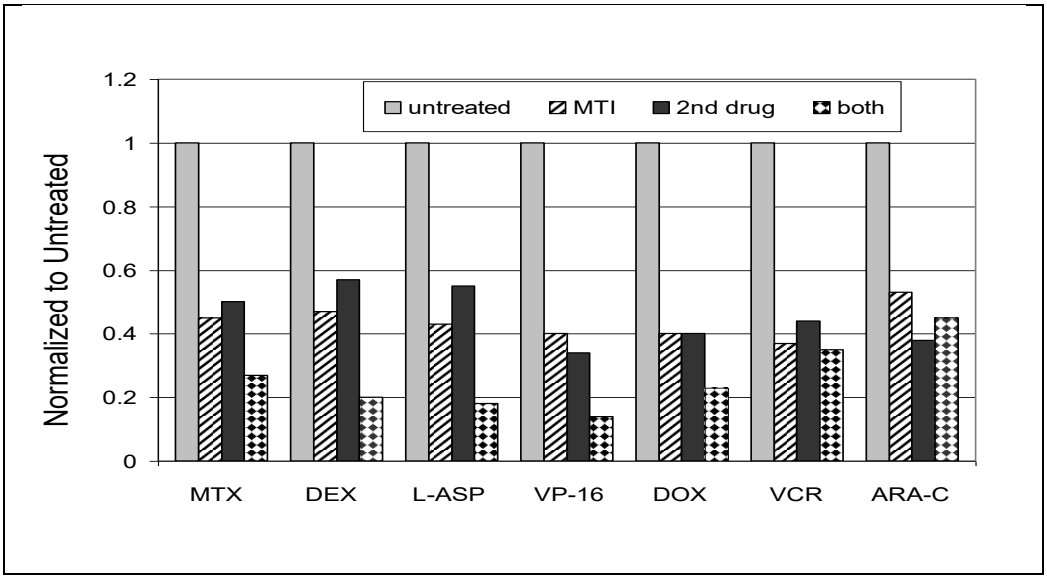
2.2.1 Antitumor Activity

Studies have shown that MTIs inhibit growth of B-precursor ALL lines *in vitro*, inducing apoptosis in both primary cell cultures 3 and NOD/SCID xenograft models.[5] Testing of the mTOR inhibitor sirolimus by the Pediatric Preclinical Testing Program (PPTP) *in vitro* showed maximal inhibition of 5 ALL cell lines ranging from 23-77%, with all 3 T-cell ALL lines having maximal inhibition greater than 70%.[7] *In vivo* rapamycin induced significant EFS differences in 5 of 8 ALL xenografts, including 2 of 2 T-cell ALL xenografts.[7]

Synergism with Chemotherapy

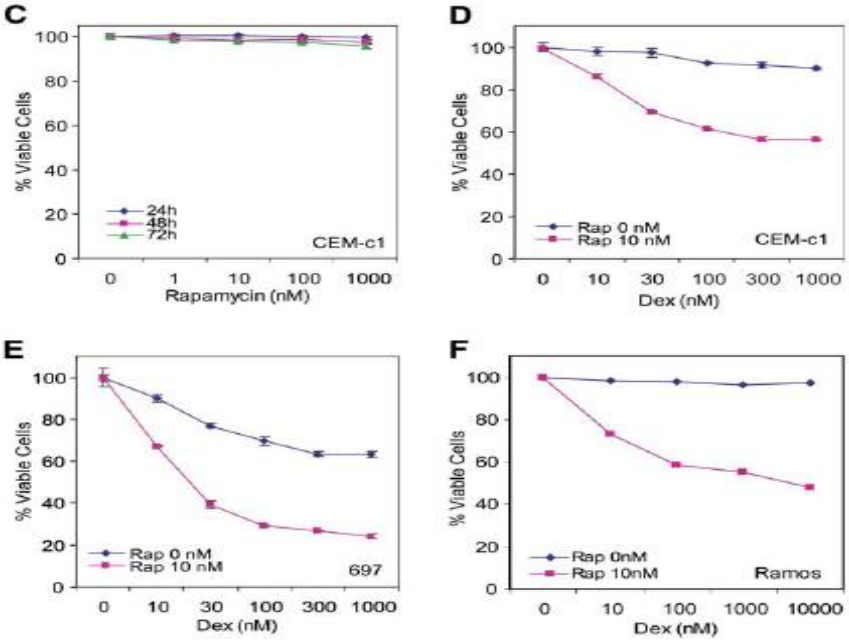
In preclinical studies, ALL cell lines treated with temsirolimus showed synergism with methotrexate, dexamethasone, L-asparaginase, etoposide and doxorubicin (Fig. 1). [6] Figure 1 depicts MTT data for each drug combination in 1 representative cell line. Grey bars represent untreated, slashed bars represent MTI alone, black bars represent cytotoxic alone, and dotted bars represent combined effect. All data is normalized to untreated baseline (Y axis). Each group of 4 bars represents a combination with a different cytotoxic. MTIs had at least an additive effect when combined with methotrexate (MTX), dexamethasone (DEX), L-asparaginase (L-ASP), etoposide (VP-16), and doxorubicin (DOX). ALL cell lines treated with vincristine or cytarabine showed neither additive benefit nor antagonism.

FIGURE 1: **Temsirolimus in Combination with Cytotoxic Therapy**



Sirolimus has also been found to reverse glucocorticoid resistance in resistant lymphoid cell lines (Fig. 2).[13] Wei et al. found that the mTOR inhibitor rapamycin sensitized lymphoid malignancy cells to glucocorticoid induced apoptosis via modulation of the antiapoptotic MCL1. MTIs also downregulate cyclin D1 leading to down-regulation of dihydrofolate reductase (DHFR) synthesis, possibly increasing sensitivity of ALL blasts to MTX.[6]

FIGURE 2: Rapamycin sensitizes lymphoid malignancy cell to GC-induced apoptosis



As described in Section 2.2.1, studies have shown that MTIs inhibit growth of B-precursor ALL lines *in vitro*, inducing apoptosis in both primary cell cultures [3] and NOD/SCID xenograft models.[5]

2.2.2 Preclinical Pharmacokinetic Studies

Repeat dose toxicity studies have demonstrated temsirolimus related changes in multiple organs and systems in rodents and non-rodents. These include lymphoid atrophy, and decreased lymphocytes, decreases in weight, size, and/or function of male reproductive

organs, increased cholesterol, and general inflammatory responses that included inflammation or rashes of the skin and increases in neutrophils, fibrinogen, and/or serum proteins. In addition, rodent-specific effects of temsirolimus included hyperglycemia, atrophy of female reproductive organs, myocardial degeneration and phospholipidosis. The pathogenesis of many of the changes is associated with the known antiproliferative effect of temsirolimus. The nature of changes and target organs were similar across studies, regardless of the duration of administration and were similar between IV and oral dosing.

The pharmacokinetic profile of temsirolimus after IV and oral administration was established based on results from *in vivo* studies in mice, rats, monkeys, and humans. Temsirolimus is extensively converted to sirolimus in mice and humans. Less conversion occurs in rats and monkeys. Temsirolimus demonstrates 85-87% binding to plasma proteins at concentrations of 10-100 ng/mL, with an apparent concentration-dependent binding to erythrocytes. The primary metabolites of temsirolimus in rats and monkeys are hydroxytemsirolimus (M10) and secotemsirolimus (M4). Oral administration of temsirolimus resulted in a more extensive number of circulating metabolites with temsirolimus as the major circulating compound-related product in rat and monkey whole blood, followed by sirolimus as the next most abundant metabolite. There is evidence of more extensive metabolism in the feces of rats compared with blood and plasma, suggesting further biotransformation during elimination. The primary oxidative metabolism is via CYP3A4, indicating that inhibitors and inducers of CYP3A4 enzyme system may alter the metabolism of temsirolimus, although temsirolimus does not induce CYP3A4.

2.3 Adult Studies

2.3.1 Phase 1 Studies

mTOR inhibitor in solid tumors:
In a Phase 1 trial of single agent temsirolimus in adults with solid tumors, doses ranged from 7.5 to 220 mg/m^2 weekly with no MTD determined.[8] At the 34 mg/m^2 and 45 mg/m^2 doses, one patient in each dose level had Grade 3 dose limiting toxicity (DLT) of thrombocytopenia; one also neutropenia and hypophosphatemia; the other had asthenia and diarrhea. Dose escalation continued to 220 mg/m^2 where the DLTs included grade 3 stomatitis, manic-depressive syndrome, transaminase elevation, and asthenia. At all dose levels, temsirolimus (and its hydrolysis product sirolimus) were in the range that inhibits cancer cell proliferation *in vitro.*[8]

mTOR inhibitor in hematologic malignancies:
A Phase 1/2 study of oral everolimus in adult patients with relapsed hematologic malignancies has also been reported.[14] Twenty-seven patients (only 1 with ALL) received either 5 mg or 10 mg daily. No DLTs were noted although grade 3 toxicities occurring in more than 5% of patients included hyperglycemia (22%), hypophosphatemia (7%) and fatigue (7%).

2.3.2 Phase 1 Studies in Combination with Chemotherapy

A variety of MTI's have been studied in combination with other therapies; including interferon in renal cell carcinoma,[15] hormonal agents in breast cancer,[16] 5-FU/LCV[17], imatinib in gastrointestinal stromal tumors, gemcitabine, and sorafenib in adult tumors. Early data on MTI/chemotherapy combinations have sometimes shown potential enhancement of expected toxicities when compared to single agent use in patients with solid tumors. In the 5-FU/LCV/temsirolimus study, grade 4 mucositis/stomatitis was observed at the 45 mg/m^2 level.[17] Grade 3 toxicities noted in adult trials with temsirolimus have been rash, nausea/vomiting, cytopenias, hypophosphatemia, hypokalemia,

hyperglycemia, asthenia/neuropathy, fatigue, mucositis, hypercholesteremia/ hypertriglyceridemia, and dyspnea.[15, 18-20]

The only published combination trial of an MTI and high-dose chemotherapy in leukemia is an adult AML Phase 1 dose escalation trial of oral daily sirolimus on a backbone of standard adult AML therapy consisting of 5 days each of mitoxantrone (8 mg/m^2/d), cytarabine (1 gm/m^2/d) and etoposide (100 mg/m^2/d). This trial did not demonstrate an increased toxicity rate with the MTI/chemotherapy combination. One patient had a DLT attributed to prolonged aplasia (120 days) at a sirolimus dose of 15 mg load and 5 mg daily. In the prior 4 dose levels, the median time to ANC >500 mm^3 was 27 days (range 16-38). Overall sirolimus was well tolerated and did not increase non-hematologic toxicity of the backbone chemotherapy.[21]

2.3.3 Phase 2 Studies

Phase 2 trials have also been conducted in a wide variety of tumors utilizing a flat dosing schedule of 25, 75 and/or 250 mg intravenously weekly with notable single agent activity in mantle cell lymphoma, breast cancer, and renal cell carcinoma. Two phase 2 studies of single agent temsirolimus in adults with relapsed or refractory mantle cell lymphoma have been conducted.[22, 23] Thirty-five patients were treated at a dose of 250 mg IV weekly. Objective responses were seen in 13 of 34 patients (one CR and 12 PR). The median time-to-progression in all patients was 6.5 months, and the median duration of response for the responders was 6.9 months. Twenty nine patients were treated at 25 mg IV weekly and responses were seen in 41% of patients (1 CR and 10 PR). Hematologic toxicities were common, and thrombocytopenia was the most frequent cause of dose reductions. The thrombocytopenia was brief, however, with resolution typically observed within one week. Treatment at the lower dose was associated with similar response but less myelosuppression[23]

A Phase 2 randomized, double-blind multicenter Phase 2 trial of temsirolimus has also been conducted in patients with advanced renal cell carcinoma. Patients (n=111) were treated with this drug as a single agent using doses of 25 mg, 75 mg or 250 mg IV weekly. The objective response rate was <10%, however time to progression was prolonged in the patients on this study compared to historical controls. Responses did not appear to be dose-dependent and the lowest dose was deemed capable of biologic activity. Therefore, the dose designated for further study was 25 mg IV weekly. The most frequently occurring drug related adverse events observed in patients on this study were rash (76%), mucositis (70%), asthenia (50%), and nausea (43%). The most frequently occurring > Grade 3 toxicities were hypophosphatemia (13%), anemia (9%), hyperglycemia (17%) and hypertriglyceridemia (6%). It has been suggested that the latter findings may be due to effects of the drug on signaling via insulin-associated pathways, and that changes in these laboratory parameters may serve as biomarkers of temsirolimus activity.[24]

Subsequently, 626 patients with metastatic RCC and 3 or more adverse risk features were treated in a randomized Phase 2I trial with temsirolimus 25 mg intravenously weekly versus 18 million units IFN-α thrice weekly versus 15 mg temsirolimus intravenously weekly + 6 million units IFN-α thrice weekly. Patients treated with temsirolimus had a statistically significant longer survival than IFN-a patients (10.9 vs. 7.3 months, P= 0.0069) with a 9% objective response rate in the temsirolimus monotherapy arm.[25] Grade 3 adverse events occurred in 67% of the temsirolimus group and included anemia, thrombocytopenia, hypercholesterolemia, hyperlipidemia, hyperglycemia, asthenia and dyspnea. Temsirolimus has also been combined with letrozole, 5 fluorouracil and leucovorin, and sorafenib with hyperglycemia, asthenia, rash, stomatitis, and hand-foot syndrome being the predominant toxicities.[17, 26, 27]

2.3.4 Pharmacology/Pharmacokinetics/Correlative and Biological Studies

In a prostate cancer clinical trial, target phosphoprotein inhibition in tumor was seen in an oral dosing regimen at a dose equivalent to 7 mg IV weekly.[28] In the oral everolimus trial nine patients had biologic samples of which 6 (including one responder) showed decreased phosphorylation of p70S6 and/or eIF4E-binding protein.[14]

2.4 Pediatric Studies

2.4.1 Prior Experience in Children

mTOR inhibitor in solid tumors:
A Phase 1 trial of single agent once weekly IV temsirolimus in children with advanced solid tumors had an MTD of 75 mg/m^2.[30] The DLT at the 150 mg/m^2 dose was grade 3 anorexia (1 pt) and grade 4 thrombocytopenia < 7 days (1 pt). Seven patients had ≥ grade 3 treatment-related adverse events: neutropenia, leukopenia, anemia, anorexia, thrombocytopenia, and increased ALT. Cmax was comparable to adult patients; temsirolimus area under the curve (AUC) was higher in children. Greater exposure to parent drug appeared balanced by shorter half-life and commensurate lower AUC of the sirolimus metabolite.

A Children's Oncology Group (COG) study of temsirolimus in combination with an antibody directed against the Type 1 insulin-like growth factor receptor (COG ADVL0813) is currently in progress. Another COG study of temsirolimus in combination with temozolomide and irinotecan (ADVL0918) is also in progress for recurrent pediatric solid tumors. In this study, both agents are being administered intravenously on a weekly basis. Results from both studies are not yet available.

On COG ADVL1114, three weekly doses of temsirolimus were given in combination with the UK ALL R3 re-induction treatment backbone. [31] Dose de-escalation of the temsirolimus from 10mg/m^2/dose to 7.5mg/m^2/dose was required due to the DLTs of hypertriglyceridemia and persistent toxicity > Grade 2 (hypertension, GGT, and mucositis in a non-responding patient) on day 42 of therapy. At the lower dose, DLTs of grade 5 sepsis, grade 3 ulcer and persistent GGT elevation at day 42 were identified. Significant infectious toxicity was noted in the relapsed ALL population. The study has been amended to decrease the number of doses of temsirolimus to 2 (on days 1 and 8) and intensive infection prevention guidelines have been added to the protocol. Of note, supportive care with g-csf was not permitted in ADVL1114.

mTOR inhibitor in hematologic malignancies:
An institutional Phase 1 trial of oral sirolimus monotherapy in 9 patients with relapsed acute leukemia has been published in abstract form.[32] The initial dose level was that used for pediatric renal transplant patients, 9 mg/m^2 load and 3 mg/m^2 daily, with one escalation to 12 mg/m^2 load and 4 mg/m^2 daily. No DLTs were noted, and 3 ALL patients met the criteria for stable disease, one receiving 3 full courses of sirolimus monotherapy.[32]

On a Dana-Farber institutional clinical trial, pediatric and adult patients with relapsed ALL were treated with a combination of sirolimus and glucocorticoids for 5 days prior to starting multiagent reinduction chemotherapy. Changes in gene expression as well as biologic markers of a pro-apoptotic state, including MCL-1 levels, were compared with samples from ALL patients who were treated with glucocorticoids alone. Of the 4 assessable patients treated with the combination of sirolimus and corticosteroid, 3 demonstrated a marked decrease in MCL-1 protein levels after initiation of therapy which was evident by 6 hours. This was accompanied by a concomitant decrease in *phospho-S6* ribosomal protein levels, suggesting successful mTOR inhibition. Of 4 patients treated with corticosteroids alone, three demonstrated no change in MCL-1 or *phospho-S6* ribosomal protein levels. All patients treated with sirolimus and corticosteroids demonstrated a decrease in circulating blasts (>80% decrease from baseline) during the 5 days of therapy.[33]

2.4.2 Pharmacology/Pharmacokinetics/Correlative Biological Studies

In the pediatric solid tumor trial Western blot analysis of phosphorylated S6 and AKT proteins in peripheral blood mononuclear cells, confirmed temsirolimus 75 mg/m^2 significantly inhibited AKT pathway signaling *in vivo.*[20] Inhibition was also seen at the 10 and 25 mg/m^2 dose levels but not in a consistently validated manner to serve as a pharmacodynamic endpoint.[30]

In the pediatric hematologic malignancy single agent sirolimus trial reductions in phosphorylation of p70S6, AKT, STAT3 and STAT5 were seen, but did not correlate with dose or response in this limited number of patients.[32] Similarly, while *in vivo* inhibition of target phosphoproteins was observed in several patients treated with temsirolimus on the COG ADVL1114 trial, these pharmacodynamic data also did not appear to correlate with clinical responses (Sarah Tasian, MD, private communication and Rheingold ASCO abstract 2015).

3.0 PATIENT ELIGIBILITY CRITERIA AND ENROLLMENT

3.1 Patient Reservation

Investigators should consult the TACL web site to determine if the study is currently open for accrual before approaching patients for participation. Before enrolling a patient on study, a reservation must be made with the TACL Operations Center. In order to make a reservation you may call [redacted] or send an email to [redacted] with the following information (if sending an email, please put "Reservation Request" in the subject line):

- Study for which you want to make a reservation:
- Name of the institution requesting reservation with contact information
- Patient Initials (Last, First)
- Patient month and year of birth

If an enrollment slot is available, you will receive an email from the TACL Operations Center to confirm your reservation. All reservations are good for five full calendar days starting with the next full day after the day the reservation is made.

3.2 Enrollment

An enrollment guide is available on the web site (https://tacl.chla.usc.edu/tacl). Patients must be enrolled prior to beginning treatment on this study. It is allowable to enroll a patient who has received IT ARA-C, IT MTX or triple IT therapy within seven days of enrollment as part of their evaluation to diagnose disease relapse. Patients will be enrolled by contacting the TACL Operations Center at Children's Hospital Los Angeles Monday through Friday, 8:30 am – 5:00 pm Pacific Time at [redacted] except holidays. You will be asked to complete the eligibility form on the TACL RDE System prior to making your call. In addition, the supporting documentation, which confirms eligibility, should be emailed or faxed to the TACL Operations Office at [redacted].

Each patient will be assigned a unique TACL registration and study subject number. The dose level of temsirolimus will be assigned by the TACL Operations Center at the time of study registration. An email confirming eligibility and assigned dose level will be sent to the treating facility and Study Co-Chairs, Patients should begin treatment within 3 calendar days of study enrollment.

Contact Information:

TACL Operations Center
Children's Hospital Los Angeles
4650 Sunset Blvd, MS #54
Los Angeles, CA 90027
[redacted]
Email: TACL@chla.usc.edu

3.3 Inclusion Eligibility Criteria

The eligibility criteria listed below are interpreted literally and cannot be waived.

3.3.1 Age

Patients must be ≥ 12 months and ≤ 21 years of age at the time of study enrollment.

3.3.2 Diagnosis

Leukemia

Patients must have relapsed or refractory acute lymphoblastic leukemia (ALL) defined as below:

a. Bone marrow involvement defined as ≥ 5% blasts (M2 or M3) with or without extramedullary involvement.
b. Refractory bone marrow involvement defined as MRD ≥ 0.1% blasts done at a COG-approved MRD testing lab after most recent treatment regimen.
c. Newly diagnosed patients (T or B-cell ALL) with refractory bone marrow involvement after Consolidation therapy are eligible.
d. First relapse B-cell ALL patients are eligible with refractory disease.
e. Second or greater relapse B-cell patients are eligible at time of relapse or with refractory disease.
f. First or greater relapse T-cell ALL patients are eligible at time of relapse or with refractory disease.
g. Isolated CNS 2 or 3 patients with < 0.1% MRD bone marrow involvement are not eligible.

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Lymphoma

Patient must have relapsed or refractory lymphoma with:

a. Lymphoblastic lymphoma or peripheral T-cell lymphoma.
b. Histologic verification of disease at original diagnosis or subsequent relapse.
c. Evaluable or measurable disease documented by clinical or radiographic criteria or bone marrow disease present at study entry.
d. Patient may have CNS 2 or 3 status.

3.3.3 Performance Level

Karnofsky ≥ 50% for patients > 16 years of age and Lansky ≥ 50 for patients ≤ 16 years of age. (See Appendix I for Performance Scales), Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score

3.3.4 Prior Therapy

Patients must have fully recovered from the acute toxic effects of all prior anti-cancer chemotherapy, defined as resolution of all such toxicities to ≤ Grade 2 or lower per the inclusion/exclusion criteria.

a. Myelosuppressive chemotherapy:

- Patients with leukemia or lymphoma who relapse while receiving maintenance chemotherapy will not be required to have a waiting period before enrollment onto this study.

- At least 14 days must have elapsed after the completion of cytotoxic therapy, with the exception of hydroxyurea. Note: Cytoreduction with hydroxyurea in patients can be initiated and continued for up to 24 hours prior to the start of protocol therapy.

- Patients who received intrathecal (IT) chemotherapy within seven days of therapy initiation remain eligible, but should not receive the day 1 IT chemotherapy. (See Section 4.1 for additional details).

b. Hematopoietic growth factors: At least 14 days after the last dose of a long-acting growth factor (e.g. Neulasta) or 7 days for short-acting growth factor (e.g., Filgrastim).

c. Biologic (anti-neoplastic agent): At least seven days after the last dose of a biologic agent. For agents that have known adverse events occurring beyond seven days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the study chair.

Immunotherapy: At least 30 days from last infusion of chimeric antigen receptor T cell (CART) therapy or tumor vaccines.

d. Monoclonal antibodies: At least three half-lives of the antibody must have elapsed after the last dose of a monoclonal antibody with the exclusion of blinatumomab. (ie: Rituximab = 66 days, Epratuzumab = 69 days). Patients must have been off blinatumomab infusion for at least 4 days and all drug-related toxicity must have resolved to grade 2 or lower as outlined in the inclusion and exclusion criteria.

e. XRT: At least 14 days after local palliative XRT (small port); At least 84 days must have elapsed if prior TBI, craniospinal XRT or if ≥ 50% radiation of pelvis; at least 42 days must have elapsed if other substantial marrow radiation.

f. Stem Cell Infusion: No evidence of active graft vs. host disease and at least 84 days must have elapsed after transplant or stem cell infusion.

3.3.6 Organ Function Requirements

3.3.6.1 Adequate Bone Marrow Function Defined as:

- Patients should not be known to be refractory to red blood cell or platelet transfusions.
- Blood counts are not required to be normal prior to enrollment on trial. However, platelet count must be ≥ 20,000/mm^3 to initiate therapy (may receive platelet transfusions).

3.3.6.2 Adequate Renal Function Defined as:

- Creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m^2 or
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	**Female**
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR [34] utilizing child length and stature data published by the CDC.

3.3.6.3 Adequate Liver Function Defined as:

- Total bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x institutional upper limit of normal for age
- . SGPT (ALT) and SGOT (AST) must be less than 3 x institutional upper limit of normal (Grade 1 or less per CTCAE 4.03).
- GGT must be less than 2.5 x institutional upper limit of normal (Grade 1 or less per CTCAE 4).
- Serum albumin ≥ 2 g/dL.
- The hepatic requirements may be waived for patients with Grade 1 or 2 elevations of hepatic transaminases clearly due to leukemic infiltration after consultation with a study Co-Chair.

3.3.6.4 Adequate Cardiac Function Defined As:

- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by gated radionuclide study/echocardiogram.

3.3.6.5 Adequate Pulmonary Function Defined as:

- Pulse oximetry > 94% on room air (> 90% if at high altitude)
- No evidence of dyspnea at rest and no exercise intolerance.
- Baseline chest x-ray with no evidence of active infectious disease or pneumonitis. (See Section 3.4.3)

3.3.6.6 Reproductive Function

- Female patients of childbearing potential must have a negative urine or serum pregnancy test confirmed prior to enrollment.
- Female patients with infants must agree not to breastfeed their infants while on this study.
- Male and female patients of child-bearing potential must agree to use an effective method of contraception approved by the investigator during the study.

3.3.6.7 Random or fasting glucose within the upper limits of normal for age. If the initial blood glucose is non-fasting and above normal limits a fasting glucose can be obtained and must be within the upper limits of normal for age.

3.3.6.8 Triglyceride/Cholesterol

Fasting or non-fasting serum triglyceride level ≤ 300 mg/dL and serum cholesterol level ≤ 300 mg/dL.

3.4 Exclusion Eligibility Criteria

3.4.1 Concomitant Medications

a. Corticosteroids: Patients receiving corticosteroids for disease control who have not been on a stable or decreasing dose of corticosteroid for at least 7 days prior to enrollment are not eligible.

b. Investigational Drugs: Patients who are currently receiving another investigational drug are not eligible. The definition of “investigational” for use in this protocol means any drug that is not licensed by the FDA, Health Canada or the Therapeutic Goods Administration to be sold in the countries they govern. (United States, Canada and Australia)

c. Anti-cancer Agents: Patients who are currently receiving or may receive while on therapy, other anti-cancer agents, radiation therapy or immunotherapy are not eligible [except leukemia patients who relapsed on Maintenance therapy or patients receiving hydroxyurea, which may be continued until 24 hours prior to start of protocol therapy].

Intrathecal chemotherapy (at the discretion of the primary oncologist) may be given up to one week prior to the initiation of study treatment (day 1 therapy).

d. Anti-GVHD or agents to prevent organ rejection post-transplant: Patients who are receiving cyclosporine, tacrolimus or other agents to prevent either graft-versus-host disease post bone marrow transplant or organ rejection post-transplant are not eligible for this trial. At least 3 half-lives must have elapsed after the last dose of GVHD meds.

e. Anticoagulants: Patients who are currently receiving therapeutic anticoagulants (including aspirin, low molecular weight heparin, and others) are not eligible. At least 3 half-lives must have elapsed after the last dose of anticoagulants.

f. Angiotensin-converting enzyme (ACE) inhibitors: Patients who are currently receiving ACE inhibitors are not eligible due to the development of angioneurotic edema-type reactions in some subjects who received concurrent treatment with temsirolimus + ACE inhibitors. At least 3 half-lives must have elapsed after the last dose of ACE inhibitors.

g. Calcium Channel Blockers: Patients who are currently receiving Calcium Channel Blockers are not eligible due to the development of angioneurotic edema-type reactions in some subjects who received concurrent treatment with temsirolimus + Calcium Channel Blockers. At least 3 half-lives must have elapsed after the last dose of Calcium Channel Blockers.

h. Enzyme inducing Anti-convulsants: Patients who are currently receiving enzyme inducing anticonvulsants (ie phenytoin, phenobarbitol, or carbamazepine) are not eligible. Stabilizing on a non-hepatic inducing metabolizing anti-convulsant (ie: gabapentin or levetiracetam) prior to study entry is acceptable. At least 3 half-lives must have elapsed after the last dose of enzyme inducing anti-coagulants.

i. Patients receiving treatment with azoles such as fluconazole or voriconazole which are potent inhibitors of temsirolimus metabolism. At least three half-lives must have elapsed after the last dose of azoles.

3.4.2 Patients must have no pre-existing Grade 1 or higher ulcerations, fistulas, mucosal lesions, or skin barrier breakdown

3.4.3 Patient with Burkitt's leukemia and/or lymphoma are not eligible.

3.4.4 Infection Criteria

Patients are excluded if they have:

- Positive blood culture within 48 hours of study enrollment;
- Fever above 38.2 within 48 hours of study enrollment with clinical signs of infection. Fever that is determined to be due to tumor burden is allowed if patients have documented negative blood cultures for at least 48 hours prior to enrollment and no concurrent signs or symptoms of active infection or hemodynamic instability.
- A positive fungal culture within 30 days of study enrollment.
- Active fungal, viral, bacterial, or protozoal infection requiring IV treatment. Chronic prophylaxis therapy to prevent infections is allowed.

3.4.5 Patients with Down syndrome and Fanconi Anemia are excluded.

3.4.6 Patients will be excluded if they have significant concurrent disease, illness, psychiatric disorder or social issue that would compromise patient safety or compliance with protocol treatment or required observations, interfere with consent, study participation, follow up, or interpretation of study results.

3.4.7 Patients with known optic nerve and/or retinal involvement (because it may not be possible to safely delay irradiation) are not eligible. Patients presenting with visual disturbances by history or physical exam should have an ophthalmological exam and, if indicated, an MRI to determine optic nerve or retinal involvement.

3.5 Regulatory

3.5.1 **Informed Consent**

All patients and/or their parents or legal guardians must sign a written informed consent. Age appropriate assent will be obtained per institutional guidelines. To allow non-English speaking patients to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

3.5.2 **Protocol Approval**

All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PROGRAM

The following sections detail the treatment plan for each course of therapy.

Due to a significantly increased risk of morbidity and mortality in children with multiply relapsed ALL undergoing intensive therapy, hospitalization from initiation of therapy until: 1) there is evidence of ANC recovery defined as ≥ 200/microliter and increasing, and 2) they are afebrile and clinically stable. See Section 5.2 for highly recommended infection prevention guidelines. Please refer to the Drug Information section for additional administration guidelines. Treatment should begin within 3 calendar days of enrollment.

4.1 Treatment Course

This is a Phase I feasibility pilot of temsirolimus in combination with five days of cyclophosphamide and etoposide. After eligibility is determined, consent obtained, and registration occurs patients receive their first dose of temsirolimus on day 1, followed immediately by their first doses of etoposide and cyclophosphamide. Etoposide and cyclophosphamide are continued for the next four days (day 1-5). A second dose of temsirolimus is given on day 8. A course is defined as 29 days. All patients should get two courses of therapy in the absence of progressive disease, dose limiting toxicity or investigator/subject preference to withdraw.

Leukemia patients who are M1 and CNS1/2 and lymphoma patients with stable disease or better after the second course of therapy, may receive up to an additional six courses (for a total of eight courses) in the absence of relapse or progressive disease.

	1	2	3	4	5	6	7	8	9	10	11	15	22	29
Temsirolimus	●							●						
Etoposide[A]	●	●	●	●	●									
Cyclophosphamide	●	●	●	●	●									
Intrathecal Therapy	See detailed instructions below													

`A-available option: substitution of Etoposide Phosphate for etoposide (at 1:1 dosing) in case of etoposide shortage

Temsirolimus

- **Dose will be assigned at study entry.** Maximum dose is based on BSA of 2 m^2 and found in Section 4.3. Give IV over 30 minutes (+/- 10 minutes) on days 1 and 8. On day 1 temsirolimus is given prior to etoposide and cyclophosphamide.
- Patients are to receive premedication with diphenhydramine (1 mg/kg, max 50 mg) prior to each temsirolimus dose (See Section 4.6.1.4 for Infusional Reactions).
- Anaphylactic precautions should be observed during temsirolimus administration.
- **A specific supply of temsirolimus is supplied by Pfizer Pharmaceuticals for use in this study. Do not use commercially available drug.**

Etoposide

- 100 mg/m^2 IV over 1-2 hours daily x 5 on Days 1-5. Dilute per institutional standards. To be given after temsirolimus on day 1 and prior to cyclophosphamide on days 1-5.
- Patients allergic to etoposide may be treated with Etoposide Phosphate (Etopophos): 100 mg/m^2 IV daily x 5 days on days 1-5.

Cyclophosphamide

- 440 mg/m^2 IV daily x 5 on Days 1-5 given over 30-60 minutes. Dilute per institutional standards, including pre and post hydration. To be given following etoposide.

Intrathecal Cytarabine (CNS 1)

COURSE 1: Give intrathecally to patients with CNS1 disease at the dose defined by age below on day 1 of course 1 if no other IT chemotherapy is given within 1 week of day 1 of course 1.

Age (yrs):	Cytarabine Dose:
1 – 1.99	30 mg
2 – 2.99	50mg
≥ 3	70 mg

Triple Intrathecal Therapy (CNS 2 and 3)

- COURSE 1: Give intrathecally to patients with CNS 2 or 3 disease at the doses defined by age below on day 1 if no other IT chemotherapy given within 1 week of day 1 of course 1. Then give weekly until the patient is CNS 1 or 2 (investigator discretion). No more than 5 weekly doses to be given in cycle 1.
- COURSES 2-8: Give intrathecally to patients who were CNS 2 or 3 at study entry on day 1 of each course.

Age (yrs):	Dose Methotrexate (MTX), Hydrocortisone (HC), Cytarabine (ARAC):		
1 – 1.99	MTX: 8 mg,	HC: 8 mg,	ARAC: 16 mg
2 – 2.99	MTX: 10 mg,	HC: 10 mg,	ARAC: 20 mg
3 – 8.99	MTX: 12 mg,	HC: 12 mg,	ARAC: 24 mg
≥ 9	MTX: 15 mg,	HC: 15 mg,	ARAC: 30 mg

Leucovorin Rescue

5mg/m^2/dose every 12 hours x 2 doses, starting at 48 hours post any Methotrexate containing IT given day 0 or after.

Intrathecal Methotrexate (Post Course 1)

COURSES 2, 4, 6, 8: Give intrathecally to patients who were CNS1 at study entry day 1 of each course.

Age (yrs):	Methotrexate Dose:
1 – 1.99	8 mg
2 – 2.99	10 mg
3 – 8.99	12 mg
≥ 9	15 mg

4.2 Disease Evaluations

4.2.1 Leukemia Patients

All patients should have a bone marrow aspirate/biopsy, lumbar puncture, and CBC to assess response on day 29 +/- 3 days as needed for scheduling reasons.

- A bone marrow procedure need not be performed if the patient has an absolute blast count (ABC) greater than or equal to 2,500/mm^3 in the peripheral blood on day 29.
- If the bone marrow is hypoplastic or M1 but counts have not recovered to ANC ≥ 500 and PLTS ≥ 50,000 (without transfusion) obtain a weekly CBC and perform a bone marrow with evidence of count recovery (ANC ≥ 500 and PLTS ≥ 50,000) or day **42**; whichever is **first**. A repeat bone marrow does not need to be performed if the patient subsequently develops an absolute blast count (ABC) greater than or equal to 2,500/mm^3
- If the marrow is M2 by aspirate (assessed by morphology and/or flow cytometry) or the bone marrow biopsy shows 5-25% residual leukemia (assessed by morphology or immunohistochemistry), then proceed to course 2 regardless of ANC and PLT count after

all grade 3 or 4 non-hematologic toxicities have returned to grade 2 or less.

- If the marrow is M3 or has more blasts than the pre-study bone marrow, has progression in any extramedullary site, or if the patient is CNS 3 at day 29, discontinue protocol therapy and proceed with alternative treatment. The one exception to this is patients who are CNS 3 solely due to chloromas or cranial nerve findings and are CNS 1/2 by LP who have had at least a partial response in that area.

- A lumbar puncture with cytospin should be done on day 29 +/- 3 days to assess CNS status. Course 2, Day 1 Intrathecal chemotherapy can be given at this time if ANC ≥ 500 and PLTS ≥ 50,000μL.

4.2.2 Lymphoma Patients

All patients should have a CBC, bone marrow aspirate or biopsy (ONLY if positive at study entry), lumbar puncture (ONLY if positive at study entry), and imaging of known sites of disease on day 29 +/- 3 days as needed for scheduling reasons.

NHL patients who were bone marrow positive at study entry

- If the marrow is hypoplastic or M1, and counts have not recovered to ANC ≥ 500 and PLTS ≥ 50,000 (without transfusion) repeat marrow and CBC weekly until recovery or progression.

- If the marrow is M2 by aspirate (assessed by morphology and/or flow cytometry) or the bone marrow biopsy shows 5%-25% residual lymphoblasts (assessed by morphology or immunohistochemistry), then proceed to course 2 regardless of ANC and PLT count after all grade 3 or 4 non-hematologic toxicities have returned to grade 2 or less.

- If the marrow is M3 or has more blasts than the pre-study bone marrow, has progression in any extramedullary site, or if the patient is CNS 3 at day 29 discontinue protocol therapy and proceed with alternative treatment. The one exception to this is patients who are CNS 3 solely due to chloromas or cranial nerve findings and are CSF negative who have had at least a partial response in that area.

4.3 Dose Escalation Schedule

The starting dose of temsirolimus (7.5 mg/m^2/dose) is lower than the FDA approved adult renal cell carcinoma dose (25 mg) and lower than the single agent DLT found in children with solid tumors (75 mg/m^2). Temsirolimus will not be escalated beyond dose level 4. If tolerated the dose will be escalated as follows:

Dose Level	Dose	Maximum Dose
1	7.5 mg/m²	15 mg
2	10 mg/m²	20 mg
3	15 mg/m²	30 mg
4	25 mg/m^2	50 mg

4.4 Criteria to begin Course 2 and subsequent courses

Patients may begin the second or subsequent courses once the following criteria are met:

4.4.1 Course 2 Requirements

- Patients must be CNS 1 or 2 in order to continue on to course 2. The one exception to this is patients who are CNS 3 solely due to chloromas or cranial nerve findings and are CNS 1/2 by LP who have had at least a partial response in that area.

- Leukemia patients with an M1 marrow after the first course may proceed to Course 2 when ANC ≥ 500/μL and platelet count ≥ 50,000 (without transfusion) as long as all grade 3 or 4 non-hematologic toxicities have resolved to grade 2 or less.

- Leukemia patients with > 25% blasts at study entry with an M2 marrow after the first course may proceed to Course 2 independent of peripheral blood counts (the minimum platelet requirement to receive temsirolimus is 20,000). Patients with leukemia and an M2 marrow or less at study entry who do not have progressive disease may continue to Course 2 at the investigators discretion, also independent of blood counts. Patients with M3 marrow should proceed to alternative therapy. To proceed to Course 2 grade 3 or 4 non-hematologic toxicities have to have resolved to grade 2 or less. The Course 2, day 1 intrathecal chemotherapy can be done up to day 8 of Course 2.

- Leukemia patients with non CNS extramedullary disease that is stable or improving and does not require radiation may proceed to course 2 per their BM status above.

- Lymphoma patients may proceed to Course 2 in the absence of clinical or radiographic progressive disease (Section 10.2.5) when ANC ≥ 500/μL and platelet count ≥ 50,000 as long as all grade 3 or 4 non-hematologic toxicities have resolved to grade 2 or less. Patients with lymphoma and marrow involvement at study entry who remain with < 25% blasts (M1 or M2) may continue to Course 2 at the investigators discretion.

- For second and subsequent courses patients must have no Grade 1 or higher ulcerations, fistulas, mucosal lesions, or skin barrier breakdown

- Organ function eligibility requirements: to proceed to course 2.

  Adequate Renal Function Defined as:
  -Creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m^2 or
  -A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR [34] utilizing child length and stature data published by the CDC.

  Adequate Liver Function Defined as:
  - Total bilirubin (sum of conjugated + unconjugated) must be less than or equal to 1.5 x institutional upper limit of normal (Grade 1 or less per CTCAE 4.03).
  - SGPT (ALT) and SGOT (AST) must be less than 3 x institutional upper limit of normal (Grade 1 or less per CTCAE 4.03).
  - GGT must be less than 2.5 x institutional upper limit of normal (Grade 1 or less per

CTCAE 4.03).
- Serum albumin ≥ 2 g/dL.

Adequate Pulmonary Function Defined as:
- Pulse oximetry > 94% on room air (> 90% if at high altitude)
- No evidence of dyspnea at rest and no exercise intolerance.

Random or fasting glucose within the upper limits of normal for age.
If the initial blood glucose is non-fasting and above normal limits a fasting glucose can be obtained and must be within the upper limits of normal for age.

Triglyceride/Cholesterol
Fasting or non-fasting serum triglyceride level ≤ 300 mg/dL and serum cholesterol level ≤ 300 mg/dL.

4.4.2 Course 3-8 Requirements

- Patients with leukemia may receive courses 3-8 if they have an M1 marrow or MRD < 5% and an ANC ≥ 500 with or without platelet recovery prior to each course. The minimum platelet requirement to receive temsirolimus is 20,000. Patients must remain CNS 1 or 2. All other extramedullary involvement must be resolved. All grade 3 or 4 non-hematologic toxicities must have resolved to grade 2 or less prior to initiation of subsequent courses.
- Patients with lymphoma may receive courses 3-8 in the absence of progressive disease and an M1 marrow status when ANC ≥ 500/μL and platelet count ≥ 50,000 as long as all grade 3 or 4 non-hematologic toxicities have resolved to grade 2 or less. The minimum platelet requirement to receive temsirolimus is 20,000. Patients must remain CNS1 or 2. All other extramedullary involvement must be resolved.
- For subsequent cycles patients must have no Grade 1 or higher ulcerations, fistulas, mucosal lesions, or skin barrier breakdown

4.5 Dose-Limiting Toxicity (DLT)

Toxicity will be graded using the CTCAE criteria, version 4.03. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (**http://ctep.cancer.gov**). Any dose-limiting toxicity (DLT) should be reported immediately through the TACL Operations Center to the Study Chair.

DLT will be evaluated during Course 1 only.

DLT will be defined as any of the following events that are at least (possibly, probably or definitely) attributable to temsirolimus. The DLT observation period for the purposes of dose-escalation will be the first cycle of therapy (max 42 days).

4.5.1 Non-hematological dose-limiting toxicity

4.5.1.1 Any grade 3, 4 or 5 non-hematologic toxicity that occurs any time after the first dose of temsirolimus and is at least possibly attributable to temsirolimus, with the following exceptions:
- Grade 3 or 4 fever, febrile neutropenia or infection, +/- hospitalization.
- Grade 3 gastrointestinal symptoms (anorexia, nausea, vomiting, and diarrhea) that resolve to ≤ grade 2 or baseline by day 36 of therapy.
- Grade 3 or 4 AST/SGOT, ALT/SGPT, or GGT that returns to ≤ Grade 2 or baseline within 36 days of the start of therapy.

- Grade 3 constitutional symptoms (fatigue, malaise, weight loss, dehydration) that resolve to ≤ grade 2 or baseline by day 36 of therapy.
- Grade 3 or 4 hyperglycemia controllable by diet, oral diabetic drug and/or insulin that resolves to ≤ grade 2 within 96 hours of an intervention. If hyperglycemia is not controllable (≤ grade 2) by diet modification, insulin therapy and an oral diabetic agent it will be considered a DLT.
- Grade 3 or 4 metabolic/laboratory abnormalities (Na, K, HCO_3, Ca++, PO_4, creatinine and uric acid) attributable to tumor lysis syndrome (including tumor lysis syndrome itself) or its treatment that resolve to ≤ grade 2 or baseline by day 36 with or without the use of supplementation or appropriate replacement therapy.
- Grade 3 or 4 asymptomatic electrolyte abnormalities that returns to ≤ Grade 2 or baseline within 36 days of the start of therapy
- Grade 3 or 4 hypofibrinogenemia
- Grade 3 albumin
- Grade 3 or 4 fasting hypertriglyceridemia or fasting hypercholesterolemia that returns to ≤ Grade 2 within 4 weeks of starting lipid lowering medication. (See Section 4.6.1.7).
- Grade 3 or 4 non-fasting hypertriglyceridemia or hypercholesterolemia. If Grade 3 or 4 non-fasting hypertriglycerdemia or hypercholesterolemia is detected when routine (non-fasting) laboratory studies are performed, the test should be repeated within 3 days in the fasting state to permit accurate grading.
- Grade 3 hypertension that resolves to (≤ Grade 2) with appropriate use of anti-hypertensives.
- Grade 3 mucositis/stomatitis. (See Section 4.6.1.6. for supportive care guidelines for mucositis).

4.5.1.3 If a patient develops grade 3 or 4 pneumonitis attributable to temsirolimus the subsequent dose of temsirolimus will be held regardless of course. This will be considered a DLT in Course 1 of therapy.

4.5.1.4 Allergic reactions that necessitate discontinuation of study drug will not be considered a dose-limiting toxicity.

4.5.1.5 Any Grade 3 or 4 toxicity that requires a dose of temsirolimus to be held in Course 1 will be considered a dose-limiting toxicity. Toxicities attributable to temsirolimus that do not resolve to ≤ Grade 2 by day 36 will be considered dose-limiting toxicities.

4.5.2 **Hematological dose limiting toxicity**

Bone marrow aplasia: > 42 days from Day 1 of therapy will be a DLT. Aplasia is defined as the failure to recover a peripheral ANC of > 500/mm^3 and PLT > 20,000/mm^3 in the absence of persistent leukemia.

4.6 Dose Modifications

4.6.1 Dose Modifications of Temsirolimus for Non-Hematological Toxicity

A Study Co- Chair must be notified of any temsirolimus dosage modification.

4.6.1.1 Patients who have any dose-limiting non-hematological toxicity (as defined in Section 4.5.1) will have subsequent doses of temsirolimus held until the toxicity resolves to ≤ grade 2 or as otherwise indicated in the specific dose modifications below . The dose of temsirolimus will be restarted at one dose level below (or 5 mg/m^2 if at dose level 1). Missed doses of temsirolimus should not be made up. The etoposide and cyclophosphamide should be continued as long as those drugs are not directly attributable to the toxicity. If a DLT occurs but the patient meets requirements to receive

a subsequent course, the dose of temsirolimus will be restarted at one dose level below (or 5 mg/m^2 if at dose level 1). If tolerated subsequently the dose can be increased back up to the original assigned dose.

4.6.1.2 If a non-hematological toxicity recurs in a second course after one dose reduction, the patient will receive no further doses of temsirolimus. Patient may complete backbone chemotherapy as long as the other chemotherapeutic agents are not implicated in the toxicity or move on to other therapy per investigator choice.

4.6.1.3 Dose modification for hyperglycemia:
Therapy modifications for patients who develop hyperglycemia (based on random, non-fasting glucose levels) should be:

Hyperglycemia Grade	Action
Grade 1-2	Continue temsirolimus
Grade 3	• Continue temsirolimus at same dose and initiate diet or TPN modification, insulin therapy and/or oral diabetic agent* as indicated. • The patient should continue to receive concomitant insulin and/or an oral diabetic agent for the management of hyperglycemia while on protocol therapy. Due to the residual effects of temsirolimus on blocking intracellular glucose uptake by inhibition of Glut1 transport and the long half life of temsirolimus, initial therapy with insulin may not work. Early use of an **oral** diabetic agent is suggested.
Grade 4	• Initiate diet or TPN modifications, insulin and/or oral diabetic therapy as indicated. • Hold temsirolimus until resolves to ≤ Grade 3 • Resume temsirolimus at same dose IF patient is asymptomatic AND serum glucose is consistently < 250 mg/dL (≤ Grade 2). The patient may continue to receive concomitant insulin and/or an oral diabetic agent for the management of hyperglycemia while receiving temsirolimus. • If Grade 4 hyperglycemia recurs despite diet modification, and a stable dose of insulin, and an oral diabetic agent, then any subsequent doses of temsirolimus should be held.

***Recommended guidelines for use of oral diabetic agents: Initiation of treatment for hyperglycemia should occur under the guidance of a pediatric endocrinologist at the local institution.** Metformin or other oral anti-hyperglycemia agent may be used per local endocrinologist's recommendations. Insulin therapy should be directed by specialists in pediatric diabetes, with the goal of normal fasting blood sugars < 126 mg/dL and HgbA1C < 8%.

4.6.1.4 Dose modification for infusional reactions to temsirolimus
Therapy modifications for patients who develop infusional reactions to temsirolimus should be:

Grade	Action
Grade 1 Transient flushing or rash, drug fever < 38◦ C (<100.4 ◦ F) And Grade 2 Rash, flushing, urticaria, dyspnea, drug fever ≥	• If a patient develops a hypersensitivity reaction despite diphenhydramine pretreatment, stop the infusion and wait 30 to 60 minutes (depending upon the reaction severity). At the physician's discretion, it may be possible to resume treatment by administering an H2 blocker approximately 30 minutes before restarting the infusion. The manufacturer recommends famotidine 0.5 mg/kg IV maximum dose 20

Grade	Action
38◦ C (≥100.4 ◦ F)	mg, rather than cimetidine, because it lacks reported drug interactions. If famotidine is unavailable, administer ranitidine 1-2 mg/kg IV maximum dose 50 mg. Re-attempt infusion at a slower rate, possibly over 60 minutes. • If grade 1-2 infusion reactions recur with subsequent dose, add dexamethasone 0.2 mg/kg (max 10mg) IV or equivalent to premedications above. (Only dose interruption/discontinuation, but not dose reduction is required for allergic/infusional reactions)
Grade 3 Symptomatic bronchospasm with or without urticaria, allergy-related edema/angioedema, hypotension	• Stop infusion immediately and remove the infusion tube • Administer diphenhydramine hydrochloride 1 mg/kg IV (max 50 mg), dexamethasone 0.2mg/kg (max 10mg) IV (or equivalent), bronchodilators for bronchospasms, and other medications as medically indicated. Hospital admission should be considered. • Discontinue temsirolimus treatment
Grade 4 Anaphylaxis	• Stop infusion immediately and remove the infusion tube • Administer diphenhydramine hydrochloride 1 mg/kg (max 50mg) IV, dexamethasone 0.2 mg/kg (max 10mg) IV (or equivalent), and other anaphylaxis medications as indicated. Epinephrine or bronchodilators should be administered as indicated. Hospital admission for observation may be indicated • Discontinue temsirolimus treatment

4.6.1.5 Dose modifications for pneumonitis

For patients who develop pneumonitis (cough, dyspnea, fever), temsirolimus should be held pending investigation. If events are considered at least possibly due to treatment, discontinue temsirolimus therapy. If the treating clinician determines that respiratory symptoms are not related to drug therapy or are infectious in nature, the patient should be retreated with the same dose of temsirolimus once symptoms resolve.

4.6.1.6 Dose modifications for mucositis or rash

The following guidelines should be used for patients who develop mucositis, or rash. **In addition, stomatitis, mucositis, and/or mouth ulcers** due to temsirolimus (inflammation or ulcers in the mouth) should be treated using local supportive care (See Section 5.2.7 for specific recommendations).

Grade	Action
Grade 1-2	Continue temsirolimus.
Grade 3	• If recovery to ≤ Grade 2 occurs prior to day 8 dose, treat with the same dose of temsirolimus. If not hold day 8 dose of temsirolimus. If this occurs in a second or subsequent course, hold temsirolimus until ≤ Grade 2 and restart in next cycle at one dose level lower (or 5mg/m2 if dose level 1)Upon retreatment, if Grade 3 or 4 toxicity recurs and persists >7 days, discontinue any further temsirolimus.

	• If mucositis develops after the second dose of temsirolimus, initiate G-CSF daily or neulasta x 1.
Grade 4	• Discontinue temsirolimus • If this occurs in a second or subsequent course, hold temsirolimus until ≤ Grade 2 and restart in next cycle at one dose level lower (or 5mg/m2 if dose level 1) • If mucositis develops after the second dose of temsirolimus, initiate G-CSF daily or neulasta x 1.

4.6.1.7 Dose modifications for elevated triglycerides or cholesterol

Temsirolimus should not be held in cycle 1 for Grade 1-3 of elevated triglyceride or cholesterol.

Dose modifications for elevated fasting cholesterol
The following guidelines should be used for patients who develop elevated fasting cholesterol.

Grade	Action
Grade 2	• Continue temsirolimus; consider treatment with an HMG-CoA reductase inhibitor depending upon recommendations of institutional hyperlipidemia consultants • If on intralipids, repeat level after intralipids have been held at least 12 hours.
Grade 3	• If on intralipids, repeat level after intralipids have been held at least 12 hours. • An HMG-CoA reductase inhibitor should be started, and dosages adjusted based upon recommendations of institutional hyperlipidemia consultants • It is expected that optimal effects of the lipid lowering medication will be observed 2-4 weeks after its initiation. Treatment with temsirolimus can continue during this time provided that hypercholesterolemia remains ≤ Grade 3
Grade 4	• Hold temsirolimus until recovery ≤ Grade 3 • An HMG-CoA reductase inhibitor should be started, and dosages should be adjusted based upon recommendations from institutional hyperlipidemia consultants • It is expected that optimal effect of the lipid lowering medication will be observed 2-4 weeks after initiation. Temsirolimus can be restarted at the same dose level in subsequent courses when recovery to ≤ Grade 3 cholesterol is observed • Upon retreatment with temsirolimus concurrent with an HMG-CoA reductase inhibitor, if Grade 4 elevations recur, temsirolimus should be held until recovery to ≤ Grade 3. Further lipid lowering medication options should be discussed with institutional hyperlipidemia consultants. Upon recovery to ≤ Grade 3 cholesterol, temsirolimus should be restarted at the next lower dose level. If the patient is being treated on the lowest dose level, temsirolimus therapy should be discontinued.

Dose modifications for elevated fasting triglycerides

The following guidelines should be used for patients who develop elevated fasting triglycerides.

Grade	Action
Grade 2-3	• If on intralipids, repeat level after intralipids have been held at least 12 hours. • Continue temsirolimus; if triglycerides are between 301 and 400 mg/dL consider treatment with an HMG-CoA reductase inhibitor depending upon recommendations of institutional hyperlipidemia consultants. HMG-CoA reductase inhibitor is highly recommended if triglycerides are > 400. • It is expected that optimal effects of the lipid lowering medication will be observed 2-4 weeks after its initiation. Treatment with temsirolimus can continue during this time provided that hypercholesterolemia remains ≤ Grade 3
Grade 4	• Hold temsirolimus until recovery to ≤ Grade 3 • An HMG-CoA reductase inhibitor should be started, and dosages should be adjusted based upon recommendations from institutional hyperlipidemia consultants • Treatment with temsirolimus can continue during this time provided that hypercholesterolemia remains ≤ Grade 3 • Upon retreatment with temsirolimus concurrent with an HMG-CoA reductase inhibitor, if Grade 4 elevations recur, temsirolimus should be held until recovery to ≤ Grade 3. Further lipid lowering medication options should be discussed with institutional hyperlipidemia consultants. Upon recovery to ≤ Grade 3, temsirolimus should be restarted at the next lower dose level. If the patient is being treated on the lowest dose level, protocol therapy should be discontinued.

4.6.2 Dose Modifications of Cyclophosphamide for Non-Hematological Toxicity

Hematuria: Omit in the presence of macroscopic hematuria. If there is a history of previous significant hematuria, hydrate before cyclophosphamide until specific gravity is < 1.015 and hydrate at 125 mL/m2/hr for 24 hours after dose. Give IV Mesna (200 mg/m2) 15 minutes before the cyclophosphamide and repeat at hours 3, 6, and 9. Mesna may also be given by continuous infusion.

Renal Dysfunction: If creatinine clearance or radioisotope GFR is < 10 mL/min/1.73 m2, reduce dose of cyclophosphamide by 50%. Prior to dose adjustment of cyclophosphamide, the creatinine clearance should be repeated with good hydration.

4.6.3 Dose Modifications of Etoposide for Non-Hematologic Toxicity

Allergic Reaction: Treat per institutional protocol. Consider premedication with diphenhydramine (1-2 mg/kg slow IV push) and/or hydrocortisone 100-300 mg/m2. Continue to use premedication before etoposide for subsequent doses. Also consider substituting an equimolar amount of Etoposide phosphate, in the face of significant allergy and/or hypotension. Etoposide phosphate is a water soluble prodrug that does not contain polysorbate 80 and polyethyleneglycol, the solubilizing agent in etoposide that may induce allergic reactions and hypotension. Etoposide phosphate is rapidly converted to etoposide *in vivo* and provides total drug exposure, as represented by AUC (0-infinity),that is statistically indistinguishable from that measured for etoposide at equimolar doses.

Hypotension: If blood pressure decreases during infusion slow infusion rate per institutional guidelines, and consider normal saline bolus or other IVF. Suggested management includes: if diastolic or systolic blood pressure (BP) falls 20 mm Hg during infusion, reduce infusion rate by 50%. Start a simultaneous

infusion of NS 10 mL/kg if BP fails to recover or falls further. Stop infusion if BP does not recover, continue NS. If the patient has had any episode of hypotension, prehydrate with 0.9% NaCl at 10 mL/kg/hr for 2 hours prior to any subsequent infusion.

Renal Insufficiency: If renal function decreases, adjust etoposide as follows: CrCl 10-50 mL/min/1.73 m2, decrease dose by 25%; if CrCl < 10 mL/min/1.73 m2, decrease dose by 50%.

Hyperbilirubinemia: If direct bilirubin is > 2 mg/dL, decrease dose by 50%. If direct bilirubin is > 5 mg/dL, hold etoposide.

4.6.4 Dose Modifications of Intrathecal Therapy

Systemic toxicity: The dosage for IT chemotherapy will not be reduced for systemic toxicity (myelosuppression, mucositis, etc.) and all patients will get leucovorin rescue post intrathecals containing methotrexate.

Dose modifications following an episode of acute neurotoxicity:
Following an acute neurotoxic event, a history and physical exam should guide the differential diagnosis. A neurology consult may be of value and should be considered. Seizures and other transient events may be linked to fever, infection, encephalitis, meningitis, hypertension, electrolyte disturbance, hypoglycemia, trauma, intracranial hemorrhage or thrombosis, narcotic withdrawal, illicit drug use, or other causes in addition to the direct side effects of chemotherapy. Appropriate laboratory studies may include, but are not limited to, blood cultures, a CBC, electrolytes, including glucose, calcium, magnesium and phosphorus, renal and liver function studies and/or an examination of the CSF. Imaging studies may include a CT scan and/or an MRI. The CT is commonly normal, in the absence of stroke, but if calcifications are present, this finding may be indicative of a more severe mineralizing leukoencephalopathy. MRI abnormalities may be pronounced, but transient. Posterior reversible encephalopathy may be present on MR with extensive diffusion abnormalities, but these do not appear to correlate with subsequent demyelination or gliosis. Additional studies, including MR angiography and/or venogram should be considered, if clinically indicated (e.g. focal deficits).

Many acute events are temporally related to the administration of intrathecal therapy, commonly 9 to 11 days after the IT administration. Following an acute event, with recovery, there are few data to support or guide therapeutic interventions. It is reasonable to hold the next dose of IT therapy, or, substitute IT Ara-C/hydrocortisone for 1 dose of IT MTX, or triple IT therapy or IT MTX if the toxicity appeared attributable to IT AraC..

Hydrocephalus, microcephaly or known abnormality of CSF flow precluding intrathecal chemotherapy via lumbar puncture: Intraventricular chemotherapy via Ommaya catheter may be used in place of intrathecal therapy delivered by LP. Intraventricular chemotherapy should be given according to the same schedule, but at 50% of the corresponding age-based doses that would be given by LP. NOTE: Obstruction to CSF flow may be a contraindication to intrathecal and/or intraventricular therapy.

Viral, bacterial, or fungal meningitis: Omit until resolved.

4.7 Drug Interactions

4.7.1 CYP3A4 Inducers and Inhibitors

The use of potent inhibitors and inducers of CYP3A4 should be avoided (see below and Appendix II). Aprepitant is an inducer, a moderate inhibitor, and substrate of CYP3A4 and should not be used as an anti-emetic. Antifungal agents should be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (voriconazole, posaconazole, itraconazole, fluconazole, etc.) should be avoided due to their strong inhibition of temsirolimus metabolism, therefore leading to higher temsirolimus exposure. Azoles may be started safely 7 days post a dose of temsirolimus, but must be stopped 5 days prior to a subsequent dose of temsirolimus. Topical antifungal agents, amphotericin, or caspofungin/micafungin are preferred if

an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed. The macrolide group of antibiotics should be avoided.

The use of enzyme inducing anticonvulsants is not permitted while on study. Neither Gabapentin nor Levetiracetam induce hepatic drug metabolizing enzymes and may be suitable alternative anticonvulsant.

The use of the following medications should be discontinued prior to initiation of protocol therapy and should be avoided during protocol therapy if reasonable alternatives exist.

Strong Inhibitors	Moderate Inhibitors	Weak Inhibitors	Other Inhibitors	Inducers
Clarithromycin Indinavir Itraconazole Ketoconazole Nefazodone Nelfinavir Posaconazole Ritonavir Saquinavir Telithromycin Losartan Voriconazole	Aprepitant Diltiazem Erythromycin Fluconazole Grapefruit Juice Verapamil	Cimetidine	Amiodarone Chloramphenicol Ciprofloxacin Delaviridine Fluvoxamine Imatinib Norfloxacin Norfluoxetine (fluoxetine)	Barbiturates Carbamazepine Efavirenz Glucocorticoids Modanfinil Nevirapine Oxcarbazepine Phenobarbital Phenytoin Pioglitazone Rifabutin Rifampin St. John's wort Troglitazone

For a more complete list of CYP 3A 4/5 Inhibitors and Inducers, see Appendix II or go to: http://medicine.iupui.edu/flockhart/

4.7.2 Other Pharmacodynamic Interactions

An increased risk of angioedema is possible in pateints taking mTOR inhibitors in combination with Ramipril and/or amlodipine. Caution should be used whem temsirolinus is given concomitantly with a calcium channel blocker (i.e., amlodipine) or ACE inhibitor (i.e.ramipril).

5.0 SUPPORTIVE CARE

Best supportive care and treatment will be given as appropriate to each patient (antiemetics, antibiotics, transfusions, oxygen therapy, nutritional support, palliative treatment for pain or cough, etc.). Patients may experience profound myelosuppression and immune suppression during this time. Caregivers must also be made aware that patients may experience very rapid clinical deterioration and hospitalization for course 1 is highly recommended (See Section 5.2.1).

5.1 Concurrent Therapy

5.1.1 Anti-cancer Therapy

Concurrent anti-cancer therapy not defined within this protocol, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered to patients receiving study drug. If these treatments are administered the patient will be removed from protocol therapy.

5.1.2 Investigational Agents

No other investigational agents may be given while the patient is on protocol therapy.

5.1.3 Growth Factors

Growth factors that support white cell number or function may be administered on day 9 or after at the discretion of the treating physician for infection, ICU level care, Grade 3/4 mucositis, ulceration, or poor wound healing. Neulasta is permitted on study.

5.2 Infection Control

Patients with relapsed ALL have a significant risk of morbidity and mortality with intensive salvage therapy. Patients in first relapse have a 4-5% induction mortality rate, the majority associated with infection. They have also been found to have a 45% rate of Grade 3 and 4 infection during re-induction on recent COG ALL trials [33] (and personal communication T. Horton, E. Raetz, and M. O'Brien), and other relapsed ALL re-induction trials.[34-36] Therefore patients with multiply relapsed ALL require a very aggressive approach to infection prevention and treatment as outlined below:

5.2.1 Hospitalization

Due to the substantial risk of infection in patients with relapsed ALL receiving intensive salvage therapy, it is strongly recommended that they remain hospitalized from the initiation of therapy until: 1) there is evidence of ANC recovery defined as ≥ 200/microliter and increasing, and 2) they are afebrile and clinically stable.

5.2.2 Bacterial Prophylaxis

It is strongly recommended that patients receive broad spectrum gram positive and negative antibiotic prophylaxis at initiation of induction therapy and until they have met the criteria for discharge outlined in 5.2.1. The specific choice of prophylactic antibiotics should be guided by the individual institution, although levofloxacin is recommended.[37,38]

5.2.3 Fungal Prophylaxis

Prophylactic anti-fungal therapy with IV caspofungin, micafungin or amphotericin is also highly recommended. The patient should remain on prophylactic anti-fungals until their ANC has fallen and is 200/microliter and rising and the patient is afebrile and clinically stable. **Note: Azole antifungals are not permitted until 7 days after the final dose of temsirolimus.** (See Section 4.7.1)

5.2.4 Fever and Neutropenia

Empiric broad spectrum antibacterial and anti-fungal therapy should be initiated immediately for patients with an ANC < 500/microliters (or < 1000 /microliters and falling) and an oral temperature > 38°C on two occasions within 12 hours or ≥ 38.5°C once or as clinically indicated. Broad-spectrum antibacterial antibiotics and anti-fungals, once started, should be continued until there is evidence of ANC recovery. If clinical symptoms or radiographic evidence of enterocolitis or typhilitis develop, broad spectrum coverage for gram negatives, anaerobes and an anti-fungal is highly recommended. Surveillance

radiographic imaging surveillance for sites of infection should also be performed as clinically indicated.

5.2.5 Empiric Management of Pulmonary Infiltrates

Consideration should be given to evaluating pulmonary infiltrates with bronchoscopy and biopsy, lavage or open lung biopsy. If a procedure cannot be tolerated, begin empiric treatment with antifungal therapy given the high likelihood of fungal disease. Empiric coverage should be initiated per local institutional guidelines with consideration of bacterial, fungal, *Pneumocystis jiroveci* pneumonia (PJP, formerly called PCP) and legionella etiologies pending culture results. If fungal pulmonary disease is documented, surveillance radiographic imaging studies of the sinuses, abdomen/pelvis and brain are indicated.

5.2.6 *Pneumocystis jiroveci* Pneumonia Prophylaxis

It is recommended that all patients receive prophylaxis for PJP (formerly called PCP). Options include trimethoprim/sulfamethoxazole (TMP-SMX) for two sequential days each week at a dose of 2.5 mg/kg/dose TMP-SMX (maximum dose of 160 mg) twice daily (BID) or according to institutional policy, unless they have a documented sulfa allergy. Options for sulfa-allergic patients include intravenous or nebulized monthly pentamidine or atovaquone per institutional standards. PJP prophylaxis should be continued throughout the entire study period.

5.2.7 Management of Mucositis/Perirectal Cellulitis

Based on pilot experience and the intensity of this regimen, episodes of Grade 3-4 mucositis and perirectal cellulitis are anticipated. Mucositis should be managed with IV hydration and hyperalimentation if indicated, effective analgesia, broad-spectrum gram-positive and gram-negative antibiotic therapy and empiric antiviral and antifungal therapy as indicated. See Section 4.6.1.6 for recommendations for early local care for mucositis/stomatitis. Management of perirectal cellulitis should include broad-spectrum antibiotic therapy with dual gram-negative coverage as well as anaerobic coverage and supportive care per institutional protocol.

5.3 Supportive Care

Appropriate antibiotics, blood products, antiemetics (except for aprepitant, see Section 4.7.1), anti-hypertensives, fluids, electrolytes, and tumor lysis precautions and general supportive care are to be used as necessary. See Section 4.6 for dose modifications of therapeutic agents based upon toxicity.

5.3.1 Recommendations for care of stomatitis, mucositis and/or mouth ulcers:

● Grade 1 - use conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.

● Grade 2 or 3 - topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, orphenol) may be used with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (e.g. Kenalog in Orabase®). Agents containing hydrogen peroxide, iodine, and thyme derivatives may worsen mouth ulcers and should not be used.

● If Grade 3 or higher mucositis develops after the second dose of temsirolimus initiate use of G-CSF or neulasta.

Topical antifungal agents (nystatin) amphotericin or echinocandins (eg, caspofungin or micafungin) are preferred if an oral infection is diagnosed. Similarly, prophylactic antiviral agents such as acyclovir should be avoided due to interaction with temsirolimus unless a viral infection is diagnosed. Systemic imidazole antifungal agents (ketoconazole, fluconazole, traconazole, etc.) should be avoided due to their strong inhibition of temsirolimus metabolism until 7 days post a dose of temsirolimus or 5 days prior.

● If due for IT therapy when any grade mucositis is present, the use of leucovorin 5 mg/m^2/dose every 12 hours x 2 doses, beginning 48 hours after the IT therapy, is encouraged.

5.3.2 Tumor Lysis:

It is recommended, but not mandated, that all subjects start allopurinol 150 mg/m^2 divided TID or urate oxidase (0.1mg/kg IV) prior to the initiation of therapy. Serum chemistries, including electrolytes, BUN, Cr, and uric acid, should be monitored at least 2 times per week for the first week. If there is no evidence of tumor lysis the patients may then get routine chemistry labs as described in Section 7.0. If a patient's peripheral leukemic blast count is $\geq$ 20,000 or if they have bulk lymphomatous disease it is highly recommended, although not mandated, that they are admitted to the hospital for IV hydration and close monitoring for metabolic abnormalities and renal insufficiency due to tumor lysis syndrome.

5.3.3 Infusional Reactions:

Anaphylactic precautions should be observed during temsirolimus administration. Patients should receive premedication with diphenhydramine (1 mg/kg, max 50 mg) prior to each temsirolimus dose. See Section 4.6.1.4 for management and dose modification guidelines for infusional reactions.

5.3.4 Blood Products

Investigators should follow institutional guidelines regarding administration of blood products. Platelets must be 20,000 in order to receive temsirolimus.

6.0 DRUG INFORMATION

6.1 Temsirolimus (CCI-779, Torisel®, Rapamycin analog, WAY-130779)
(NSC#683864, IND#61010)

6.1.1 Nomenclature and Molecular Structure

The chemical name is Sirolimus 42-ester with 2,2-bis (hydroxymethyl)-propionic acid. The molecular formula is $C_{56}H_{87}NO_{16}$. The molecular weight is 1030.30 daltons.

6.1.2 Mode of Action and Pharmacology

Temsirolimus [an ester of the immunosuppressive compound sirolimus, (rapamycin, Rapamune®)] blocks cell cycle progression from the G1 to the S phase by binding to the intracellular cytoplasmic protein, FK506 binding protein (FKBP)12. This complex inhibits activity of the enzyme mTOR (mammalian target of rapamycin), inhibiting translation of several key proteins that regulate progression through the G1 phase in response to growth factors. Sirolimus, temsirolimus's major metabolite, also binds to FKBP12.

Classification: mTOR inhibitor

Approximate Solubility (at 25ºC): Very soluble in ethanol. Soluble in phosal 50 PG, octanol, propylene glycol, polyethylene glycol (PEG) 400, triacetin, polyethylene glycol (PEG) 200, labrosol, and polysorbate 80. Insoluble in miglyol 812, olive oil and water.

Incompatibilities: Avoid contact of the diluted product with polyvinyl chloride (PVC) equipment or devices that are plasticized with di- (2-ethylhexyl)pthalate (DEHP) to prevent DEHP leaching. Store diluted temsirolimus solutions in bottles (glass) or plastic bags (polyolefin or polypropylene).

Infusion sets containing polyvinyl chloride should not be used to administer temsirolimus to avoid leaching of plasticizer.

The following are examples of in-line filters that are compaitible with temsirolimus:

- IV 6200 Disposable I.V. Filter 0.2 micron by EPS®, Inc
- IV 6120 Disposable I.V. Filter 1.2 micron by EPS®, Inc
- LV 5000 Large Volume 5 micron Conical Filter by B.Braun
- Baxter Paclitaxel Set with 0.22 micron filter
- Codan 0.2 micron monofilter

Other polyethersulfone filters may be used.

Potential Drug Interactions: Temsirolimus is a CYP3A4 substrate. Avoid concomitant treatment of temsirolimus with potent CYP3A4 inhibitors and agents that have CYP3A4 induction potential (See Appendix II).

The combination of temsirolimus and sunitinib resulted in dose limiting toxicity at low doses of both agents. Avoid concomitant sunitinib during temsirolimus treatment.

In vitro, temsirolimus inhibited CYP2D6, CYP3A4, and CYP3A5 activity. It inhibited paclitaxel metabolism, but did not significantly affect 5-fluorouracil metabolism.

Temsirolimus and warfarin may interact to increase INR.

Patient Care Implications: For hypersensitivity prophylaxis, see Section 5.6.1.4.

Vaccinations: Avoid the use of live vaccines during temsirolimus treatment.

6.1.3 Toxicity/Adverse Events

The toxicity and adverse events in the table below are derived from the Prescribing Information for Torisel® (temsirolimus) and Investigator's Brochure. The following events were reported in at least 10% of patients who received Torisel in a clinical trial.

Adverse Events with Possible Relationship to Temsirolimus (CTCAE 4.0 Terms)		
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious**
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
• Anemia		
GASTROINTESTINAL DISORDERS		
• Diarrhea • Gastrointestinal disorders – Other (Mucositis/stomatitis)[1] • Nausea • Anorexia • Abdominal Pain	• Constipation • Vomiting	• Gastrointestinal disorders – Other (Perforation, GI)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
• Asthenia • Generalized Pain • Edema[2] • Pyrexia	• Weight Loss • Headache • Chills • Chest pain	
IMMUNE SYSTEM DISORDERS		
		• Allergic reactions, anaphylaxis
INFECTIONS AND INFESTATIONS[5]		
•	• Infections[3] • Urinary tract infection[4] • Pharyngitis • Rhinitis	
INVESTIGATIONS		
• Platelet count decreased • Lymphopenia • Elevated alkaline phosphatase • Elevated serum creatinine • Elevated AST • Leukopenia • Hemoglobin decreased • Total cholesterol increased •	• ALT increased • Neutrophil count decreased • Weight loss • Total bilirubin increased	
METABOLISM AND NUTRITION DISORDERS		
• Anorexia • Hyperglycemia • Metabolism and nutrition disorders- Other (hyperlipidemia)	• Decreased potassium	

Adverse Events with Possible Relationship to Temsirolimus (CTCAE 4.0 Terms)		
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious**
• Hypertriglyceridemia • Hypophosphatemia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	• Arthralgia • Back pain • Myalgia	
NERVOUS SYSTEM DISORDERS		
	• Dysgeusia[5]	• Intracerebral hemorrhage
PSYCHIATRIC DISORDERS		
	• Depression • Insomnia	
RENAL AND URINARY DISORDERS		
		• Renal Failure
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
• Dyspnea • Cough	• Epistaxis	• Interstitial lung disease • Pulmonary thromboembolism
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
• Rash[6]	• Dry skin • Nail disorder • Pruritus • Acne	

[1] Includes aphthous stomatitis, glossitis, mouth ulceration, mucositis and stomatitis
[2] Includes edema, facial edema, and peripheral edema
[3] Includes infection, not otherwise specified (NOS) and the following infections that occurred infrequently as distinct entities: abscess, bronchitis, cellulitis, herpes simplex and herpes zoster
[4] Includes cystitis, dysuria, hematuria, urinary frequency, and urinary tract infection
[5] Includes taste loss and taste perversion
[6] Includes eczema, exfoliative dermatitis, maculopapular rash, puritic rash, pustular rash, rash NOS and vesiculobullous rash

The following selected adverse reactions were reported less frequently (<10%).

- **Gastrointestinal Disorders** – Fatal bowel perforation occurred in 1 patient (1%)

- **Eye Disorders** - Conjunctivitis (including lacrimation disorder) occurred in 15 patients (7%).

- **Immune System** - Allergic/Hypersensitivity reactions occurred in 18 patients (9%).

- **Angioneurotic edema**-type reactions (including delayed reactions occurring two months following initiation of therapy) have been observed in some patients who received TORISEL and ACE inhibitors concomitantly.

- **Infections** - Pneumonia occurred in 17 patients (8%); upper respiratory tract infection occurred in 14 patients (7%).

- **General Disorders and Administration Site Conditions** - Impaired wound healing occurred in 3 patients (1%).

- **Respiratory, Thoracic and Mediastinal Disorders** – Interstitial lung disease occurred in 5 patients (2%), including rare fatalities.

- **Vascular -** Hypertension occurred in 14 patients (7%); venous thromboembolism (including deep vein thrombosis and pulmonary embolus [including fatal outcomes]) occurred in 5 patients (2%); thrombophlebitis occurred in 2 patients (1%).

6.1.4 Formulation and Stability

- TORISEL (temsirolimus) is supplied as a commercially labeled kit consisting of the following: TORISEL (temsirolimus) injection (25 mg/mL). The TORISEL vial includes an overfill of 0.2 mL. Inert ingredients in the drug vial include dehydrated alcohol, d,l-alpha-tocopherol, propylene glycol, and anhydrous citric acid.
- DILUENT for TORISEL. The DILUENT vial includes a deliverable volume of 1.8 mL. The diluent vial contains polysorbate 80 NF, polyethylene glycol 400 NF, and absolute alcohol USP.

Stability

- The 10 mg/mL drug solution/diluent mixture is stable for 24 hours at room temperature
- Administer within 6 hours of the final dilution in 0.9% NaCl. Store at room temperature (20º-25ºC) and protect from light.

Storage

- Refrigerate intact TORISEL kit at 2º-8ºC and protect from light.

6.1.5 Preparation and Administration

Solution Preparation

These mixing instructions apply to commercial TORISEL only (as is provided for this study). The investigationally labeled product used in other studies is mixed differently.

Protect from excessive light.

Follow this two steps dilution process (TORISEL should only be diluted with the supplied diluent):

Step 1

Inject 1.8 mL of DILUENT for TORISEL into the vial of TORISEL injection (25 mg/mL). Due to the intentional 0.2 mL overfill in the TORISEL injection vial, the resulting drug concentration will be 10 mg/mL. A total volume of 3 mL will be obtained. Mix well by gentle inversion of the vial. DO NOT SHAKE. Allow sufficient time for air bubbles to subside.

Step 2
Withdraw the required amount of TORISEL from the 10 mg/mL drug solution/diluent mixture prepared in Step 1. Further dilute with 0.9% sodium chloride injection immediately in glass or polyolefin containers to a final concentration between 0.04 mg/mL and 1 mg/mL.

Route of Administration
Administer temsirolimus as an intravenous infusion over 30 minutes (+/- 10 minutes) with an appropriate in-line filter (i.e. 0.2 to 5 micron) for all temsirolimus doses equal to or greater than 10 mg. To avoid drug loss, do not use an inline filter for temsirolimus doses less than 10 mg. Protect from light during administration.

6.1.6 Supplier: Pfizer Pharmaceuticals, Inc

6.1.7 Drug Ordering Information:

Once you have IRB approval, contact TACL Operations at TACL@chla.usc.edu to arrange for a starter supply of temsirolimus.

Sites receiving temsirolimus will verify the drug disposition and storage conditions on receipt and document this verification in the pharmacy records. When a definitive expiration date is determined, study drug will be removed from inventory at each investigative site and replaced with a new supply of study drug. Questions regarding drug shipments to and from TACL sites can be addressed to TACL Operations at 323-361-5429 or TACL@chla.usc.edu.

6.2 Etoposide (Vepesid®, Etopophos®,VP-16) NSC #141540

6.2.1 Source and Pharmacology:
A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase 2 and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t½ is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma Etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide.

The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%.

Cmax and AUC values for orally administered etoposide capsules consistently fall in the same range as the Cmax and AUC values for an intravenous dose of one-half the size of the oral dose. The overall mean value of oral capsule bioavailability is approximately 50% (range 25-75%).

Etoposide phosphate is a water soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses.

6.2.2 Toxicity:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting	Anorexia	Transient hypotension during infusion; anaphylaxis (chills, fever, tachycardia, dyspnea, bronchospasm, hypotension)
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (anemia, leukopenia), alopecia	Thrombocytopenia, diarrhea, abdominal pain, asthenia, malaise, rashes and urticaria	Peripheral neuropathy, mucositis, hepatotoxicity, chest pain, thrombophlebitis, congestive heart failure, Stevens-Johnson Syndrome, exfoliative dermatitis
Delayed: Any time later during therapy, excluding the above conditions			Dystonia, ovarian failure, amenorrhea, anovulatory cycles, hypomenorrhea, onycholysis of nails
Late: Any time after completion of treatment			Secondary malignancy (preleukemic or leukemic syndromes)
Unknown Frequency and Timing: Fetal toxicities and teratogenic effects of etoposide have been noted in animals at 1/20th of the human dose. It is unknown whether the drug is excreted in breast milk.			

6.2.3 Formulation and Stability

Etoposide for Injection is available as a 20 mg/mL solution in sterile multiple dose vials (5 mL, 25 mL, or 50 mL each). The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of etoposide are stable until expiration date on package at controlled room temperature (20˚-25˚C or 68˚-77˚F). Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide, 32.7 mg sodium citrate *USP*, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration (2°-8°C or 36°- 46°F). Unopened vials of etoposide phosphate are stable until the expiration date on the package.

6.2.4 Guidelines for Administration

Etoposide: Dilute etoposide to a final concentration ≤ 0.4 mg/mL in D5W or NS. Etoposide infusions are stable at room temperature for 96 hours when diluted to concentrations of 0.2 mg/mL; stability is 24 hours at room temperature with concentrations of 0.4 mg/mL. The time to precipitation is highly unpredictable at concentrations > 0.4 mg/mL. Use in-line filter during infusion secondary to the risk of precipitate formation. However, the use of an in-line filter is not mandatory since etoposide precipitation is unlikely at concentrations of 0.1-0.4 mg/mL. **Do not administer etoposide by rapid intravenous injection.** Slow rate of administration if hypotension occurs. Leaching of diethylhexyl phthalate (DEHP) from polyvinyl chloride (PVC) bags occurred with etoposide 0.4 mg/mL in NS. To avoid leaching, prepare the etoposide solution as close as possible, preferably within 4 hours, to the time of administration or alternatively as per institutional policy; glass or polyethylene-lined (non-PVC) containers and polyethylene-lined tubing may be used to minimize exposure to DEHP.

Etoposide Phosphate: Reconstitute the 100 mg vial with 5 or 10 mL of Sterile Water for Injection, D5W, NS, Bacteriostatic Water for Injection with Benzyl Alcohol, or Bacteriostatic Sodium Chloride for Injection with Benzyl Alcohol for a concentration equivalent to 20 mg/mL or 10 mg/mL etoposide equivalent (22.7 mg/mL or 11.4 mg/mL etoposide phosphate), respectively. **Use diluents without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol.** When reconstituted as directed, etoposide phosphate solutions can be stored in glass or plastic containers under refrigeration for 7 days. When reconstituted with a diluent containing a bacteriostat, store at controlled room temperature for up to 48 hours. Following reconstitution with SWFI, D5W, or NS store at controlled room temperature for up to 24 hours. Following reconstitution, etoposide phosphate may be further diluted to a concentration as low as 0.1 mg/mL of etoposide with D5W or NS.

6.2.5 Supplier: Commercially available. See package insert for further information.

6.3 Cyclophosphamide (Cytoxan) NSC #26271

6.3.1 Source and Pharmacology

Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P450 isoenzymes (CYP2B6, CYP2C9, and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent *in vitro* than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. The plasma half-life ranges from 4.1 to 16 hours after IV administration.

6.3.2 Toxicity

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Anorexia, nausea & vomiting (acute and delayed)	Abdominal discomfort, diarrhea	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: Within 2-3 weeks, prior to the next course	Leukopenia, alopecia, immune suppression	Thrombocytopenia, anemia, hemorrhagic cystitis (L)	Cardiac toxicity with high dose (acute – CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail Changes, impaired wound healing, infection secondary to immune suppression
Delayed: Any time later during therapy, excluding the above conditions	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)	Amenorrhea[1]	Gonadal dysfunction: ovarian failure[1] (L), interstitial pneumonitis, pulmonary fibrosis[2] (L)
Late: Any time after completion of treatment			Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use > 2 years), bladder fibrosis

Unknown Frequency and Timing: Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.

[1] *Dependent on dose, age, gender, and degree of pubertal development at time of treatment.*
[2] *Risk increased with pulmonary chest irradiation and higher doses.*
(L) Toxicity may also occur later.

6.3.3 Formulation and Stability

Cyclophosphamide for injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 g, and 2 g vials. The powder for injection contains 82 mg sodium bicarbonate/100 mg cyclophosphamide and the lyophilized powder for injection contains 75 mg mannitol/100 mg cyclophosphamide. Storage at or below 25ºC (77ºF) is recommended. The product will withstand brief exposures to temperatures up to 30ºC (86ºF).

6.3.4 Guidelines for Administration

If the drug will be administered as undiluted drug at the 20 mg/mL concentration, then reconstitute to 20 mg/mL with NS ONLY to avoid a hypotonic solution. If the drug will be further diluted prior to administration, then first reconstitute with NS, SWFI, or Bacteriostatic Water for Injection (paraben preserved only) to a concentration of 20 mg/mL. Following reconstitution further dilute in dextrose or saline containing solutions for IV use.

6.3.5 Supplier: Commercially available. See package insert for further information.

6.4 IT Methotrexate (MTX, amethopterin, Trexall®) NSC #000740

6.4.1 Source and Pharmacology:

A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 µmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. After oral administration, approximately 60% of a 30 mg/m2 dose is rapidly absorbed from the GI tract, with peak blood levels at 1 hour. At doses > 30 mg/m² absorption decreases significantly. Even at low doses absorption may be very erratic, varying between 23% and 95%. The elimination of MTX from the CSF after an intrathecal dose is characterized by a biphasic curve with half-lives of 4.5 and 14 hours. After intrathecal administration of 12 mg/m², the lumbar concentration of MTX is ~100 times higher than in plasma. (Ventricular concentration is ~ 10% of lumbar concentration). MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

6.4.2 Toxicity

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate:	Nausea, headache	Arachnoditis: (headache,	Anaphylaxis, vomiting,

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Within 1-2 days of receiving drug		fever, vomiting, meningismus, nuchal rigidity, and pleocytosis)	seizures(L), confusion, back pain, rash, bleeding into subarachnoid or subdural space (risk > with platelet counts < 20,000),
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia, somnolence, cranial nerve palsy, subacute myelopathy (paraparesis/paraplegia), speech disorders, pain in the legs, bladder dysfunction
Delayed: Any time later during therapy, excluding the above conditions		Learning disability (L), Cognitive disturbance	Leukoencephalopathy[1] (L)
Late: Any time after completion of treatment			Progressive CNS deterioration[1]

[1] May be enhanced by HDMTX and/or cranial irradiation.
(L) Toxicity may also occur later.

6.4.3 Formulation and Stability

Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative. Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.

6.4.4 Guidelines for Administration:

See Treatment and Dose Modifications sections of protocol. Leucovorin rescue may be necessary with certain doses of methotrexate.

For Intrathecal use: Use **preservative free** 25 mg/mL solution. For intrathecal administration, dilute with 5-10 mL preservative free NS, lactated Ringer's, or Elliot's B solution as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.

Patient Age (years)	Methotrexate Dose	Recommended volume	10% CSF volume	CSF Volume *
1 – 1.99	8 mg	5 – 10 mL	5 mL	50 +/- 10 mL (babies)
2 – 2.99	10 mg	5 – 10 mL	8 mL	80 +/- 20 mL (younger children)
3 – 8.99	12 mg	5 – 10 mL	10 mL	100 +/- 20 mL (older children)
9 or greater	15 mg	5 – 10 mL	13 mL	130 +/- 30 mL (adults)

*Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on TACL studies.

Intrathecal cytarabine mixed in NS, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.

6.4.5 Supplier: Commercially available. See package insertfor further information.

6.5 Leucovorin Calcium (LCV, Wellcovorin®, citrovorum factor, folinic acid) NSC #003590

6.5.1 Source and Pharmacology:
Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active compound of the mixture is the (-)- l-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of —one-carbon|| moieties. Administration of leucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase. In contrast, leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5-fluorouracil. Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid (an active metabolite of 5-FU) to thymidylate synthase and thereby enhances the inhibition of this enzyme. Peak serum levels of 5-methyl THF (an active metabolite) were reached at approximately 1.3-1.5 hours (IV/IM) and 2.3 hours for the oral form. The terminal half-life of total reduced folates was approximately 6.2 hours. Following oral administration, leucovorin is rapidly absorbed and expands the serum pool of reduced folates. At a dose of 25 mg, almost 100% of the *l*-isomer (the biologically active form) but only 20% of the *d*-isomer is absorbed. Oral absorption of leucovorin is saturable at doses above 25 mg. The apparent bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg doses. Both oral and parenteral leucovorin raise the CSF folate levels.

6.5.2 Toxicity

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug			Anaphylaxis, urticaria, seizure
Unknown Frequency and Timing: Fetal toxicities and teratogenic effects of leucovorin in humans are unknown. It is unknown whether the drug is excreted in breast milk.			

6.5.3 Formulation and Stability:
Leucovorin calcium for injection is supplied as a sterile ready to use liquid and a sterile powder for injection. The 10 mg/mL preservative free liquid is available in 50 mL vials containing sodium chloride 400 mg/vial. Store preservative free liquid in the refrigerator at 2°-8°C (36°-46°F) protected from light. The powder for injection is available in 50 mg, 100 mg, 200 mg, and 350 mg vials. Store at room temperature 15°-25°C (59°-77°F) protected from light. Reconstitute the sterile powder with sterile water for injection or bacteriostatic water for injection to a concentration of 10 mg/mL leucovorin calcium. **Do not use diluents containing benzyl alcohol for doses > 10 mg/m² or in infants < 2 years of age or patients with allergy to benzyl alcohol.** When Bacteriostatic Water is used, the reconstituted solution is good for 7 days. If reconstituted with SWFI, use solution immediately as it contains no preservative. One milligram of leucovorin calcium contains 0.004 mEq of leucovorin and 0.004 mEq of calcium. The oral form of leucovorin is available as 5 mg, 10 mg, 15 mg, and 25 mg tablets. Inactive ingredients vary depending on

manufacturer but tablet formulations may include: corn starch, dibasic calcium phosphate, magnesium stearate, pregelatinized starch, lactose, microcrystalline cellulose, and sodium starch glycolate.

6.5.4 Guidelines for Administration:
See Treatment and Dose Modifications sections of the protocol.

- Injection: Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL solution per minute). IV leucovorin and sodium bicarbonate are incompatible.

- Oral: Oral leucovorin should be spaced evenly (e.g., every six hours) throughout the day and may be taken without regard to meals. Doses > 25 mg should be given IV due to the saturation of absorption. Leucovorin should not be administered < 24 hours after intrathecal injections which contain methotrexate unless there are special circumstances.

Supplier: Commercially available from various manufacturers. See package insertfor further information.

6.6 IT Cytarabine

6.6.1 Source and Pharmacology:
Cytarabine appears to act through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the nucleotide triphosphate (Ara-CTP), an effective inhibitor of DNA polymerase. Ara-CTP is inactivated by a pyrimidine nucleoside deaminase, which converts it to the nontoxic uracil derivative (Ara-U). It appears that the balance of kinase and deaminaselevels may be an important factor in determining sensitivity or resistance of the cell to cytarabine. It has an initial distributive phase t½ of about 10 minutes, with a secondary elimination phase t½ of about 1 to 3 hours. Peak levels after intramuscular or subcutaneous administration of cytarabine occur about 20 to 60 minutes after injection and are lower than IV administration. Intrathecally administered doses are metabolized and eliminated more slowly with a t½ of about 2 hours.

6.6.2 Toxicity:

Cytarabine: Single Agent, Intrathecal			
	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headach	Arachnoiditis	Rash, somnolence, meningismus, convulsions, paresis
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia
Delayed: Any time later during therapy, excluding the above conditions			Necrotizing leukoencephalopathy, paraplegia, blindness (in combination with XRT & systemic therapy)
Late: Any time after			

completion of treatment			
Unknown Frequency and Timing: This drug may be harmful to an unborn/developing child. The development of an unborn baby can be affected by this drug (either alone or in combination with some other drugs). Birth defects can include abnormal genetic changes, abnormal development of body structures, abnormal reduction of blood cells, and low birth weight.			

6.6.3 Formulation and Stability:
Cytarabine for Injection is available in vials of 100 mg, 500 mg, 1 g, and 2 g containing a sterile powder for reconstitution. It is also available at a 20 mg/mL concentration with benzyl alcohol (25 mL per vial) or as a preservative free solution (5 mL, 50 mL per vial), and at a 100 mg/mL concentration with benzyl alcohol (20 mL vial) or as preservative free solution (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Cytarabine solutions should be protected from light.

6.6.4 Guidelines for Administration:

Intrathecal:
Intrathecal: For intrathecal administration, dilute with 5-10mL (or volume per institutional practice) preservative free 0.9% sodium chloride injection, lactated Ringer's injection, Elliot's B solution. The volume of CSF removed should be equal to at least ½ the volume delivered.

Patient Age (years)	Recommended volume	10% CSF volume	CSF Volume *
1 – 1.99	5 – 10 mL	5 mL	50 +/- 10 mL (babies)
2 – 2.99	5 – 10 mL	8 mL	80 +/- 20 mL (younger children)
3 – 8.99	5 – 10 mL	10 mL	100 +/- 20 mL (older children)
9 or greater	5 – 10 mL	13 mL	130 +/- 30 mL (adults)

*Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8.

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on TACL studies.

Intrathecal cytarabine mixed in NS, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.

6.6.5 Supplier: Commercially available. See package insert for further information.

6.7 Intrathecal Triples (Methotrexate/Hydrocortisone/Cytarabine, ITT)

6.7.1 Source and Pharmacology:
The intrathecal route of administration of a drug produces more consistent CSF drug concentrations at relatively smaller doses because of the volume difference between the CSF and blood compartments (140 mL vs. 3500 mL in an adult). (The CSF volume of children after the first 3 years is equivalent to that of an adult). Drug half-lives are longer as well because clearance is related to flow rather than metabolism or protein binding. Intrathecal methotrexate has a biphasic elimination curve from the CSF with a $t_{½}$ of 4.5 and 14 hours respectively. Following IT injection of cytarabine the elimination of the drug from the CSF is biphasic with a $t_{½}$ of 1 and 3.4 hours respectively which is 8-fold longer than the clearance from plasma. The elimination of hydrocortisone is similarly prolonged.

6.7.2 Toxicity

Intrathecal Triple Therapy (Methotrexate/ Hydrocortisone/Cytarabine)

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoditis: (headache, fever, vomiting, meningismus and pleocytosis)	Rash, anaphylaxis (L), paresis, bleeding into subarachnoid or subdural space (risk > with platelet counts <20,000), confusion, fatigue, disorientation, seizures
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, somnolence, ataxia, cranial nerve palsy, transient and rarely permanent paraplegia (L), speech disorders
Delayed: Any time later during therapy, excluding the above condition		Cognitive disturbances (L), learning disabilities (L)	Demyelinating leukoencephalopathy[1] (L), blindness[1]
Late: Any time after the completion of treatment			Progressive CNS deterioration[1]

[1] *May be enhanced by systemic therapy such as high dose methotrexate or cytarabine and/or cranial irradiation.*
(L) Toxicity may also occur later.

6.7.3 Formulation and Stability:

Methotrexate 25 mg/mL 2 mL vial **preservative free** or methotrexate 20 mg sterile powder for injection vial. Cytarabine 100 mg vial sterile powder for injection. Hydrocortisone sodium succinate100 mg vial sterile powder for injection.

6.7.4 Guidelines for Administration:

See Treatment and Dose Modification sections of the protocol. For intrathecal administration, dilute with 5-10 mL preservative free normal saline, lactated ringers or Elliot's B solution or as per institutional standard of practice. The volume of CSF removed should be equal to at least half the volume delivered.

Patient Age (years)	Doses (MTX/HDC/Ara-C)	Recommended volume	10% CSF volume	CSF Volume [35]
1 – 1.99	8 mg / 8 mg / 16 mg	5–10 mL	5 mL	50 ± 10 mL (babies)
2 – 2.99	10 mg / 10 mg / 20 mg	5-10 mL	8 mL	80 ± 20 mL (younger children)
3 – 8.99	12 mg / 12 mg / 24 mg	5-10 mL	10 mL	100 ± 20 mL (older children)
9 or greater	15 mg / 15 mg / 30 mg	5-10 mL	13 mL	130 ± 30 mL (adults)

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Intrathecal triples are stable in normal saline for 24 hours at 25ºC but contain no preservative and should be administered as soon as possible after preparation.

6.7.5 Supplier**:** Methotrexate, cytarabine and hydrocortisone are commercially available from various manufacturers. See package insert for further information

7.0 REQUIRED OBSERVATIONS/MATERIAL AND DATA TO BE ACCESSIONED

All protocol-specified hematology, blood chemistries, and bone marrow aspirations and/or biopsies are to be performed under the auspices of the local laboratory at each investigational site. Additional assessments may be obtained as needed for good patient care.

7.1 Required Clinical, Laboratory and Disease Evaluation

Laboratory values used to assess eligibility (see Section 3.0) must be no older than seven (7) days at the time of enrollment.

Imaging studies must be obtained within 14 days prior to enrollment (repeat the tumor imaging if necessary). Chest x-ray for eligibility, per section 3.3.6.5, may also be obtained within 14 days of enrollment. The bone marrow aspirate (and/or biopsy) must be obtained within 14 days prior to the start of protocol therapy. Lumbar puncture must be done within 7 days prior to the start of protocol therapy or on day 1 of therapy.

During the courses of therapy, any abnormal laboratory tests must be re-checked by day 36 to assess resolution.

Patients who are removed from protocol therapy during course 1 should continue to have the required observations in Section 7.1 until the originally planned end of the course or until all adverse events have resolved, whichever happens LATER. The only exception is with documentation of the patient's withdrawal of consent.

STUDIES TO BE OBTAINED	Pre-Study	During Course 1	End of Course 1	Subsequent Courses
History	X[1]	---	---	---
Physical Exam with vital signs	X[1]	Weekly	X	Once per cycle
Height, weight, BSA	X	---	X	Once per cycle
Performance Status	X[1]	---	X	Once per cycle
CBC, differential, platelets	X[1]	Every 3-4 days	X	Every 7-14 days
Urinalysis	X	---	---	---
Tumor Lysis Labs[2]	X	Every 3-4 days in Week 1	X	---
Electrolytes including glucose, Ca^{++}, PO_4, Mg^{++}	X	Weekly	X	Every 7-14 days
Creatinine, ALT, AST, GGT, total bilirubin, BUN	X[1]	Weekly	X	Once per cycle
Albumin	X[1]	---	X	---
Triglyceride/Cholesterol (Total, HDL, LDL)	X[1]	Weekly	X	Once per cycle
Lipase/Amylase	X	---	---	---
PT/PTT/Fibrinogen	X	---	---	---
Pregnancy Test	X[1]	If pregnancy is suspected		
Pulse Ox and Chest X-ray	X[1]	---	---	---
ECHO/MUGA	X[1]	---	---	---
Ophthalmologic Exam[3]	X[1]	---	---	---
Bone marrow aspirate and/or	X[1]	---	Day 29 +/- 3 days[4]	Day 29 of 2nd

STUDIES TO BE OBTAINED	Pre-Study	During Course 1	End of Course 1	Subsequent Courses
Biopsy for routine morphology			(if positive at study entry)	cycle and If not in CR at end of prior cycle or if suspicion of relapse
Tumor imaging (lymphoma patients only)	X[1]	---	Day 29 +/- 3 days	If not in CR at end of prior cycle or if suspicion of relapse[5]
Lumbar puncture with cell count & cytospin- for all leukemia patients and lymphoma patients with CNS disease at study entry	X[1]	Perform at the time the lumbar puncture for IT therapy is done	Day 29+/- 3 days. May give IT for start of Course 2	Perform at the time the lumbar puncture for IT therapy is done
Minimal residual disease (MRD) – for leukemia patients and lymphoma patients with BM involvement at study entry	---	---	Day 29 +/- 3 days	End of course 2: Day 29 +/- 3 days[7]
Correlative bone marrow biology Studies[6]	X	---	Day 29 +/- 3 days	X[1,4]
Correlative peripheral blood biology studies[6]	X	Once, day 3-5	Day 29 +/- 3 days	---

1. Required for verification of eligibility. Send all results to TACL Operations Center with study registration eligibility.

2. Tumor lysis labs (electrolytes, BUN, Cr, Ca, PO_4, and uric acid) should be obtained two times in the first week . If there is no evidence of tumor lysis, obtain chemistries as outlined above.

3. Patients presenting with visual disturbances should have an ophthalmological exam and, if indicated, an MRI to determine optic nerve or retinal involvement prior to enrollment per Section 3.4.6.

4. If the bone marrow is hypoplastic or M1 but counts have not recovered to ANC ≥ 500 and PLTS ≥ 50,000 (without transfusion) obtain a weekly CBC and perform a bone marrow with evidence of count recovery (ANC ≥ 500 and PLTS ≥ 50,000) or day **42**; whichever is **first**. If still delayed repeat a bone marrow every 7-14 days after day 42 until count recovery or relapse. A repeat bone marrow does not need to be performed if the patient subsequently develops an absolute blast count (ABC) greater than or equal to 2,500/mm^3

5. On subsequent cycles perform BM and tumor imaging at the end of each cycle until in a CR. Once in CR can perform every other cycle.

6. See Section 8.0 for timing of correlative biology studies.

7. Obtain sample for MRD at end of course 2 if bone marrow M1 post Course 2 but MRD was > 0.01% at end of Course 1.

7.2 Required Observations Following Completion of Protocol Therapy

When a patient discontinues the study, a Final Visit will be conducted. A History and PE and labs should be obtained.

- CBC, differential, platelets
- Electrolytes including glucose, Ca^{++}, PO_4, Mg^{++}
- Creatinine, ALT, bilirubin, BUN

- Triglyceride/Cholesterol (Total, HDL, LDL)

Following discontinuation of the study drug, the patient will be treated in accordance with the investigator's best clinical judgment. If a patient discontinues from the study due to an adverse event considered possibly or probably related to study drug, a Follow-up Visit must be conducted no later than 30 days after the last dose of anti-cancer therapy administered as part of this protocol. Safety assessments will be conducted at least every 30 days, until all toxicities resolve, return to baseline or become clinically satisfactory, stable, or are considered irreversible.

Patients will be followed for life for any delayed toxicities related to protocol therapy, and secondary malignancies. Survival information, including disease status, alternative therapies, sites of relapse and the date and cause of death will be collected after the last study visit until the patient is considered off study.

8.0 CORRELATIVE AND PHARMACOKINETIC STUDIES

Correlative Study	Sample Type	Volume per Sample	Tube Type	Timing of Sample Collection		
				Baseline	Cycle 1	Subsequent Cycles
Bone Marrow MRD	bone marrow	6 mL for patients ≥12 kg 3 mL for patients < 12 kg	conical tubes (SM) containing EDTA/RPMI as the anticoagulant and media diluent or EDTA tubes	---	Day 29+/- 3 days	Once CR is obtained
Bone Marrow Correlative Biology Study	bone marrow	10 mL for patients ≥10kg 3-5 mL for patients <10kg	green top (heparinized) tubes	X	Day 29+/- 3 days	Until CR obtained and at recurrence
PBMC* Correlative Biology Study	blood	5 mL for patients ≥ 10 kg 2 mL for patients < 10 kg	lavender-top (K_2EDTA) tubes or CPT tubes	X	*Once Day 3-5 and 29	

***** Leukemia patients only; send baseline and subsequent samples if patient has any level of detectable peripheral blasts at study entry (no minimum).

8.1 Bone Marrow MRD (Required for leukemia patients)

If a patient has <5% bone marrow blasts (morphologically) on their end of Course 1 and/or 2 bone marrow, and is not aplastic, bone marrow will be sent to determine minimal residual disease by immunoflow. These data will provide important correlative data regarding MRD and early response to combined therapy. If aplastic with subsequent non-blasts recovery MRD should be sent with the bone marrow that documents recovery

Samples requested:	**Bone Marrow** • Course 1, Day 29 +/- 3 days if < 5% blasts by local morphology • Course 2, Day 29 +/- 3 days if < 5% bone marrow blasts post Course 2 and MRD > 0.01% at end of Course 1
Bone Marrow Collection Procedure:	• Collect 6 mL for patients ≥12 kg and 3 mL for patients < 12 kg of marrow into a syringe and transfer the specimen immediately into a conical tube with 15 ml of RPMI/EDTA. If the shipping media tubes are unavailable, place the marrow into large purple EDTA tubes that are commonly used in all hospitals. The viability would be enhanced if put into RPMI/EDTA for shipping. • Mix sample well. Up to 5 ml of marrow can be placed in one 15 cc tube with RPMI/EDTA. • Use multiple syringes and tubes as needed. Reposition marrow aspirate needle as least once during procedure to ensure the maximum quality of marrow.
Specimen Labeling:	Each tube must be labeled with the study ID number and TACL ID along with the date and time the sample was obtained. No personal identifying patient information should be included on the specimen or transmittal form.

Specimen Packaging and Shipping:	Samples are to be sent at room temperature. Include the completed TACL Specimen Shipping Form with shipment and email (tacl@chla.usc.edu) or FAX a copy to TACL Operations Center (877-904-2166). Samples can be shipped to arrive Monday-Friday. For Saturday arrivals, please call the lab ahead of time: Phone: 206-288-7060 Please send copy of Flow Cytometry histogram done locally with day 29 bone marrows.  For specimen shipping, TACL will provide a FED-EX account. Please call the TACL Operations Center for any questions.

8.2 Bone Marrow Correlative Biology Studies (Optional)

Participation in the collection of these specimens is encouraged, but is voluntary, and patients may still participate in the therapeutic part study if they decline correlative biology studies. Institutions are encouraged to talk to their patients about participation.

The following description of studies applies for the bone marrow and PBMC correlative studies described in Section 8.2 and Section 8.3 of the protocol.

1. **Evaluating effect of temsirolimus on mTOR pathway proteins**
   The effects of temsirolimus upon ALL cells will be assessed by comparing expression of a number of key mTOR signaling proteins before and after initiating treatment. The changes in activation (phosphorylation) of the PI3K/mTOR pathway and other signaling proteins will be determined by leukemia cell-specific phosphoflow cytometry analyses +/- immunoblotting using published techniques..[5, 6, 21] Baseline and subsequent PBMC samples will be collected at designated timepoints from patients regardless with any level of peripheral ALL blasts present at time of study entry.

2. **Investigating the impact of temsirolimus on proteins involved in resistance to chemotherapy.**
   ALL blasts from blood and/or bone marrow will also be studied to assess changes in proteins involved in chemotherapy resistance. Proteins will be studied by immunoblot using published techniques and may include markers of methotrexate resistance (DHFR, Rb, and cyclin D1) and corticosteroid resistance (MCL1, RIP-1, CYLD, beclin-1, BRG1, and GSK3).[5, 6, 13 21, 42] If necessary, samples will be flow sorted to enrich for ALL blasts. These studies will be performed pre-study, once between Day 3-5 if peripheral blast count is ≥2500/mm^3. If peripheral blasts count is not ≥ 2500/mm^3, pre-study bone marrow will be used and compared to end of cycle 1 bone marrow.

3. **Investigating the impact of temsirolimus on membrane transporters.** mTOR inhibitors, including temsirolimus, have the potential to inhibit a number of membrane transporters, including ABCG2 (BCRP), Pgp, and MRP. Inhibition of these transporters may affect metabolism of other medications

and chemotherapeutic agents.[43,44] ABCG2, Pgp, and MRP levels, will be measured in ALL blasts by flow cytometry, using published techniques.[44] These studies will be performed on peripheral blood if peripheral blasts count is ≥2500/mm^3 or bone marrow if less than 2500/mm^3 with time points as described in resistance to chemotherapy experiments. See section 8.3 for further details on peripheral blood biology studies. These studies will only be performed on samples that are shipped fresh.

4. **Studies of ALL blasts treated with temsirolimus alone and in combination with cytotoxics in pre-clinical models of ALL**
   ALL blasts from pre-treatment samples will be injected into xenograft models of ALL for expansion of blast numbers and to assess *in vivo* response to treatment with temsirolimus with and without cytotoxic agents, using published techniques.[6, 18]

   If cells (peripheral blood and/or bone marrow) are limited, priority will be the following order: (1) MRD assessment, (2) studies assessing changes in mTOR pathway; (3) xenograft experiments, (4) studies assessing proteins involved in chemotherapy resistance, and (5). studies assessing impact of temsirolimus on membrane transporters

Samples requested:	**Bone Marrow** • Pre-treatment • Course 1, Day 29 +/- 3 days • Course 2, Day 29 +/- 3 days (if patient was not in CR after course 1) • Any subsequent bone marrows while on study or with relapse/recurrence.
Bone Marrow Collection Procedure:	10 mL for patients ≥10 kg, 3-5 mL for patients <10 kg of bone marrow should be collected in one to three green top (sodium heparin) tubes. No processing is required, Samples should be shipped the same day they are obtained.
Specimen Labeling:	Each tube must be labeled as bone marrow with the study ID number, TACL ID, and the date and time the sample was obtained. No personal identifying patient information should be included on the specimen or transmittal form.
Specimen Packaging and Shipping:	▪ Sample tubes should be placed in polypropylene tubes (for secondary containment) and shipped in a styrofoam box as appropriate for biologic material. ▪ Ship the sample at room temperature (with a cold pack if possible; NO DRY ICE) on the same day it was obtained with Federal Express overnight priority delivery for arrival on a Monday through Friday only. Samples must be received **within 24 hours** of obtaining the sample. **Do not ship samples for delivery on a weekend or holiday**. Bone marrow and blood samples that cannot be delivered on a weekday should be stored refrigerated at 4°C and shipped the next business day. **Ship samples to:** [redacted]

	[redacted] [redacted] For specimen shipping, TACL will provide a FED-EX account. Please call the TACL Operations Center for any questions.

8.3 PBMC Correlative Biology Studies (Optional)

Participation in the collection of these specimens is encouraged, but is voluntary, and patients may still participate in the therapeutic part study if they decline. Institutions are encouraged to talk to their patients about participation.

The description of these studies is described in full detail under Section 8.2.1.

Samples requested:	**Peripheral Blood** • Pre-treatment • Course 1, Day 3-5* • Course 1 Day 29. *Send one sample obtained between day 3-5 (day 3 preferred if acceptable for the patient). Do not batch.
Blood Collection Procedure:	Blood samples (5 mL for patients ≥ 10 kg, 2 mL for patients < 10 kg) will be collected in lavender-top (K_2EDTA) tubes.
Sample Processing:	Blood samples do not require any processing by the site as long as they are collected on Monday through Thursday (not including holidays) and will be shipped to the laboratory on the **same day** of collection. Please contact Dr. Tasian if other arrangements are needed.
Specimen Labeling:	Each tube must be labeled as peripheral blood with the study ID number, TACL ID, and the date and time the sample was obtained. No personal identifying patient information should be included on the specimen or transmittal form.
Specimen Packaging and Shipping:	**FRESH SPECIMENS** ▪ Sample tubes should be placed in polypropylene tubes (for secondary containment) and shipped in a styrofoam box as appropriate for biologic material. ▪ Ship the sample at room temperature on the same day it was obtained with Federal Express overnight priority delivery for arrival on a Monday through Friday only. Samples must be received **within 24 hours** of obtaining the sample. **Do not ship samples for delivery on a weekend or holiday**. **Ship samples to:** Tasian Laboratory The Children's Hospital of Philadelphia CTRB, Room 3100 3501 Civic Center Blvd. Philadelphia, PA 19104

	[redacted] For specimen shipping, TACL will provide a FED-EX account. Please call the TACL Operations Center for any questions.

9.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY, OFF STUDY CRITERIA AND STUDY TERMINATION

9.1 Criteria for Removal from Protocol Therapy

a. Patient is not CNS 1 or 2 after the first course of therapy (this excludes patients who are CNS 3 due to chloromas or cranial nerve involvement with evidence of improvement).
b. Patient remains M3 after the first course of therapy
c. Patient remains M2 after the second course of therapy
d. Relapse in any site following remission
e. Progressive disease
f. Completion of protocol therapy
g. Second malignant neoplasm
h. Patient/parent withdrawal or refusal after beginning protocol therapy
i. Patient/parent withdrawal or refusal before beginning protocol therapy
j. Patient off treatment for other complicating disease
k. Non-compliance with protocol regimen and procedures
l. Investigator determination
m. Female patient becomes pregnant or begins breast-feeding

Pregnancy during research participation: Information may only be collected on a research patient who becomes pregnant and is ≥ 18, a partner of a research participant who becomes pregnant and is ≥18 at the time of the pregnancy and/or the newborn infant provided that the proper IRB-approved, consent forms and data collection forms contain provisions for collection of information on the pregnancy and outcome.

Incidents of exposure during pregnancy should be reported as an SAE using the IIR Serious Adverse Event Report Form (and Reportable Event Fax Cover Sheet) along with the Exposure During Pregnancy Supplemental Form. A second Exposure During Pregnancy Supplemental Form should be submitted to report the outcome of the pregnancy.

If the **outcome of the pregnancy meets any of the criteria for seriousness, report it to Pfizer** as an SAE if permitted by your IRB on this trial. Otherwise a separate consent will need to be obtained to report pregnancy outcome data.

Examples of pregnancy outcomes that are SAEs:
- Spontaneous abortion (includes miscarriage and missed abortion)
- Stillbirth
- Congenital anomaly (including in an aborted fetus, a stillborn infant, or neonate that dies shortly after birth)
- Neonatal death (within 1 month of birth) should be reported, without regard to causality
- Any infant death that is assessed as possibly related to the in utero exposure to temsirolimus should be reported as an SAE

9.2 Off Study Criteria

a. Death
b. Patient Lost to follow-up
c. Patient withdraws consent, refuses follow-up

9.3 Termination of the Study by TACL

The TACL Consortium may terminate this study prematurely, either in its entirety or at an investigative site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns.

9.4 Reproductive Risks

In male animal studies, fertility was decreased or absent. These effects on male fertility were accompanied by testicular tubular degeneration, decreased sperm concentration and motility, decreased reproductive organ weights, and prostate atrophy. In female animal fertility studies, there were decreased number of corpora lutea and increased incidences of pre- and post-implantation loss resulting in decreased number of live fetuses, and decreased fetal weights.

In developmental animal studies, there were increased embryo/fetal mortality and decreased fetal growth (decreased fetal weight and delayed skeletal ossification). Also there was an increased incidence of intestinal protrusion through the abdomen.

However, there are no adequate and well-controlled studies in pregnant women using temsirolimus nor is there any information available for labor and delivery. Additionally, many drugs are excreted in human milk but lactation studies of intravenous Temsirolimus have not been conducted and the effects of temsirolimus excretion in human milk have not been evaluated.

The potential risk for humans is unknown.

10.0 STATISTICAL CONSIDERATIONS

10.1 Statistical Considerations for Dose Escalation Criteria

The primary endpoint for dose escalation is the occurrence of a dose-limiting toxicity (DLT). A standard 3+3 patient cohort escalation design, as described below, will be used based on the toxicity experienced in the first course of treatment:

1. Three patients are entered at the first dose level
2. If 0/3 experiences DLT at a given dose level, then the dose is escalated to the next higher level, if a higher dose level exists, and three patients are enrolled. If a higher dose level does not exists, up to three more patients are accrued at the same dose level.
3. If 1/3 experiences DLT at current dose, then up to three more patients are accrued at the same dose level.
4. If 2 or more DLTs are observed in a three-patient or six-patient cohort at a given dose level, then the MTD has been exceeded, dose escalation will be stopped, and up to three additional patients will be enrolled at the next lower dose level, if a lower dose level exists (unless six patients have already been treated at that prior dose).

10.1.1 Maximum Tolerated Dose (MTD)/Highest Tested Dose (HTD)

The MTD is the highest dose level tested at which 0/6 or 1/6 patients experience DLT with at least 2/3 or 2/6 patients encountering DLT at the next higher dose. If the highest specified dose level (Dose Level 4) in this study is reached with 0/6 or 1/6 patients experiencing DLT, i.e., the MTD has not been reached, this dose level will be referred to as the Highest Tested Dose (HTD). If at least 2/3 or 2/6 patients encountering DLT at the lowest dose, then no MTD exists. See also section 10.2.

10.1.2 Definition of a Patient Evaluable for Toxicity

Any patient who receives at least one dose of temsirolimus and experiences DLT at any time during protocol therapy is considered evaluable for Adverse Effects. Patients are evaluable for toxicity assessment if they terminate treatment for toxicity or intolerability, or they experience a dose limiting toxicity, or they receive 85% of the required dose of and other agents without dose limiting toxicity during the first course of therapy. Patients who do not satisfy one of these conditions are not evaluable for toxicity and will be replaced.

10.1.3 Definition of a Patient Evaluable for Response

A patient enrolled on the trial who receives any prescribed therapy (day 1 and later) is considered evaluable for response.

10.2 Patient Accrual and Study Duration

The proposed study will accrue a minimum of 2 and maximum of 24 patients. Estimated accrual is 1-2 patients a month. This is expected to take 12-36 months. Allowing for time to evaluate patients for DLT, slow initial accrual rate, and opening of other relapsed ALL studies, it is expected that this study will be open to accrual for 2-3 years.

10.3 Interim Monitoring of Toxic Death

The occurrence of toxic death (TD) at any time will be a primary endpoint for safety monitoring. When a patient expires while undergoing treatment, within 30 days of receiving protocol therapy, or during follow-up where the death is directly attributed to toxicity experienced on the protocol, the study committee and statistician will be notified to review the event. No formal statistical rule will be employed. Rather, if the crude proportion of patients experiencing a toxic death exceed 5% at any time, the cause and circumstances of these deaths will be reviewed with the study committee and with the Data and Safety Monitoring Committee to determine whether modifications to or termination of the study is warranted.

10.4 Inclusion of Women and Minorities

The study is open to all participants regardless of gender or ethnicity. Review of accrual to past studies of new agents demonstrates the accrual of both genders and all NIH-identified ethnicities to such studies. The small number of patients entered into this trial will obviate any analysis of variation in response rate with gender or ethnicity.

10.5 Correlative Studies and Response Analysis

A descriptive analysis of the pharmacodynamic (PD) effects of temsirolimus will be performed to assess inhibition of mTOR and downstream signaling in patients' lymphoblasts. The PD data will be summarized with simple summary statistics, including means, medians, ranges, and standard deviations (if numbers and distribution permit).

While the primary aim of this study is to evaluate the toxicity of temsirolimus, patients will have disease evaluations performed at end of therapy. Disease response will be assessed according to RECIST criteria for patients with lymphoma, and will be reported descriptively.

All these analyses will be descriptive and exploratory and hypotheses generating in nature.

11.0 RESPONSE CRITERIA

11.1 Bone Marrow Status Definitions

M1 Marrow
Less than 5% blasts in a bone marrow aspirate or biopsy if aspirate not available.

M2 Marrow
5-25% blasts in a bone marrow aspirate or biopsy if aspirate not available.

M3 Marrow
Greater than 25% blasts in a bone marrow aspirate or biopsy if aspirate not available.

11.2 Response Criteria for Patients with Leukemia

11.2.1 **Complete Remission (CR)**
Attainment of M1 bone marrow with no evidence of circulating blasts or extramedullary disease and with recovery of peripheral counts (ANC ≥ 500/μL and PLT count ≥ 50,000 μL). Qualifying marrow and peripheral counts should be performed within 1 week of each other.

11.2.2 **Complete Remission with Incomplete Blood Count Recovery (CRi)**
Attainment of M1 bone marrow with no evidence of circulating blasts or extramedullary disease, but with insufficient recovery of absolute neutrophil count (ANC < 500/μL) or platelets (< 50,000/ μL).

11.2.3 Minimal Residual Disease negative subset of CR/CRi
The attainment of a bone marrow with MRD < 0.01% blasts while meeting criteria for CR or CRi.

11.2.4 Partial Remission (PR)
Complete disappearance of circulating blasts and achievement of M2 marrow status if M3 originally without new sites of extramedullary disease, and with recovery of absolute neutrophil counts (ANC ≥ 500/μL). Complete response in the marrow without resolution of extramedullary sites is a PR.

11.2.5 **Stable Disease (SD)**
Patient does not satisfy the criteria for PD, or has recovery of ANC ≥ 500/μL and fails to qualify for CR, CRi, or PR.

11.2.6 **Progressive Disease (PD)**
An increase of at least 25% in the absolute number of bone marrow leukemic cells morphologically, development of new sites of extramedullary disease, or other laboratory or clinical evidence of PD, with or without recovery of ANC or platelets. Patients with MRD-only level disease (< 5% blasts) at study entry who develop ≥ 10% disease morphologically will be considered to have progressive disease.

11.2.7 **Induction Death (ID)**
Any patient who dies prior to receiving subsequent therapy.

11.2.8 **Not evaluable (NE)**
Patient does not satisfy the criterion for PD or ID, and either did not have a marrow evaluation, had inadequate marrow cell count, or had insufficient recovery of ANC to be classified as CR, CRi, PR, or SD.

11.3 CNS Response Criteria

11.3.1 **CNS Status Definitions**

CNS 1: In cerebral spinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs).

CNS 2: In CSF, presence < 5/µL WBCs and cytospin positive for blasts, or ≥ 5 /µL WBCs but negative by Steinherz/Bleyer algorithm:

CNS 2a: < 10/µL RBCs; < 5/µL WBCs and cytospin positive for blasts;
CNS 2b: ≥ 10/µL RBCs; < 5/µL WBCs and cytospin positive for blasts; and
CNS 2c: ≥ 10/µL RBCs; ≥ 5/µL WBCs and cytospin positive for blasts but negative by Steinherz/Bleyer algorithm (see below)

CNS 3: In CSF, presence of ≥ 5/µL WBCs and cytospin positive for blasts **and/or** clinical signs of CNS leukemia:

CNS 3a: < 10/µL RBCs; ≥ 5/µL WBCs and cytospin positive for blasts;
CNS 3b: ≥ 10/µL RBCs, ≥ 5/µL WBCs and positive by Steinherz/Bleyer algorithm (see below)
CNS 3c: Clinical signs of CNS leukemia/lymphoma (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome).

METHOD OF EVALUATING INITIAL TRAUMATIC LUMBAR PUNCTURES:
If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains ≥ 5 WBC/µL and blasts, the following Steinherz/Bleyer algorithm should be used

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} > 2X \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

A patient with CSF WBC ≥ 5/µL blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis.

Example: CSF WBC = 60/µL; CSF RBC = 1500/µL; blood WBC =46000/µL;
blood RBC = 3.0×10^6/µL:

CSF calculation: $\frac{60}{1500} = 0.04$

Blood calculation: $\frac{46000}{3.0 \times 10^6} = 0.015 \times 2 = .03$

Therefore this patient has CNS disease because 0.04 > 0.03

11.4 Response Criteria for Patients with Lymphoma

Response and progression will be evaluated in this study using the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[45] Key points are that 5 target lesions are identified and that changes in the *largest* diameter (unidimensional measurement) of the tumor lesions but the *shortest* diameter of malignant lymph nodes are used in the RECIST v 1.1 criteria.

11.4.1 Definitions

11.4.1.2 Evaluable for objective response: Patients who exhibit objective disease progression prior to the end of cycle 1 will be considered evaluable for response. For all other patients, only those patients who have measurable disease present at baseline, have received at least one

cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response.

11.4.1.2 Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.4.2 Disease Parameters

11.4.2.1 Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

11.4.2.2 Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

11.4.2.3 Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

11.4.2.4 Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be

included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

11.4.2.5 Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.4.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

11.4.3.1 Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

11.4.3.2 Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

11.4.3.3 Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

11.4.3.4 PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

11.4.3.5 Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

11.4.3.6 Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare

cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

Cytology should be obtained if an effusion appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease.

11.4.3.7 FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: A 'positive' FDG-PET scan lesion means one that is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.4.4 Evaluation of Target Lesions

11.4.4.1 Complete Response (CR): Disappearance of all target and non-target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. If immunocytology is available, no disease must be detected by that methodology.

11.4.4.2 Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

11.4.4.3 Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions). Note: in presence of SD or PR in target disease but unequivocal progression in non-target or non-measurable disease, the patient has PD if there is an overall level of substantial worsening in non-target disease such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy

11.4.4.4 Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

11.4.5 Evaluation of Non-Target Lesions

11.4.5.1 Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

11.4.5.2 Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

11.4.5.3 Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

11.4.5.4 Overall Best Response Assessment
Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in Section 11.5 from a sequence of overall response assessments.

11.4.6 **Response Criteria for Patients with Lymphoma and Evaluable (but not Measurable) Disease**

11.4.6.1 Evaluable Disease
The presence of at least one lesion, with no lesion that can be accurately measured in at least one dimension. Such lesions may be evaluable by nuclear medicine techniques, immunocytochemistry techniques, tumor markers or other reliable measures.

11.4.6.2 Complete Response
Disappearance of all evaluable disease.

11.4.6.3 Partial Response
Partial responses cannot be determined in patients with evaluable disease

11.4.6.4 Stable Disease
That which does not qualify as Complete Response (CR), Partial Response (PR), or Progressive Disease.

11.4.6.5 Progressive Disease
The appearance of one or more new lesions or evidence of laboratory, clinical, or radiographic progression.

11.4.6.6 Overall Best Response Assessment
Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in Section 11.5 from a sequence of overall response assessments.

11.5 Best Response

11.5.1 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 2: For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥28 days Confirmation**
CR	Non-CR/Non-PD	No	PR	≥28 days Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥28 days from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

Table 3: For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

Table 4. Sequences of overall response assessments with corresponding best response.

1st Assessment	2nd Assessment	Best Response
Progression		Progressive disease

Stable, PR, CR	Progression	Progressive disease
Stable	Stable	Stable
Stable	PR, CR	Stable
Stable	Not done	Not RECIST classifiable
PR	PR	PR
PR	CR	PR
PR, CR	Not done	Not RECIST classifiable
CR	CR	CR

11.5.2 **Duration of Response**

11.5.2.1 Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

11.5.2.2 Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.0 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Definitions

Adverse Event: An adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction (SAR): Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not available, is not consistent with the risk information described in the general investigational plan.

Serious Adverse Events (SAE) or Serious Suspected Adverse Reactions (SSAR): An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

Death of Patient	An event that results in the death of a patient.
Life-Threatening	An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
Hospitalization	An event that results in an admission to the hospital for any length of time. This does not include an emergency room visit or admission to an outpatient facility.
Prolongation of Hospitalization	An event that occurs while the study patient is hospitalized and prolongs the patient's hospital stay.
Congenital Anomaly	An anomaly detected at or after birth, or any anomaly that results in fetal loss.
Persistent or Significant Disability/ Incapacity	An event that results in a condition that substantially interferes with the activities of daily living of a study patient. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (*e.g.,* sprained ankle).
Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome	An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the patient and may require medical or surgical intervention to prevent any of the outcomes listed above (*i.e.,* death of patient, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an 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.

12.2 Data Collection

Adverse events and suspected adverse reactions will be collected and reported on the electronic CRFs beginning with the first dose of study therapy until 30 days following the last dose of study therapy. The investigator will evaluate all adverse events and suspected adverse reactions as to their severity and relationship to temsirolimus as well as the regimen as a whole. **Serious adverse events and serious suspected adverse reactions will require expedited reporting to the TACL Operations Center as described below.**

12.3 Reporting Serious Adverse Events or Serious Suspected Adverse Reactions

12.3.1 The following serious adverse events or serious suspected adverse reactions requires expedited reporting:

- All Grade 5 events regardless of causality.
- All Grade 4 events that are possibly, probably or definitely related to temsirolimus or the regimen as a whole. Exclude reporting of hematologic toxicity as a serious adverse event unless the event meets the criteria of hematologic dose limiting toxicity per protocol section 4.5.2: Bone marrow aplasia: > 42 days from Day 1 of therapy will be a DLT. Aplasia is defined as the failure to recover a peripheral ANC of ≥ 500/mm^3 and PLT ≥ 20,000/mm^3 in the absence of persistent leukemia.

- All unexpected Grade 3 events that are possibly, probably or definitely related to temsirolimus or the regimen as a whole.
- Any reported pregnancy in a female study patient or partner of a male study patient

12.3.2 Steps for Reporting

Step 1: Identify the adverse event or suspected adverse reaction using the NCI Common Toxicity Criteria (CTC), version 4.03.

The CTC provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTC can be downloaded at **http://evs.nci.nih.gov/ftp1/CTCAE/About.html**.

Step 2: Grade the event using the NCI CTCAE version 4.03.

Step 3: Determine if the adverse event or suspected adverse reaction meets the criteria of being "serious".

Step 4: Determine the relationship of Temsirolimus and the regimen as a whole to the event
The investigator will assess the causal relationship between the investigational product and the regimen as a whole and the adverse event. The investigator will use his/her clinical expertise and judgment to select the attribution category below that best fits the circumstances of the AE.

> **Related:** There is a reasonable possibility that the drug/regimen caused the adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.
>
> **Unrelated:** There is not a reasonable possibility that the drug/regimen caused the adverse event. There is not evidence to suggest a causal relationship between the drug and the adverse event.

Step 5: Determine if the adverse event or suspected adverse reaction is "unexpected".

Step 6: Notify the TACL Operations Center by telephone or email

The following information should be submitted within 24 hours of event notification by either phone [redacted], or Email TACL@chla.usc.edu

1. Patient TACL study ID and initials
2. Event description
3. Severity (NCI Grade)
4. Onset date
5. Reason event is considered serious
6. Dose of study drug and dates of administration
7. Investigator opinion of relationship to DRUG and the regimen as a whole
8. Name and phone number of physician in charge of the case
9. Name and phone number of CRA or Research nurse working with the case

Step 7: Submit a written report to the TACL Operations Center

Complete the TACL SAE Notification Form within 72 hours of learning of the event. The completed form is email to TACL@chla.usc.edu The form may also be faxed to (877) 904-2166. A follow-up SAE Notification Form must be submitted upon resolution of the event or as requested by the TACL Operations Center. Please confirm via email or phone that the TACL Operations Center has received this notification.

12.4 Institutional Reporting to the IRB

All SAE's should be reported to the treating institutions IRB or Ethics board. The TACL Operations Center will also report all SAE's to the TACL IRB. The TACL Operations Center will distribute SAE's to all TACL sites as appropriate for submission to their own IRB's.

13.0 DATA AND SAFETY MONITORING

13.1 Data Submission

All study data will be submitted via electronic data capture forms using the DataLabs/TACL Website. Please refer to the TACL web site (https://tacl.chla.usc.edu/tacl) for T2014-001 CRF Completion Guide or contact the TACL Operations Center at [redacted] if you need assistance.

The following are required to be submitted to the Operations Center for all patients entered:
Paper copy of following: Roadmaps and Bone Marrow Reports (include both aspirate and biopsy reports). These forms are to be emailed or faxed to the TACL Operations Office at 877-904-2166 at the end of each course during which the bone marrows are done.

13.2 Weekly Safety Review

The TACL Operations Center (TOC) conducts weekly (as needed) patient safety and review meetings with the protocol co-chairs, , research coordinators and other administrative TACL team members to review all data submitted, non-serious adverse events and other correspondence pertaining to patients. Serious adverse events will be immediately evaluated by the study team and determination regarding notification of participating sites will be made. All serious adverse events will be sent to the CHLA IRB and DSMC if required. Any interim results that would affect patient safety would be immediately communicated to all participating TACL sites. All correspondence with sites will be done via email, with all information also being posted on a member's only section of the TACL website.

13.3 Data Safety and Monitoring Committee (DSMC)

The TACL Operations Center utilizes the Children's Hospital Los Angeles Hem/Onc Data Safety and Monitoring Committee. DSMC meetings will occur every 6 months. Every 6 months, a DSMC report for each protocol will be prepared by the study statistician and study PI detailing patient accrual, toxicities, deaths on study, current study status, responses (responses will be blinded until study completes accrual), summary of amendments to protocol/consent, lists of any publications from study, and plans for study in coming year. Any publications from the study (abstract or manuscript) will be attached to the DSMC report. After DSMC review, the DSMC will issue a confidential report for each study to the study PI and TACL Operations Center.

Not more than eight weeks after the DSMC meeting, a DSMC public review report will also be created for each protocol after approval of the confidential report and resolution of any issues by the PI The public report will then be emailed to the participating sites for each study. These can be filed at the IRB at each site if required per local IRB guidelines.

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15.0 SAMPLE INFORMED CONSENT

Local IRB changes to this document are allowed. Changes within the document should not substantially alter the meaning or intent of the consent document. If the institution or IRB insists on making deletions or more substantive modifications to the consent, especially in the risks sections, they should be reviewed and approved by the TACL Operations Center.

INFORMED ASSENT*/CONSENT DOCUMENT / PARENTAL PERMISSION FOR PARTICIPATION IN RESEARCH

TACL Protocol

A Phase I Trial of Temsirolimus (CCI-779, Pfizer, Inc.) in Combination with Etoposide and Cyclophosphamide in Children with Relapsed Acute Lymphoblastic Leukemia and Non-Hodgkin Lymphoma

Subject Name:	
Medical Record #:	
Physician:	

When we say "you" in this consent form, we mean you or your child; "we" means the doctors and other staff.

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

This study is being carried out by the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Consortium. TACL is a group of Universities and Children's Hospitals that are working together to find treatments for children with leukemia and lymphoma.

You are being asked to take part in this research study because your ALL or Non-Hodgkin's Lymphoma has come back (relapsed).

This is a phase I study of a drug called temsirolimus in combination with intensive cytotoxic chemotherapy consisting of cyclophosphamide and etoposide. Temsirolimus is considered experimental because it has not been proven to work in a situation like yours. We are using temsirolimus because it seems to work against cancer in cells and animals. We do not know if temsirolimus will work in people. Temsirolimus has been used in only a small number of adults and children and there is a lot that we do not know about it yet. This is called a Phase I study because the goal is to find the highest dose of temsirolimus that we can give safely in combination with intensive chemotherapy.

Patients with multiply relapsed ALL have a significant risk of severe side effects and serious infections with intensive salvage therapy. In an attempt to provide your child the safest care possible it is highly recommended that your child receive their initial course as an inpatient and be started on preventative antibiotics and anti-fungal medications during their stay.

Why is this study being done?

We are testing new experimental drug combinations such as the combination of temsirolimus, cyclophosphamide and etoposide in the hopes of finding a drug combination that may be effective against relapsed leukemia and non-hodgkin's lymphoma.

The goals of this study are:

- To find the highest safe dose of temsirolimus that can be given with intensive chemotherapy without causing severe side effects;
- To learn what kind of side effects temsirolimus can cause;
- To learn more about the pharmacology (how your body handles the drug) of temsirolimus;
- To learn how temsirolimus affects specific molecules on leukemia and lymphoma cells;
- To determine whether temsirolimus, combined with intensive chemotherapy is a beneficial treatment for your tumor.

How many people will take part in this study?

It is expected that up to 24 children and young adults will take part in this study.

What will happen if I take part in this research study?

Before you begin the study…

You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of your regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. The results of these tests will be reviewed. It is possible that after these tests are reviewed, you will not be able to take part in the study. If you are not able to take part in the study, your doctor will discuss with you the reasons why.

- A medical history
- Physical exam with vital signs(blood pressure, heart rate, temperature, oxygen levels)
- Bone marrow test to check your cancer (see Tests on the Bone Marrow, below)
- Lumbar puncture to test the fluid in your spinal cord (see Lumbar Punctures, below)
- Blood tests to check your organ function, including liver and kidneys
- Tests to make sure you are not pregnant (if you are a female and old enough to become pregnant)
- Urine Test
- Imaging study (CT, MRI, PET/CT) to determine the extent of your cancer (if you have lymphoma)
- Echocardiogram (ECHO) which uses sound waves to test heart function

<u>Tests on the Bone Marrow</u>
Examinations of the bone marrow will be performed routinely and may be done at the discretion of your study doctor. You have already had many tests of your bone marrow for your previous treatment of ALL. Many children receive some form of sedation or anesthesia during this procedure. A small area over your hip bone on the back will be cleaned and numbed with lidocaine and/or with an anesthetic cream. Approximately 2-3 teaspoons of bone marrow will be withdrawn through a needle inserted into the bone. The test is painful, especially when the bone marrow is withdrawn. There is also a small risk of bleeding or infection from this procedure.

<u>Lumbar Punctures ("L.P.s", "spinal taps")</u>

You are familiar with spinal taps since they were done during your initial therapy for ALL. Whether you decide to participate or not on this study, additional spinal taps will need to be done to give medicines, which are necessary to prevent the leukemia from spreading to the spinal fluid. Many children receive some form of sedation or anesthesia during this procedure. Spinal taps are painful and may cause headaches. The skin at the site of needle insertion is usually numbed with an anesthetic cream or lidocaine before the procedure is performed. Approximately 1 teaspoon of spinal fluid will be withdrawn prior to injection of the medicine.

During the study …

If the exams, tests and procedures show that it is safe for you to be enrolled on the study, and you will be given temsirolimus by intravenous (IV) infusion on days 1 and 8. Cyclophosphamide and etoposide will be given by intravenous (IV) infusion on days 1-5. You will then recover from the combined therapy. A cycle last approximately 4 weeks and you may get up to 8 cycles as long as you have stable or improving cancer.

We highly recommend you remain in the hospital from the time you start the therapy (or from Day 1) until blood work and your medical team determine it is safe for you to be sent home. We also highly recommend you are started on preventative antibiotics and anti-fungals while you are hospitalized.

Various methods will be used to give drugs:

- IV – Drug is given using a needle or tubing inserted into a vein, It can be given by IV push over several minutes or by infusion over minutes to hours
- IT – Drug used to treat the brain and spinal cord is given using a needle inserted into the spinal canal

Treatment

Drug	Days	Dose
Temsirolimus	1 and 8	As assigned, IV
Cyclophosphamide	1-5	440 mg/m^2 IV daily
Etoposide	1-5	100 mg/m^2 IV daily
Triple IT (patients with CNS) (Methotrexate, Cytarabine, Hydrocortisone)	Weekly during Course 1, then day 1 of each subsequent course	IT dose depends on age
Methotrexate IT (patients without CNS disease	Day 1 of Courses 1, 2, 4, 6 and 8	IT dose depends on age
Leucovorin	Starting 48 hours after any Methotrexate containing IT given day 0 or after.	5mg/m^2/dose every 12 hours x 2 doses

Medical Tests During Treatment

Whether you are on this study or not the following medical tests will be done to monitor for response to treatment as well as side effects related to treatment. These include:

- Physical exams with vital signs
- Blood tests to check your organ function including:
    - CBC to look at your blood cells
    - Chemistries to look at elements and minerals in your blood
    - Blood tests to look at your liver, pancreas and kidney function
- Bone Marrow Tests
- Lumbar Punctures
- Radiology scans such as a CT, MRI or X-ray, if indicated

Tests for Research Purposes

We would also like to do some extra tests called biologic studies. These tests will help us learn more about temsirolimus on leukemia cells and may help children who receive this drug in the future. The information learned would not change the way you are treated, and the results of these tests will not be returned to you. Some of these tests are required and others are optional. Although these tests are very important part of how we will better learn to use this drug, it is your decision as to whether or not you agree to participate.

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Bone Marrow Samples for MRD testing

When bone marrow testing is being done as part of your standard evaluation(s) on Day 29 (or a later cycle if not quite in a complete remission on day 29), we would like to request an extra sample [6 mL (slightly more than 1 teaspoon) from patients greater than or equal to 12 kg (26.5 pounds), or 3 mL (less than 1 teaspoon) from patients less than 12 kg (26.5 pounds)] to be collected for minimal residual disease (MRD) testing. This type of testing is a more sensitive way to evaluate whether there are any leukemia cells present after therapy. *These bone marrow samples are required from all patients who had > 5% blasts in their bone marrow at study entry.*

Biology Studies using Bone Marrow Samples (Optional)

When bone marrow testing is being done as part of your standard evaluation(s) before you begin therapy and on Day 29, we would like to request an extra sample [10 mL (about 2 teaspoons) from patients greater than or equal to 10 kg (22 pounds), 3-5 mL (about 1 teaspoon) from patients less than 10 kg (22 pounds)] to be collected to evaluate the effect of temsirolimus on your cells in the bone marrow and if sample permits to grow your cancer in a mouse to further study the effect of temsirolimus and intensive reinduction chemotherapy on proteins in those cells.

The tests may help us to better learn how this drug may work. Please indicate by initialing below whether you choose to participate in the biology studies.

_____/_____ Yes, I agree to participate in the biology studies using bone marrow samples.

_____/_____ No, I do not agree to participate in the biology studies using bone marrow samples.

Biology Studies using Blood Samples (Optional)

During the study, additional blood samples will be collected to evaluate the effect of temsirolimus and intensive reinduction chemotherapy in your blood. The blood samples are 5 mL (about 1 teaspoon) for patients greater than or equal to 10 kg (22 pounds), and 2 mL (about ½ teaspoon) from patients less than 10 kg (22 pounds) and will be obtained prior to therapy, once between day 3 and 5 and at the end of cycle 1 of therapy on Day 29. This amount of blood is safe to draw even from small children.

The tests may help us to better learn how this drug may work. Please indicate by initialing below whether you choose to participate in the biology studies.

_____/_____ Yes, I agree to participate in the biology studies using blood samples.

_____/_____ No, I do not agree to participate in the biology studies using blood samples.

What are my responsibilities?

- During the study you will be asked to take all your chemotherapy drugs as prescribed. It is very important that you follow your doctor's instructions regarding when and how to take your study medications. Be sure to ask your study doctor or nurse if you have any questions about taking your study medications.

- If you experience any unusual side effects as explained by your study doctor, you should contact the study center immediately. You should also contact your study doctor if you are hospitalized for any reason during the study or within 30 days after completion of therapy.

How long will I be in the study?

If you have stable or responding disease you may receive up to 8 cycles of therapy for up to 1 year. After completing the study therapy, your doctor will discuss with you the options for additional treatment. These options will vary depending on whether or not your leukemia or Non-Hodgkin Lymphoma responded to the therapy.

After completing the treatment on this study we would like to continue to collect some medical information about how you are doing for as long as you are willing to let us. We will collect information on how your ALL or Non-Hodgkin Lymphoma is doing, what kind of therapy you may be getting and if you have any long term side effects.

Can I stop being in the study?

Yes. You can decide to stop at any time. Your clinical care will not be affected by your decision to withdraw. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely. You will be asked to visit the hospital or clinic for some follow-up tests to make sure all the side effects you may have experienced have gone away.

It is important to tell your doctor if you are thinking about stopping so any risks from the study treatment and chemotherapy drugs can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

Your doctor may stop you from taking part in this study at any time without your permission if he/she believes it is in your best interest; your tumor gets worse; if the side effects of temsirolimus or combination chemotherapy are too harmful for you; you need a treatment that is not allowed on this study; new information becomes available; if you do not follow the study rules; if you become pregnant or begin to breast feed; or if the study is stopped.

What side effects or risks can I expect from being in the study?

All people who receive cancer treatment are at risk of having side effects. In addition to killing cancer cells, chemotherapy drugs can damage normal tissues and produce side effects. Side effects are usually reversible when the medication is stopped, but occasionally can persist and cause serious complications or death. The therapy used in this protocol is made stronger so that it can kill cancer cells quickly before they can become resistant to treatment. Protocols used to treat relapsed leukemia or Non-Hodgkin lymphoma are more intensive than are those to treat newly diagnosed disease. It is not possible to predict whether the side effects listed below, or other rare side effects may occur. Side effects can be increased when chemotherapy drugs are combined. It is possible that this re-induction therapy will prove better than past therapies for relapsed ALL or Non-Hodgkin lymphoma, but it may be no better or worse than other treatments. It may be associated with more side effects, which may be life threatening. There also is a risk of death. We will tell you if we learn any new information that may affect your health, welfare, or decision to stay in this study. You should talk to your study doctor about any side effects that you have while taking part in the study.

Common side effects include nausea, vomiting, hair loss and fatigue. The risks from having your blood taken are minimal, but can include an infection or a blood clot. There may be pain and or bruising at the site where the needle is inserted to draw your blood. Chemotherapy causes temporary bone marrow depression. Bone marrow depression means that your bone marrow may make:

- Less red cells causing anemia;
- Less platelets, causing bruising and an increased chance of bleeding;
- Fewer white blood cells, causing a risk for serious infections.

Red blood cell and platelet transfusions may be required.

Each drug will have a unique set of side effects. Side effects related to drugs occur in people at different rates or frequencies. The following kinds of risks and side effects may be observed from the drugs used in this protocol are divided into More Likely, Less Likely and Rare but Serious categories:

Temsirolimus

More Likely	Less Likely	Rare but Serious
• Decrease in red blood cells • Decrease in the number white blood cells • Decrease in the number of a type of white blood cell called lymphocytes • Decreased hemoglobin • Diarrhea • Nausea • Decrease in platelets which can cause bruising and bleeding • Sores in your mouth or other parts of your digestive tract • Increased Cholesterol level • Loss of appetite • Rash • Swelling of face, arms, legs • Increase of fat in the blood • Increase blood level of a form of fat called triglyceride • Increased blood level of a liver enzyme (AST/SGOT) • Decreased blood level of phosphate • Increased blood level of creatinine (a substance normally eliminated by the kidneys into the urine) • Stomach pain • Fever • Muscle weakness • Infections • Increased blood level of a live or bone enzyme (alkaline	• Increased blood level of a liver enzyme (ALT/SGPT) • Increased blood level of a liver enzyme (bilirubin) • Urinary tract infection • Decreased blood level of potassium or other salts in the blood • Sore throat • Constipation • Vomiting • Chills • Non-cardiac chest pain • Abnormal wound healing • Weight loss • Joint pain, Back pain, Muscle pain • Flu-like symptoms, including body aches • Changes in taste • Headache • Feeling sad or depressed • Having trouble sleeping • Sneezing, runny nose, itchy and water eyes due to allergies • Nose bleed • Dry skin, itchy skin, acne • Nail loss, change in nail texture and color • High blood pressure • Low blood pressure • Decrease in the number of a type of white blood cell called neutrophils • Pneumonia or other respiratory tract infections • Swelling or infection of the eye • Bloating, fluid collection including in lungs which may cause shortness of breath • Allergic reaction by your body to the drug that can occur immediately or be delayed. The reaction may include hives, low blood pressure, wheezing, swelling of the throat and difficulty breathing	• A tear or hole in the wall of your stomach or intestines • A blood clot that has dislodged from another part of the body and travelled to the lungs where it blocks oxygen from the body and may lead to death. • Kidney failure • Rupture of a blood vessel in the brain • Damage to the lungs which can cause scarring in the lungs and affect the ability of your body get oxygen

More Likely	Less Likely	Rare but Serious
phosphatase) • Cough and shortness of breath • Tiredness/fatigue • Increased glucose (sugar) in the blood		

Cyclophosphamide

More Likely	Less Likely	Rare but Serious
• Loss of appetite • Nausea • Vomiting • Fewer white blood cells in the blood • Hair loss • Decreased ability of the body to fight infection • Absence or decrease in the number of sperm which may be temporary or permanent which may decrease the ability to have children	• Abnormal hormone function which may lower the level of salt in the blood • Abdominal pain • Diarrhea • Fewer red blood cells and platelets in the blood • Bleeding and inflammation of the urinary bladder • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Temporary blurred vision • Nasal stuffiness with IV infusions • Skin rash • Darkening of areas of the skin and finger nails • Slow healing of wounds • Infections	• Heart muscle damage which may occur with very high doses and which may be fatal • Abnormal heart rhythms • Damage and scarring of lung tissue which may make you short of breath • A new cancer or leukemia resulting from this treatment. • Damage or scarring of urinary bladder tissue • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Infertility which is the inability to have children

Etoposide

More Likely	Less Likely	Rare but Serious
• Nausea and vomiting • Hair Loss • A feeling of weakness or tiredness • Fewer red and white blood cells and platelets in the blood • A low number of red blood cells can make you feel tired and weak • A low number of white blood cells can make it easier to get infections • A low number of platelets causes you to bruise and bleed more easily	• Loss of appetite • Decreased blood pressure during the infusion which may require treatment • rashes • Diarrhea • Pain in the abdomen • Mouth sores • Tingling sensation or loss of sensation in fingers or toes • A feeling of extreme tiredness or weakness • The finger or toe nails may loosen from their nail beds • Inflammation of the vein through which the medication was given • Chest pain	• Damage to the liver • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • A new cancer or leukemia resulting from this treatment • Severe rashes which can result in loss of skin and damage to mucous membranes • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Damage to the heart muscle which may make you feel tired, weak, feel short of breath, and retain fluid

More Likely	Less Likely	Rare but Serious

Cytarabine

Risks and side effects related to cytarabine when given into the spinal fluid include:

More Likely	Less Likely	Rare but Serious

• Nausea
• Vomiting
• Fever
• Headache
	• Inflammation of the lining that covers the brain which could lead to a severe headache, a stiff neck, fevers, abnormally high number of white blood cells in the spinal fluid	• Rash
		• Feel tired, hard to stay awake
		• Irritation of the lining of the brain leading to headache and neck stiffness
		• Convulsions
		• Slight or partial paralysis
		• Fewer red and white blood cells and platelets in the blood
		  ○ a low number of red blood cells can make you feel tired and weak
		  ○ a low number of white blood cells can make it easier to get infections
		  ○ a low number of platelets causes you to bruise and bleed more easily
		• Difficulty controlling your muscles
		• Damage to brain tissue which can lead to difficulty speaking, loss of mental function, lack of coordination, seizures, coma and death
		• Loss of feeling and movement in your legs
		• Blurred vision with a chance of blindness

Methotrexate

Risks and side effects related to methotrexate when given into the spinal fluid include:

More Likely	Less Likely	Rare but Serious
• Nausea • Headache	• Inflammation of the lining that covers the brain which could lead to a severe headache, a stiff neck, fevers, abnormally high number of white blood cells in the spinal fluid • Difficulty learning or thinking clearly • Confusion or a sense of not knowing where you are. • Difficulty with speaking	• Seizures • Temporary loss of function or feeling in the lower part of the body (partial paralysis) or which may affect bladder function • Severe damage to brain tissue which could lead to difficulty carrying out normal

More Likely	Less Likely	Rare but Serious

	• Vomiting
	• Rash
	• Sleepiness
	• A feeling of extreme tiredness
	• Unsteady walk
	• Leg pain
	• Fewer red and white blood cells and platelets in the blood
	  ○ a low number of red blood cells can make you feel tired and weak
	  ○ a low number of white blood cells can make it easier to get infections
	  ○ a low number of platelets causes you to bruise and bleed more easily
		daily tasks or could lead to a coma.
		• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever
		• Bleeding into the space in the spine where the injection is given. You may need to receive a platelet transfusion before your injection to prevent this.

Combined Intrathecal Therapy (given into the spinal fluid)

The following toxicities may occur when methotrexate, cytarabine ± hydrocortisone are given together into the spinal fluid:

More Likely	Less Likely	Rare but Serious
• None	• Headache • Abnormally high number of cells in the spinal fluid • Learning disability • Nausea and Vomiting • Fever • Rash • Seizures • Drowsiness • Stiff neck • Irritation of tissues in the brain/spinal cord • Unsteady walk	• Partial paralysis • Damage to brain tissue • Increasingly poor nervous system function • Fewer red and white blood cells and platelets in the blood

Leucovorin

Risks and side effects related to leucovorin include:

More Likely	Less Likely	Rare but Serious
NONE	• Hives	• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Seizure

Risks associated with Disease Evaluation Imaging

X-rays and CT scans expose you to radiation. For PET-CT scan you will get a radioactive sugar (FDG) by vein. The radiation exposure for the PET scan is about the same to about half that of a standard CT scan.

You will also get x-rays from the CT portion of the PET-CT scan. The total amount of radiation you get from the combined PET and CT scan will not be more than 2 to 6 times the amount of radiation that everyone gets in a year from natural sources like the atmosphere. The risk from this amount of radiation is considered to be small when compared with other everyday risks.

Reproductive risks

Because the drugs in this study can affect an unborn baby, you should not become pregnant or father a baby while on this study. You should not nurse a baby while on this study. It is a condition of this study that adequate birth control methods be used by all participants and/or their sexual partners while enrolled in the study. Examples of these include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), Levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these methods of birth control cannot be used, contraceptive foam with a condom is recommended. Ask your doctor about counseling and more information about preventing pregnancy.

If you are a female patient and become pregnant or a male patient and your partner becomes pregnant during this study, contact your study doctor immediately. Female patients who become pregnant or male patients whose partner becomes pregnant will be given instructions for discontinuation of study medication and will be removed from study. The maker of temsirolimus (Pfizer, Inc) will be notified if study patients become pregnant while taking temsirolimus as part of this study. Adult patients (≥18 years old at the time of enrollment or their adult partners (≥18 years old at the time of pregnancy) will be approached about consenting to provide more information about the pregnancy if they were taking temsirolimus during any part of the pregnancy. A separate pregnancy consent will be obtained at the time to report any data about the pregnancy.

Confidential Information

Because the sponsor of this study is outside of your medical center, there is the potential small risk of accidental release of confidential information when sending out any research documentation. Please see the "Will my medical information be kept private?" section of this document for more details.

Developing a Second Cancer

It is possible that you may develop a second form of cancer as a result of this treatment. Experience so far suggests that the chance of this happening is very small. Not enough information has been gathered in children to be able to give an accurate prediction, although it may be in the range of one in every 50 to 800 children treated.

Are there medications, herbal remedies or foods that I should not take while on this study?

The use of the following medications, foods and herbal remedies should be avoided while on temsirolimus as it can affect how your body processes the drug. If you are taking any of these drugs or are prescribed these drugs, please let your study doctor know. Your doctor will help find reasonable alternatives if you are on one of these drugs.

Amiodarone
Aprepitant
Atazanavir
Barbiturates
Carbamazepine
Chloramphenicol
Cimetidine
Ciprofloxacin
Clarithromycin
Delaviridine
Diltiazem
Efavirenz
Erythromycin
Fluconazole
Fluvoxamine
Glucocorticoids
Grapefruit
Grapefruit Juice
Imatinib
Indinavir
Itraconazole
Ketoconazole
Modanfinil
Nefazodone
Nelfinavir
Nevirapine
Norfloxacin
Norfluoxetine (fluoxetine)
Oxcarbazepine
Phenobarbital
Phenytoin
Pioglitazone
Posaconazole
Rifabutin
Rifampacin
Rifampin
Ritonavir
Saquinavir
St. John's wort

Telithromycin	Verapamil
Troglitazone	Voriconazole

The following types of medicines used to treat high blood pressure should be avoided while taking temsirolimus:

- Angiotensin converting enzyme inhibitor (ACE inhibitor)
- Calcium Channel Blockers (CCB)

Your study doctor can review your medications and tell you if you are currently taking an ACE inhibitor or a calcium channel blocker.

Are there benefits to subjects taking part in the study?

Participation on this study may or may not benefit you. Participating in this study will not cure your relapsed leukemia/lymphoma. Based on experience with the drugs used in the treatment plan, researchers believe this therapy may cause your leukemia/lymphoma to stop growing or go into remission for a period of time. Your cancer may not have any response to the therapy received while participating in this study.

Are there benefits to society?

It is hoped that the information learned from this study may help future children or young adults with relapsed ALL/ Non-Hodgkin Lymphoma.

What other choices do I have if I do not take part in this study?

You do not have to participate in this study to receive treatment for recurrent leukemia. There is no "standard" therapy for recurrent leukemia. Most treatment plans have used drugs similar to those used in this protocol, although these drugs may be given in different combinations, and at different times. You can receive other combinations of chemotherapy without participating in this study.

As an alternative to this study, you may decide you don't want additional treatment for your relapsed leukemia or lymphoma. You will always receive medicines to help you feel more comfortable and deal with problems caused by your cancer or treatment whether you participate in this study or not.

Talk to your doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private?

Members of the research team and, if appropriate, your primary care physicians and nurses will know that you are a research subject. All results will be kept confidential, but may be made available to you, and/or your physician if you wish. Because this study involves the treatment of a medical condition, a copy of this consent form will be placed in your medical record. This will allow the doctors that are caring for you to obtain information about what medications or procedures you are receiving in the study and treat you appropriately. You may read your medical record. The records are available to those caring for you at this hospital.

Organizations that may inspect/or copy your research records for quality assurance and data analysis include:

- Therapeutic Advances in Childhood Leukemia & Lymphoma Consortium (TACL)
- Pfizer, Inc. (the makers of temsirolimus) and their designated representatives
- The U.S. Food and Drug Administration (FDA)

- The U.S. Department of Health and Human Services (DHHS)
- The Institutional Review Board (IRB) of CHLA
- Health Canada

As a result, these organizations may see your name; but they are bound by rules of confidentiality not to reveal your identity to others.

Reasonable steps will be taken to protect your right to privacy. No information about you, or provided by you during the research will be shared with others without your written permission, except as explained below:

If necessary to protect your rights or welfare (for example, if you are injured and need emergency care); or if required by law (i.e., child abuse, reports of certain infectious diseases).

The information collected will be used to meet the purpose of this clinical study. In addition, this information may be used to support applications to market the studied drug in the United States and in other countries. It may also be used in reports of the study or for scientific publications and presentations that will not identify study participants by name.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov , as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What are the costs of taking part in this study?

The health care costs during your participation in this study that are considered part of the standard treatment of your disease will be billed to your insurance or other third-party payer. This includes blood tests, hospitalizations, procedures that will be done and medications with the exception of the medication Temsirolimus. The drug company, Pfizer, will provide the medication, Temsirolimus, free of charge for this study. Pfizer does not cover the cost of getting the Temsirolimus ready and giving it to you, so you or your insurance company may have to pay for this.

You will not have to pay for the following tests that will be done for research purposes only:
- Bone Marrow Biology Studies
- Peripheral Blood Biology Studies

Your family is responsible for other costs which may result from your participation in the study, such as, but not limited to, time off of work, car fare, baby sitter fees, food purchased while at the hospital, etc. You will not receive any type of payment for participating in this study. Taking part in this study may lead to added costs to your insurance company. Please ask about any expected added costs or insurance problems.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at *http://cancer.gov/clinicaltrials/understanding/insurance-coverage* . You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call her at _____.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is voluntary. You may choose not to participate in this study. If you decide not to participate, you will not be penalized and you will still receive the standard treatment.

If you choose to participate, you may discontinue your participation in the study at any time. If you discontinue participation in the study, physicians and hospital personnel will still take care of you.

You also have the right to know about new information that may affect your health, welfare, or your willingness to participate in the study. You will be provided with this information as soon as it becomes available.

Whether you participate or not, you will continue to get the best medical care this hospital can provide.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ at _____.

For questions about your rights while taking part in this study, call the Institutional Review Board at _____

Where can I get more information?

Call the National Cancer Institute's Cancer Information Service:
1-800-4-CANCER (1-800-422-6237) OR 1-800-332-8615 (for the hearing impaired)

- You will be given a copy of this consent form.
- You will be given a copy of this treatment plan upon request.
- Visit the TACL Consortium Website at https://tacl.chla.usc.edu/tacl

SIGNATURE OF RESEARCH SUBJECT

Your signature below indicates:

- You have read this document and understand its meaning;
- You have had a chance to ask questions and have had these questions answered to your satisfaction;
- You agree to participate in this research study; and
- You will be given a copy of the signed permission form.

_____	_____
Name of Subject	Signature & Date

SIGNATURE OF PARENT(S)/GUARDIAN

Your signature below indicates that you have read this document; understand its meaning; have had a chance to ask questions; have had these questions answered to your satisfaction; and agree to your child's participation in this research study. You have been given a signed copy of this assent/permission form.

_____	_____
Name(s) of Parent(s)/Guardian	Name(s) of Parent(s)/Guardian
_____	_____
Signature of Parent (Guardian) & Date	Signature of Parent (Guardian) & Date

SIGNATURE OF INVESTIGATOR

I have explained the research to the subject and/or the subject's parent(s)/guardian(s) and have answered all of their questions. I believe that they understand the information described in this document and freely give consent/permission/assent to participate.

_____
Name of Investigator

_____	_____
Signature of Investigator	Date (must be same as the subject's)

SIGNATURE OF WITNESS (if a translator is used)

My signature as witness certified that the subject and/or the subject's parent(s)/guardian(s) signed this permission form in my presence as their voluntary act and deed.

_____

Name

_____	_____
Signature of Witness	Date (must be the same as the subject's)

Please check appropriate box and sign below.

☐Investigator's Statement of Certification for Subjects less than Seven Years of Age (Assent)

The undersigned investigator, _____, hereby certifies that he/she has discussed the information contained in the study consent with the subject, including any risks that may reasonably be expected to occur. The undersigned further certifies that the subject was encouraged to ask questions, that all questions were answered, and that assent was obtained.

☐ Assent was not obtained for a subject under 18 years of age. *(Please state the reason. Examples include: child is an infant; child is comatose; child lacks cognitive abilities to understand the information.)*

_____
_____
_____

Date:_____Time:_____

Signature_____

INFORMED CONSENT: ATTACHMENT 1

EXPOSURE DURING PREGNANCY (EDP) SUPPLEMENTAL FORM

Complete whenever an embryo or fetus has been exposed to study drug. Send as soon as EDP has been diagnosed, together with the SAE Report Form with the appropriate fields completed. If more space is needed, use additional copies of this page.

Pregnancy

First Day of Last Menstrual Period - - Estimated Date of Conception - -
DD-MMM-YYYY DD-MMM-YYYY

Gestation at time of initial exposure _____ weeks **Or, if number of weeks unknown:** ☐ First trimester? ☐ Second trimester? ☐ Third trimester?

Relevant History/Exposure to Products

Risk factors for adverse pregnancy outcomes including environmental or occupational exposures, e.g. hypertension, diabetes, etc. Family history of congenital abnormality/genetic diseases, consanguinity (or any family relation or lineage) between parents (specify degree):

1) Did the mother smoke during this pregnancy? ☐ No ☐ Yes: Number per day? _____

2) Did the mother drink alcohol during this pregnancy? ☐ No ☐ Yes: Frequency? _____

3) Did the mother use illicit drugs during this pregnancy? ☐ No ☐ Yes: Frequency? _____

Obstetrical History *(Check the box if not applicable)*

☐ Not Applicable: No previous pregnancy

Number of previous pregnancies _____ Number of other children _____

Outcome of previous pregnancies (live birth, miscarriage, elective termination, late fetal death, ectopic pregnancy, molar pregnancy). Previous maternal pregnancy complications. Previous fetal/neonatal abnormalities and type. History of sub-fertility.

OUTCOME OF PREGNANCY

Complete and send after the end of pregnancy in all cases when an embryo or fetus has been exposed to study drug

Date of outcome of pregnancy - -
DD-MMM-YYYY

Pregnancy outcome

Check one ☐ Full term live birth ☐ Preterm live birth ☐ Stillbirth* ☐ Spontaneous abortion/miscarriage* ☐ Induced abortion ☐ Unknown

Gestational age at birth in weeks, (if known): _____

Complete also the Serious Adverse Event section of the report

Infant

Check one ☐ Normal ☐ Congenital Malformation/Anomaly** ☐ Other neonatal problem** ☐ Unknown

Other neonatal problem/abnormality (include dysmaturity, neonatal illness, hospitalization, drug therapies) Specify:

Apgar Score 1min _____ 5min _____

☐ Male ☐ Female Birthweight _____ grams ***Or, if birthweight in grams unknown:*** Birthweight ☐ lb ☐ oz

Length at birth: _____ ☐ in ☐ cm Head Circumference at birth: _____ ☐ in ☐ cm

****Complete also the Serious Adverse Event section of the report, specifying the diagnosis as the Serious Adverse Event***

Paternal Information *(Check the box if not applicable)*

☐ Not Applicable

Age _____ (years) **Date of Birth** - - **Occupation** _____

DD-MMM-YYYY

Relevant History

Risk factors including environmental or occupational exposures, e.g. AIDS, toxins. Family history of congenital abnormality/genetic diseases, consanguinity (or any family relation or lineage) between parents (specify degree):

Exposure to Products

Where any drugs (e.g., OTC, medical prescription) taken by the father during the mother's pregnancy? ☐ No ☐ Yes, *please specify*

Product	Indication	Start Date \ Stop Date	Reason for stopping	Dose	Formulation	Frequency
		- - DD-MMM-YYYY - - DD-MMM-YYYY				
		- - DD-MMM-YYYY - - DD-MMM-YYYY				
		- - DD-MMM-YYYY - - DD-MMM-YYYY				
		- - DD-MMM-YYYY - - DD-MMM-YYYY				

Exposure to Products - Recreational Drug Use

1) Did the father smoke during the mother's pregnancy? ☐ No ☐ Yes: Number per day? _____

2) Did the father drink alcohol during the mother's pregnancy? ☐ No ☐ Yes: Frequency? _____

3) Did the father use illicit drugs during the mother's pregnancy? ☐ No ☐ Yes: Frequency? _____

APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX II: CYP3A4 INDUCERS AND INHIBITORS

The use of the following medications should be discontinued prior to initiation of protocol therapy and should be avoided during protocol therapy if reasonable alternatives exist. This is not an inclusive list; please refer to other resources such as http://medicine.iupui.edu/clinpharm/ddis/table.aspx for additional information.

Strong Inhibitors	Moderate Inhibitors	Weak Inhibitors	Other Inhibitors	Inducers
Clarithromycin Indinavir Itraconazole Losartan Ketoconazole Nefazodone Nelfinavir Posaconazole Ritonavir Saquinavir Telithromycin Voriconazole	Aprepitant Diltiazem Erythromycin Fluconazole Grapefruit Juice Verapamil	Cimetidine	Amiodarone Bocepravir Chloramphenicol Ciprofloxacin Delaviridine Fluvoxamine Imatinib Mifepristone Norfloxacin Norfluoxetine (fluoxetine) Star fruit Telaprevir	Barbiturates Carbamazepine Efavirenz Glucocorticoids Modanfinil Nevirapine Oxcarbazepine Phenobarbital Phenytoin Pioglitazone Rifabutin Rifampin St. John's wort Troglitazone

DRUGS	POTENTIAL INTERACTION	ACTION TO BE TAKEN
Anticonvulsants	Induction of drug metabolizing enzymes Lowered EFS	AVOID phenytoin, Phenobarbital, carbamazepine Consider Gabapentin or Levetiracetam (Keppra) as alternative
Rifampin	Induction of drug metabolizing enzymes	DO NOT USE
Azole Antifungals (fluconazole, itraconazole*, voriconazole, posaconazole, ketoconazole)	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS such as echinocandins or amphotericin.
Macrolide Antibiotics (erythromycin, clarithromycin, azithromycin, roxithromycin, telithromycin)	Inhibition of drug metabolizing enzymes	CONSIDER ALTERNATIVE MEDICATIONS

For a more complete list of CYP 3A 4/5 Inhibitors and Inducers, go to:
http://medicine.iupui.edu/flockhart/