Title:	A PHASE 2 TRIAL OF SIROLIMUS WITH METHOTREXATE IN RELAPSED/REFRACTORY LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA
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Table of Contents ..... **iii**
Abbreviations and Definitions of Terms ..... **vii-viii**
Protocol Synopsis ..... **ix--x**
Table 1: Schedule of Clinical and Laboratory Studies ..... **xi**

BACKGROUND INFORMATION AND RATIONALE **1**
1.1 Introduction 1
1.2 Name and Description of Investigational Product or Description of Intervention 1
1.3 Findings from Non-Clinical and Clinical Studies 2
1.3.1 Pre-Clinical data on mTOR Inhibitors (MTI) 2-3
1.3.2 Pre-clinical data on temsirolimus combined with chemotherapy 3-6
1.3.3 Adult Neoplastic Studies 6
1.3.4 Pediatric Neoplastic Studies 7
1.3.5 Combination therapy of MTIs and methotrexate 7
1.4 Selection of Drugs and Dosages 7
1.4.1 Sirolimus 7
1.4.2 Methotrexate 7
1.5 Compliance Statement 8
1.6 Pharmacokinetics of Sirolimus 8

2 STUDY OBJECTIVES **9**
2.1 Primary Objective (or Aim) 9
2.2 Secondary Objectives (or Aim) 9

3 INVESTIGATIONAL PLAN 9
3.1 General Schema of Study Design 10
3.1.1 Screening Phase 11
3.1.2 Treatment Phase 11
3.1.3 Follow-up Phase 11
3.2 Randomization and Blinding 11
3.3 Study Duration, Enrollment and Number of Sites 11
3.3.1 Duration of Study 11
3.3.2 Total Number of Study Sites/Total Number of Subjects Projected 12
3.4 Study Population 12
3.4.1 Inclusion Criteria 12
3.4.2 Organ Function Requirement 12
3.4.2.1 Bone Marrow Function 12
3.4.2.2 Adequate Renal Function 12-13
3.4.2.3 Adequate Liver Function 13
3.4.2.4 Adequate Cardiac Function 13
3.4.2.5 Adequate Pulmonary Function 13
3.4.3 Exclusion Criteria 13-15

4 STUDY PROCEDURES 15
4.1 Pre-Study / Screening Visit 15
4.2 Treatment Plan 15
4.3 Definition of cycle 16
4.4 Required Observations/Material And Data To Be Accessioned During Study 17
4.5 Changes to the study protocol 18
4.6 Study visits for all cohorts - Cycle 1 and subsequent cycles 18
4.6.1 Planned follow-up Visits will be scheduled according to the roadmap 18
4.6.2 Unscheduled Visits 18
4.7 Supportive Care/Concomitant Therapy 18
4.7.1 General 18
4.7.2 Tumor Lysis Syndrome 18
4.7.3 Growth Factor 18
4.7.4 Hypercholesteremia/Hypertriglyceridemia 19
4.7.5 Interactions 19
4.7.6 PCP Prophylaxis 19
4.8 Extramedullary Disease 19
4.9 Dose Modifications 19
4.9.1 Myelosuppression 20
4.9.2 Aplastic marrow 20
4.9.3 Organ system toxicity 20
4.9.4 Stomatitis 20
4.9.5 Liver Dysfunction 20
4.10 Rescue Medication Administration 21
4.11 Subject Completion Withdrawal 21
4.11.1 Early Termination Study Visit 21

5 STUDY ENDPOINTS AND EVALUATIONS 21
5.1 Primary Endpoint 21
5.2 Secondary Endpoints 21
5.3 Screening and Baseline and On-study Evaluation 21
5.3.1 Physical Examination and current medication 21-22
5.3.2 Demographic information collected 22
5.3.3 Vital Signs 22
5.3.4 Laboratory Evaluations 22
5.4 Efficacy Evaluations 22
5.4.1 Response criteria (Leukemia) 22-23
5.4.2 Response criteria (for NHL patients) 23-24
5.4.3 Response Assessment 24
5.4.3.1 Quantification of Disease Burden 24
5.4.3.2 Complete Response (CR) 24
5.4.3.3 Partial Response (PR) 24
5.4.3.4 Progressive Disease (PD) 24

5.4.3.5 Stable Disease (SD) 24
5.4.3.6 Response Assessment 24
5.4.3.7 Best Response 24-25
5.5 Pharmacokinetic Evaluation..... 25
5.5.1 Description of Assay..... 25
5.5.2 Sampling Schedule..... 25-26
5.5.3 Sample Collection and Handling Instructions..... 26
5.6 Biology Studies. 26
5.6.1 Peripheral Blood Mononuclear Cells/ Peripheral Blood Lymphoblast..... 26
5.6.2 Bone Marrow 26
5.6.3 Collection of Bone Marrow or Peripheral Blood..... 26-27
5.6.4 Documenting specimen delivery 27

6 STATISTICAL CONSIDERATIONS..... 27
6.1 Sample Size and Study duration..... 28
6.2 Statistical Analysis..... 28
6.2.1 Clinical Trial Analysis..... 28
6.2.2 Biology Study Analysis 28
6.3 Definitions..... 29
6.3.1 Evaluable for Toxicity..... 29
6.4 Inclusion of Women and Minorities..... 29
6.5 Statistical Methods..... 29
6.5.1 Baseline Data..... 29
6.5.2 Efficacy Analysis - Clinical Trials Analysis..... 29
6.5.3 Pharmacokinetic Analysis..... 29
6.5.4 Biology Study / Pharmacodynamic Analysis..... 29
6.5.5 Safety Analysis..... 30

7 STUDY MEDICATION..... 30
7.1 Sirolimus..... 30
7.1.1 Toxicity..... 31-32
7.1.2 Formulation and Stability..... 32-33
7.1.3 Sirolimus Administration, Monitoring and Dose Adjustments..... 33
7.2 Methotrexate..... 34
7.2.1 Toxicity..... 34-35
7.2.2 Formulation and Stability..... 35-36
7.3 Drug Accountability..... 36

8 SAFETY MANAGEMENT..... 36

8.1 Clinical Adverse Events..... 36
8.2 Adverse Event Reporting..... 36
8.2.1 General Guidelines For All Adverse Events..... 36-37

8.2.2 Persistent Adverse Events 37
8.2.3 Baseline Adverse Events 37
8.3 Definition of an Adverse Event 37-38
8.4 Definition of a Serious Adverse Event (SAE) 38
8.4.1 Relationship of SAE to study drug or other intervention 38
8.5 IRB/IEC Notification of SAEs 39
8.5.1 Follow-up Report 38-39
8.6 Investigator Reporting of a Serious Adverse Event to Sponsor 39
8.7 Medical Emergencies 39

9 STUDY ADMINISTRATION 39

9.1 Treatment Assignment Methods 39
9.2 Data Collection and Management 39-40
9.3 Regulatory and Ethical Considerations 40
9.3.1 Data and Safety Monitoring Plan 40
9.3.2 Risk Assessment 41
9.3.3 Potential Benefits of Trial Participation 41
9.3.4 Risk-Benefit Assessment 41
9.4 Recruitment Strategy 41-42
9.5 Informed Consent/Assent 42
9.6 Payment to Subjects/Families 42

10 PUBLICATION 42

Appendix I: Eligibility Criteria Form 43-44
Appendix II: Roadmap for Therapy 45-46
Appendix III: Medication Diary 47-48
Appendix IV: Relevant Drug Iinteractions 49
Appendix V: Karnofsky/Lansky 50

11 References 51-53

ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myelogenous Leukemia
ANC	Absolute Neutrophil Count
AUC	Area under the curve
BM	Bone Marrow
BMI	Body Mass Index
BMT	Bone Marrow Transplant
BSA	Body Surface Area
Ca++	Calcium
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHOP	The Children's Hospital of Philadelphia
CCG	Children's Cancer Group
CCI-779	Temsirolimus
cc	Cubic centimeter
C_{max}	Maximum Concentration
CNS	Central Nervous System
Cr	Creatinine
CR	Complete Response
CRA	Clinical Research Assistant
CRp	C-Reactive Protein
CSF	Cerebrospinal fluid
CT	Computed tomography
CTC v3	Common Terminology Criteria Version 3
DEX	Dexamethason
DHFR	dihydrofolate reductase
DLT	Dose limiting toxicity
EDTA	Diaminoethanetetraacetic acid
EFS	Event free survival
ELISA	Enzyme-linked immunosorbent assay
ENT	Ears, nose and throat
°F	Degrees Fahrenheit
FK506	Tacrolimus
5-FU/LCV	5-Fluorouracil and Leucovorin
G-CSF	Growth Colony Stimulating Factor
GVHD	Graft verses host disease
GI	Gastrointestinal
g/dl	Grams per deciliter
HDMTX	High dose methotrexate
Hgb	Hemoglobin
HPLC	High-performance (pressure) liquid chromatography
HPLC-MS	High pressure liquid chromatography - conduction microcalorimetry
HSCT	Hematopoetic stem cell transplant
Ht	Height
ICH	International Conference on Harmonisation
ID	Identification
IRB	Institutional Review Board
IT	Intrathecal
IV	Intravenous
kg	Kilogram
LD	longest diameter
LFT's	Liver function tests
M1	<5% blasts in BM
M2	5-25% blasts in BM
m2	Meter squared
mEq	Milliequivalents
mg	Milligram
Mg++	Magnesium
MRD	Minimal Residual Disease
MRI	Magnetic Resonance Imaging
MTD	Maximum tolerated dose
MTI	Mammalian target inhibitors
mTOR	Mammalian target of rapamycin
MTX	Methotrexate
NG / NJ	Nasogastric / Nasojejunal
Ng/ml	Nanogram per millileter
NHL	Non Hodgkins Lymphoma
NIH	National Institute of Health
PD	Progressive Disease
PI	Principal Investigator
PK	Pharmakokinetic
PLT	platelet
PO	Per Os (Oral)

PO_4	phosphorous
POG	Pediatric Oncology Group
RNAs	Riboneucleic Acid
Rapa	Rapamycin
SAE	Severe Adverse Event
SCT	Stem cell transplant
SOC	Standard of Care
SD	Stable disease
ULN	Upper limit of normal
µL	Microliter
VCR	Vincristine
VS	Vital signs
Wt	Weight
XRT	Radiation

PROTOCOL SYNOPSIS

Study Title	A PHASE 2 TRIAL OF SIROLIMUS WITH METHOTREXATE IN RELAPSED/REFRACTORY LEUKEMIA AND NON-HODGKIN'S LYMPHOMA	
Sponsor	Leukemia/Lymphoma Society Translational Research Grant	
Clinical Phase	Phase 2	
Study Rationale	• At present children who have bone marrow or combined bone marrow and extramedullary relapses of acute leukemia while on therapy have 5-20% of long-term survival. • Newer, targeted agents need to be identified and integrated into the present cytotoxic chemotherapy regimens. Biologically targeted cancer agents, including signal transduction inhibitors like mammalian target of rapamycin inhibitors (MTIs), have shown great promise in treating hematologic malignancies. • A Phase 1 trial of sirolimus (an MTI) alone performed at CHOP has been well tolerated with no DLTs and has evidence of hitting the biologic target. • 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 other cytotoxic agents. • Based upon pre-clinical mouse models we propose to study the toxicity and efficacy of adding sirolimus to oral methotrexate in relapsed and refractory patients.	
Study Objective(s)	Primary Objectives	• Determine the efficacy of oral sirolimus when given in combination with methotrexate in children with refractory/relapsed ALL or NHL. • Determine the safety and tolerability of oral sirolimus given in combination with methotrexate.
	Secondary Objectives	• To characterize the trough levels produced by administration of oral sirolimus in children with refractory/relapsed lymphoblastic leukemia or lymphoma. • To evaluate the effect of sirolimus on intracellular targets, including ribosomal protein s6 (a marker of mTOR inhibition), AKT, P27kip1, DHFR, cyclin D1, Rb, and STAT5 in peripheral blood mononuclear cells, peripheral blood lymphoblasts, and bone marrow lymphoblasts.

Test Article(s) (if applicable)	Sirolimus
Study Design	Phase 2 study looking at efficacy and toxicity of oral sirolimus in combination with methotrexate.
Subject Population Key criteria for inclusion and exclusion:	Inclusion Criteria: • Patients ≤ 25 years, at time of enrollment, with second or greater relapse of ALL or NHL. • Patients with lymphoblastic lymphoma or peripheral T-cell lymphoma must have radiologic or physical evidence of recurrence. Standard exclusion criteria for oncology protocols will be used.
Number of Subjects/Sites	9 to 17 enrollees primarily at CHOP, possible extension to other sites.
Study Duration	Total study duration is expected to be 2-2.5 years.
Study Phases	Subject participation will be for a 28 day cycle. They can be enrolled for subsequent cycles as long as they maintain stable disease or better. Follow-up for 30 days once therapy is stopped or upon enrollment on another study.
Efficacy Evaluations	The day 28 bone marrow in the initial cycle will determine response. If a patient has stable disease or better, at day 28 of cycle 1, they may continue to receive subsequent cycles as long as they maintain a stable disease or better. The fall in absolute peripheral blasts count will also be monitored.
Pharmacokinetic Evaluations	Trough levels of sirolimus.
Safety Evaluations	SAE's as delineated in the protocol will be reported to the IRB.
Statistical and Analytic Plan	This study will be conducted using a two staged Simon design.
Data and Safety Monitoring Plan	An independent medical monitor will review SAE's and response data at least twice yearly with the study team.

Table 1: Schedule of Clinical and Laboratory Studies

All entry/eligibility studies must be performed within 1 week prior to entry onto the trial (unless otherwise specified). Imaging studies are required within 1 month prior to study entry.

STUDIES TO BE OBTAINED	Pre-Study	Cycle 1	Subsequent Cycles	Off Study
Informed Consent/Assent	X			
Demographics/Medical History	X			
Physical Exam (Ht, Wt, BSA, VS)	X	Weekly	Once a cycle	X
Performance Status	X	Once a cycle	Once a cycle	X
CBC, differential, platelets[1]	X	Weekly	Once a cycle	X
Review Eligibility Criteria	X			
TROUGH sirolimus Level[2]		Day 5-8, then weekly	Day 1 of a cycle, or with any addition of a CYP3A4 agonist	
Prior/Concomitant Medications	X	X	X	
Urinalysis	X			
Electrolytes including Ca++, PO_4, Mg++, Cr, ALT, AST, Total bilirubin[3]	X	1-3 X per week	Once a cycle	X
Cholesterol/Triglyceride levels	X	X	Once a cycle	X
Pregnancy Test[4]	X			
Tumor Disease Evaluation	X	D 28	End of every other cycle	X
Bone Marrow Aspirate or Biopsy (Response)[5]	X	D 28	X	X
Spinal Tap[5]	X			
Echocardiogram[6]	X			
Bone Marrow Biology Studies[7]	X	Day 28	X	X
Peripheral Blood Biology Studies[7]	X	D 1, 8, 28	X	X
Oral Drug Diary		Once a cycle	Once a cycle	
Adverse Event/Serious Adverse Event Assessment		X	X	X

OBTAIN OTHER STUDIES AS NEEDED FOR GOOD PATIENT CARE

[1] These are the minimum labs required for the study. CBCs and Chemistries should be obtained as frequently as dictated by good medical care.

[2] See Section 4.10 for timing of sirolimus levels. Trough levels will also be checked 5-8 days after any dose adjustments are made.

[3] Tumor lysis labs (electrolytes, BUN, Cr, Ca, PO_4, and uric acid) should be obtained two times in the first week (Section 4.7.2). If there is no evidence of tumor lysis, obtain chemistries as outlined above. On subsequent cycle chemistries should be obtained at least once per cycle but more as dictated by good patient care.

[4] Patients of childbearing potential require a negative pregnancy test prior to starting treatment and per institutional standards.

[5] Response will be evaluated at day 28 of cycle 1 and then at the end of every 28 day cycle until a CR/CRp is obtained. (Section 5.4) If a patient obtains a CR or CRp on the cycle 1, day 28 bone marrow, bone marrows only need to be performed if there is suspicion/evidence of relapse. The on-study bone marrow and LP may be up to 14 days prior to enrollment.

[6] On study echocardiogram needs to be obtained within 6 months of study initiation.

[7] Section 5.6 for timing of biology studies

BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Children with acute leukemia, the most common pediatric malignancy, have benefited greatly from dramatic advances in therapy over the past two decades. Stepwise improvements in treatment have led to five-year event free survival rates approaching, and in some cases exceeding, eighty percent [1-4]. However, one in five children will develop recurrent disease despite strategies of risk stratification and modern intensified chemotherapy, and their prognosis is poor [5, 6]. At present children who have bone marrow or combined bone marrow and extramedullary relapses while on therapy have a 5-20% of long term survival [5]. For children whose disease relapses off therapy the prognosis is marginally improved with a 20-50% rate of long-term survival [5]. In addition, as therapy for initial disease is intensified, there is concern that recurrent disease may be more difficult to treat, and be more refractory to traditional chemotherapy. The number of children with relapsed acute leukemia equals or exceeds the incidence of most other pediatric tumors [7, 8]. Only newly diagnosed cases of ALL, astrocytoma and neuroblastoma exceed the number of patients with recurrent acute leukemia.

The primary modality of treatment for childhood NHL is intensive multiagent chemotherapy. The outcome in childhood NHL has improved significantly over the past two decades, especially in those with disseminated disease (Stage III/IV Murphy classification and/or Group B/C – FAB Classification) (80-90% 2 year EFS) [9-13]. Unfortunately, the long-term survival rate in children with relapsed NHL is dismal, with less than 20% achieving long term survival [11, 14]. New treatment modalities are additionally required to improve the reinduction rate and durability of response in children with relapsed NHL.

Thus, effective therapy for relapsed acute leukemia has emerged as one of the top therapeutic priorities for pediatric oncologists. Newer, targeted agents need to be identified and integrated into the present cytotoxic chemotherapy regimens. Biologically targeted cancer agents, including signal transduction inhibitors like mammalian target of rapamycin inhibitors (MTIs), have shown great promise in treating hematologic malignancies [15-17]. While signal transduction inhibitors may be efficacious as single agents, it is more likely that these targeted agents in will demonstrate greater efficacy in combination with other cytotoxic agents.

1.2 Name and Description of Investigational Product or Description of Intervention

Sirolimus was initially developed as an immunosuppressive agent because it inhibits T-lymphocyte proliferation that occurs in response to antigenic and cytokine stimulation. In vivo, sirolimus binds to other proteins to generate an immunosuppressive complex that binds to and inhibits the activation of the mammalian target of sirolimus (mTOR). The inhibition of mTOR's protein kinase activity inhibits a variety of signal transduction pathways including production of proteins that regulate the cell cycle. Sirolimus inhibits the progression of

lymphocytes from the G1-to-S phase. This may provide synergy with other cytotoxic agents which inhibit activity at alternative points in the cell cycle. Preclinical data show that sirolimus inhibits the growth of B-precursor ALL lines in vitro and has activity in a murine model of leukemia/lymphoma. A Phase 1 study of sirolimus alone has shown evidence of stable disease, but use with combined therapy needs to be studied.

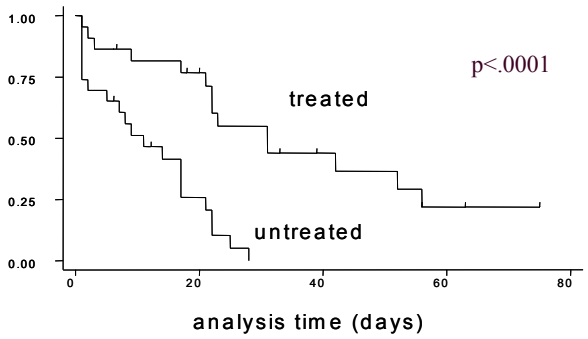
1.3 Findings from Non-Clinical and Clinical Studies

1.3.1 Pre-Clinical data on mTOR Inhibitors (MTI)

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase involved in signal transduction pathways which regulate cell growth and proliferation [16, 18]. 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 [16, 18]). While mTOR itself has not been found to be mutated directly, constitutive activation of signal transduction proteins related to the mTOR pathway (AKT, S6K1, PI3-K) or loss of proteins linked to mTOR (PTEN) are found in numerous malignancies [18, 19]. Studies have shown that MTIs inhibit growth of B-precursor ALL lines in vitro, inducing apoptosis in both primary cell cultures [15] and NOD/SCID xenograft models [17]. Testing of the mTOR inhibitor sirolimus by the Pediatric Preclinical Testing Program 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% [20]. In vivo rapamycin induced significant EFS differences in 5 of 8 ALL xenografts, including 2 of 2 T-cell ALL xenografts [20]. Sirolimus has also been found to reverse glucocorticoid resistance in resistant lymphoid cell lines [21]. Present MTI's under study include sirolimus, temsirolimus, everolimus, and deforolimus. The MTI sirolimus was chosen for use in this study as it is already FDA approved in children, comes in both a liquid and tablet oral formulation, and has readily available trough levels at all Children's Hospitals.

Work at CHOP using sirolimus in precursor B cell malignancies and both human and murine pre-B ALL cell lines have shown both inhibition of growth in culture as well as induction of apoptosis in these cells. The active drug concentration in these studies was well below the achievable serum level in patients. Furthermore, sirolimus is active as a single agent in a murine model of B-precursor leukemia/ lymphoma. Eμ-Ret transgenic mice are a model of precursor B cell malignancy. These mice develop B-precursor malignancies between 4 and 7 months of life as a

Single-agent rapamycin extends mean survival in leukemic mice from 10 to 30 days



result of the activated tyrosine kinase expressed in the B lineage. In these experiments, leukemic mice with significant disease burdens were treated with sirolimus. When compared to untreated littermates, sirolimus-treated mice survived almost 3 fold longer (see Kaplan-Meier analysis of survival in treated and untreated mice in Figure above). In addition to extending survival, sirolimus also normalized the significantly elevated peripheral white blood cell counts in treated mice. There was no statistically significant difference between treated and untreated mice with respect to the hemoglobin or platelet counts.

1.3.2 Pre-clinical data on temsirolimus combined with chemotherapy

In preclinical studies, ALL cells lines treated with the temsirolimus or sirolimus showed synergism as determined by Chou and Talalay mathematical synergy models with methotrexate, dexamethasone, L-asparaginase, etoposide and doxorubicin [22] (Figure 1). ALL cell lines treated with vincristine or cytarabine showed neither additive benefit nor antagonism. The combination of methotrexate and either sirolimus or temsirolimus was tested in 9 ALL cell lines (pre-B and T) and was synergistic in all cell lines (Combination Indices <1). NOD/SCID mice xenografted with primary human ALL were treated with either placebo, weekly MTX alone (5 -10 mg/kg), weekly temsirolimus alone (20mg/kg), temsirolimus 5 days/wk (5mg/kg/dose) or a combination of the two using both temsirolimus doses [22]. Kaplan-Meyer analysis of time to progression demonstrated a statistically significant difference, comparing all treatment arms to control ($p < 0.01$), and comparing the combination of drugs to methotrexate only and MTI.

Figure 1.

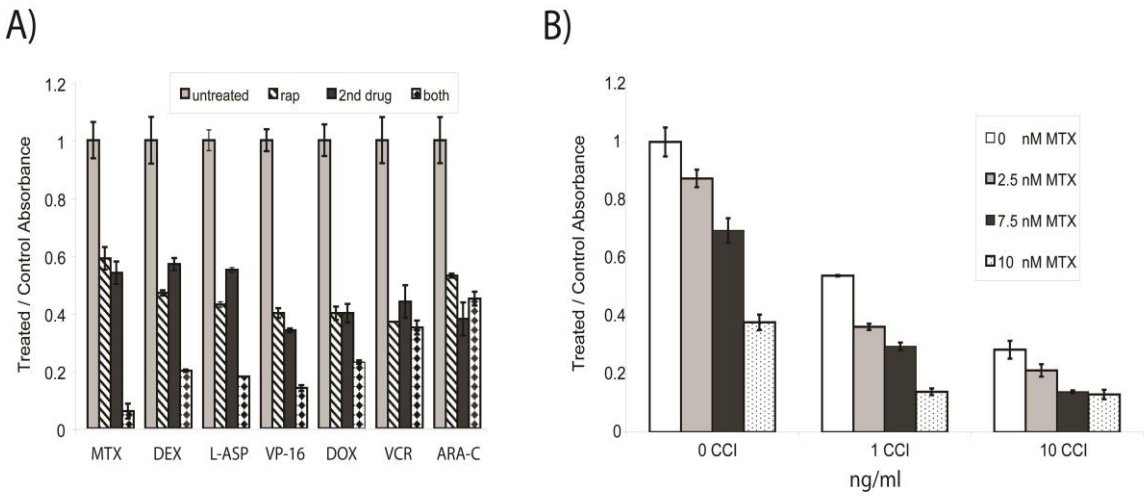


Figure 1. MTIs can be effectively combined with chemotherapy. **Aliquots of 2 x 10^4 cells from 3 ALL lines were treated with sirolimus (rap) and 7 chemotherapeutics. Figure 1a depicts MTT data for each drug combination in 1 representative cell line. Grey bars represent untreated, slashed bars represent MTI alone, black bars represent cytotoxic alone, and dotted bars represent combined effect. All data is normalized to untreated baseline (=1) with a value >1 representing relative cell proliferation and <1 inhibition. Each group of 4 bars represents a combination with a different cytotoxic agent. MTIs had at least an additive effect when combined with methotrexate (MTX), dexamethasone (DEX), L-asparaginase (L-ASP), etoposide (VP-16), and doxorubicin (DOX). The combination of MTIs with vincristine (VCR) and Ara-C (cytarabine) did not add a benefit over either single agent alone. Doses depicted in figure: sirolimus (0.3ng/ml), MTX (5nM), DEX (5uM), L-ASP (1ug/ul), VP-16 (1nM), DOX (1nM), VCR (1nM), ARA-C (0.1ug/ml). Next, aliquots of cells from 6 ALL lines were treated with methotrexate and 2 MTIs (temsirolimus (CCI) and sirolimus). Figure 1b depicts MTT data for temsirolimus and methotrexate in 1 cell line (289), demonstrating a synergistic effect at multiple drug doses. The other cell lines tested showed similar results.**

Figure 2. A majority of mice treated with MTX + temsirolimus were cured of their disease, while only short-term disease control was seen with either drug alone.

Figure 2.

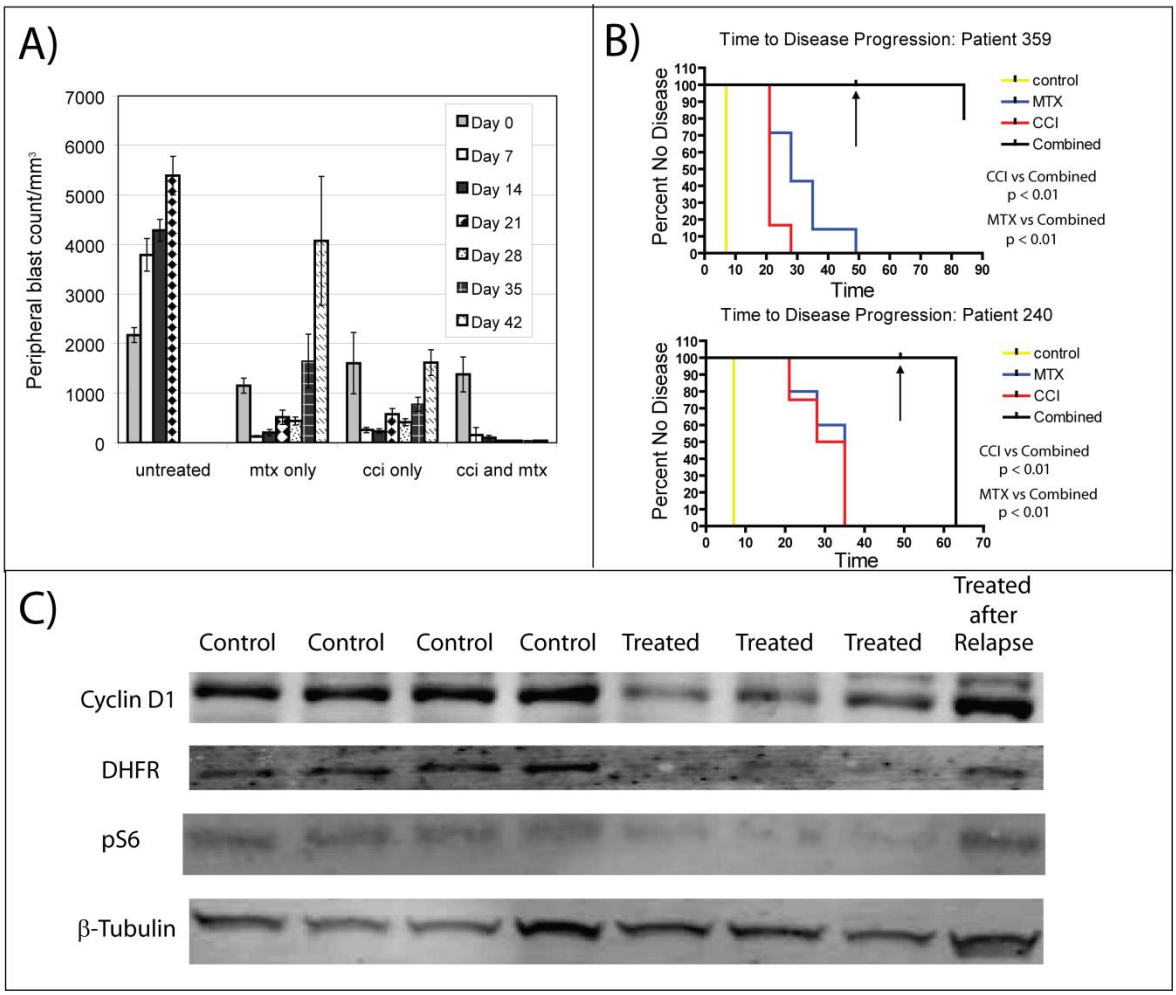


Figure 2. Temsirolimus and methotrexate are synergistic and produce durable remission. NOD/SCID mice were xenografted with human ALL from patient samples. After establishment of ALL, mice were randomized to treatment with vehicle, temsirolimus (CCI), methotrexate, or a combination of temsirolimus and methotrexate. Temsirolimus was dosed with two schedules, 5mg/kg 5 days a week and 20mg/kg weekly. Disease was evaluated at weekly intervals by FACS of peripheral blood for anti-human CD19 and anti-human CD45. Figure 2A depicts comparison of arms from xenografts generated from sample 359 showing weekly changes in blast count (WBC in mm3 x % blasts). As similar results were found for both dosing schedules only the 20mg/kg weekly dosing is depicted in figure 2A. Each series of vertical bars represents average blast count in animals for a particular treated arm at a given timepoint. Control animals died after 3 weeks. Mice treated with temsirolimus or methotrexate alone had improvement in disease but eventual progression. Mice treated with both drugs had complete resolution of peripheral blasts by Day 21. After 49 days (depicted by black arrow in figure 2B) all drugs were stopped. One half of the mice were sacrificed and no mouse receiving combination therapy had measurable disease. The remaining mice were followed for 2 months and sacrificed. Figure 2B depicts time to progression on different arms by Kaplan-Meier analysis from sample 359 (top) and 240 (bottom). Figure 2C depicts immunoblots of splenocytes from sample 240 mice treated with temsirolimus ("Treated") or vehicle control ("Control") for varying lengths of time, showing decreased cyclin D1 (top row), DHFR (second row), and phospho-S6 (pS6) (third row), comparing treated to control animals. In addition, mice that were treated with temsirolimus until relapse ("Treated after Relapse") had increased expression of cyclin D1 after relapse. Depicted 240 samples in 2C from left to right: Control 7 days, Control 14 days, Control 21 days, Control 30 days, Treated 7 days, Treated 14 days, Treated 21 days, and Treated 30 days.

MTIs have been shown to prevent activation and increase degradation of cyclin-dependent kinases, including cyclin D1 [23]. Cyclin D1 is involved in Rb phosphorylation and release E2Fs which are involved in dihydrofolate reductase (DHFR) synthesis [24-26]. Resistance to methotrexate has been shown in tumors that have high DHFR expression [27, 28]. MTIs were demonstrated to increase the sensitivity of ALL to methotrexate by decreasing cyclin D1, which in turn decreased DHFR (Figure 2C).

1.3.3 Adult Neoplastic Studies

In a Phase I trial of single agent temsirolimus in adults with solid tumors doses ranged from 7.5 to 220mg/m2, with no MTD identified [29]. At the 34mg/m2 and 45mg/m2 doses one patient in each dose level had Grade 3 dose limiting toxicities (DLT) of thrombocytopenia; one also neutropenia and hypophosphatemia; the other had asthenia and diarrhea. Dose escalation continued to 220mg/m2 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 [29].

A variety of MTI's have been studied in combination with other therapies; including interferon in renal cell carcinoma [30], hormonal agents in breast cancer [31], 5-FU/LCV [32], imatinib in gastrointestinal stromal tumors, gemcitabine, and sorafanib in adult tumors. Early data on MTI/chemotherapy combinations may have 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 IV mucositis/stomatitis was observed at the 45mg/m2 level [32]. Grade 3 toxicities noted in adult trials with temsirolimus (solo and in combination) have been rash, nausea/vomiting, cytopenias, hypophosphatemia, hypokalemia, hyperglycemia, asthenia/neuropathy, fatigue, mucositis, hypercholesteremia / hypertriglyceridemia, and dyspnea [29-34].

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 (8mg/m2/d), cytarabine (1gm/m2/d) and etoposide (100mg/m2/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 15mg load and 5mg daily. In the prior 4 dose levels the median time to ANC >500 was 27 days (range 16-38). Overall sirolimus was well tolerated and did not increase non-hematologic toxicity of the backbone chemotherapy [35].

1.3.4 Pediatric Neoplastic Studies

To date 9 children have been enrolled in a sirolimus only Phase 1 study at CHOP (CHP-755). Four of 7 patients on Dose level 1(9mg/m^2 load, 3mg/m^2 daily) were evaluable for toxicity and none had a DLT. One of 2 patients on Dose level 2 (12mg/m2 load, 4mg/m2 daily) have been evaluable for toxicity. There have been no DLT's to date on any cycle. Average trough levels for the 3mg/m^2 daily dose level on day 7 and 22 were 10.9 and 8.5. At the 4mg/m^2 daily level (2 pts) the trough at day 7 and 22 were 16.1 and 7.6

A Phase I trial of single agent once weekly IV temsirolimus in children with advanced solid tumors had an MTD of 75mg/m^2. The DLT at the 150mg/m^2 dose was grade 3 anorexia (1 pt) and grade 4 thrombocytopenia < 7 days (1 pt). Seven pts had ≥ grade 3 treatment-related adverse events: neutropenia, leukopenia, anemia, anorexia, thrombocytopenia, and increased ALT. C_{max} was comparable to adult pts; 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. 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 [34]. Inhibition was also seen at the 10 and 25mg/m^2 dose levels (personal communication, S. Grupp).

1.3.5 Combination therapy of MTIs and methotrexate.

A combination of the mTOR inhibitor everolimus and methotrexate was studied in a randomized placebo-controlled trial in patients with rheumatoid arthritis. One-hundred twenty-one patients were randomized to receive everolimus and methotrexate (mean methotrexate dose 17.5mg) or methotrexate and placebo. The combination was found to be effective and well tolerated. Only 2 SAEs were reported in the combination therapy arm (1 patient developed epiglottis and obstructive airway disorder and the second developed cardiac failure). One patient on the combination arm died of a myocardial infarction. The SAEs and death were not thought to be caused by the combination of agents. Mucositis was rare, transient, and low grade (I-II) (9 patients in combination arm and 2 in methotrexate alone). [36]

1.4 Selection of Drugs and Dosages

1.4.1 Sirolimus - The dose of sirolimus is based upon the approved daily dose used for pediatric solid organ transplant patients (9 mg/m^2 bolus day 1 and 3 mg/m^2/day) and the lack of toxicity seen in our single agent Phase 1 trial at similar dose levels. Dose will then be altered to maintain a sirolimus trough level between ≥ 8 and ≤ 13.

1.4.2 Methotrexate - The starting dose of oral methotrexate is the once weekly dose of methotrexate used in children with ALL during their maintenance therapy on present day COG trials. [37]

1.5 Compliance Statement

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 22 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report adverse events in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

1.6 Pharmacokinetics of Sirolimus

The pharmacokinetics of sirolimus has been studied in healthy subjects, pediatric dialysis patients, hepatically-impaired adult patients, and adult renal transplant patients [38, 39]. Oral doses of both liquid and solid sirolimus are rapidly, though variably, absorbed. Mean time-to-peak concentrations range from 1 hour in healthy subjects to 2 hours in renal transplant recipients. Half-life is upwards of 2½ days. Metabolism is by the intestinal and hepatic CYP3A4 enzyme family and 91% of the elimination of the drug is via the GI tract. The AUC correlates well with trough and peak concentrations. Patients who ingested the drug after a high fat breakfast did have delayed C_{max} and it is recommended to consistently take sirolimus with or without food.

In a Phase I pharmacokinetic study conducted in renal transplant patients doses ranging from 0.5 to 6.5 mg/m^2 were administered every 12 hours [38]. Phase III studies to date have had concomitant use of cyclosporine, steroid, or both. At a dose of 2 mg/day the sirolimus trough concentration was 8.58 +/- 4.0ng/ml and at 5mg/day the trough was 17.3 +/- 7.4 ng/ml. Sirolimus concentrations in stable renal transplant patients are dose proportional between 3 and 12 mg/m^2. Also, in this population a loading dose of 3 times the maintenance dose provided near steady-state concentrations within 1 day in most patients. Stable renal transplant recipients have received single doses of up to 22mg/m^2. No toxicity has been observed in any of several single dosing studies with sirolimus doses ranging from 3-22 mg/m^2.

In pediatric dialysis patients, young patients 5-11 years exhibited greater oral clearances and shorter half-lives. This suggests that they may need higher doses per body weight or surface area than adults. Patients 12 to 18 years had similar PK parameters as adult renal transplant patients. The variability of bioavailability in the pediatric group may be overcome by obtaining trough serum levels, which are readily available at CHOP.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- To determine the efficacy of oral sirolimus when given in combination with oral methotrexate in children with refractory/relapsed lymphoblastic leukemia or lymphoma.

- To determine the safety and tolerability of oral sirolimus when given with oral methotrexate.

2.2 Secondary Objectives

- To characterize the trough levels produced by administration of oral sirolimus in children with refractory/relapsed lymphoblastic leukemia or lymphoma.

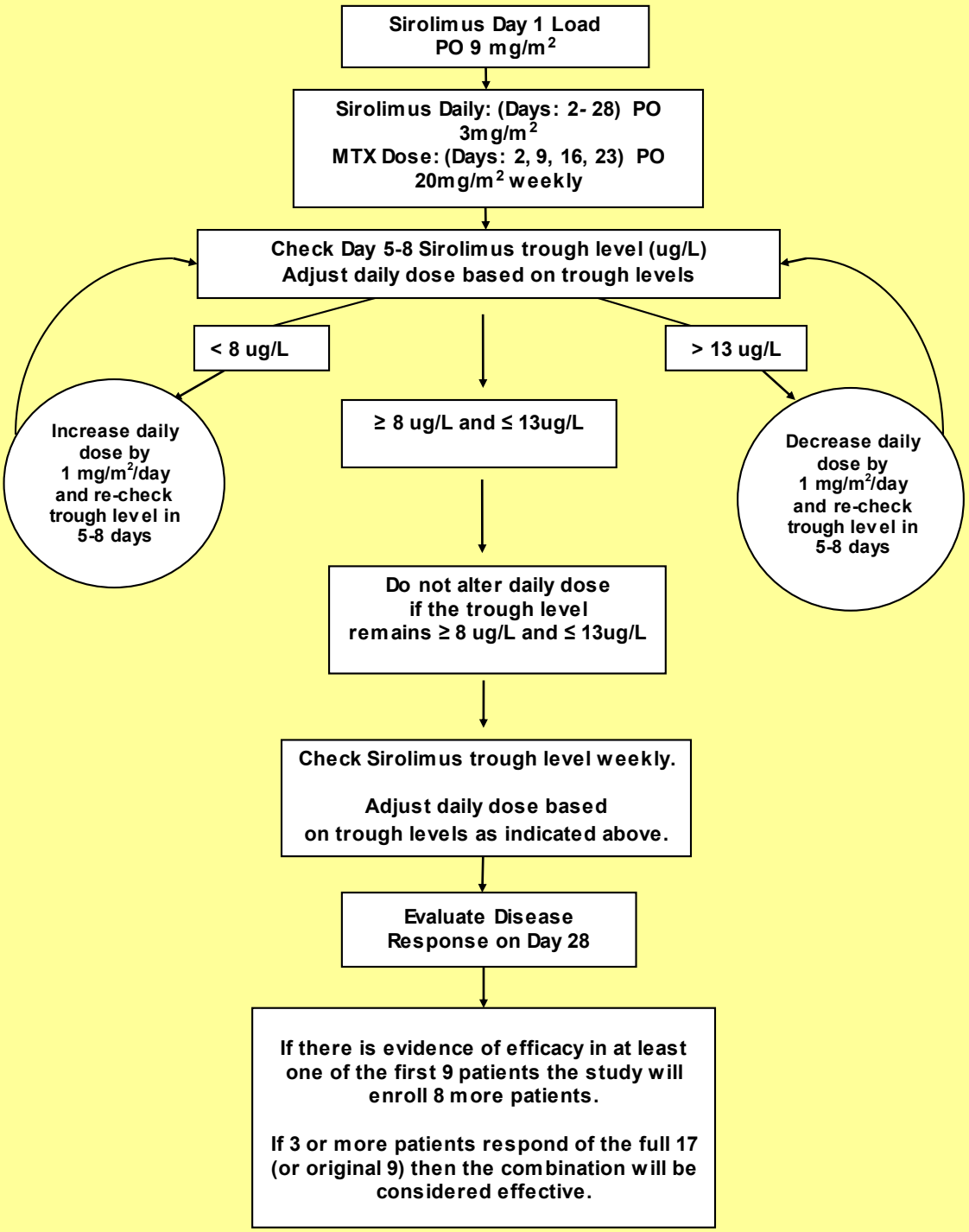
- To evaluate the effect of sirolimus on intracellular targets, including ribosomal protein s6 (a marker of mTOR inhibition), AKT, P27kip1, cyclin D1, DHFR, Rb, and STAT5 in peripheral blood mononuclear cells, peripheral blood lymphoblasts, and bone marrow lymphoblasts.

3 INVESTIGATIONAL PLAN

This is a Phase 2 study of a daily regimen of oral sirolimus combined with once weekly oral methotrexate in patients with relapsed and refractory ALL and NHL. Patients will receive a loading dose of sirolimus followed by daily dosing through day 28. This will be combined with oral methotrexate weekly starting on day 2.

To evaluate the primary aim of this study, the efficacy of sirolimus and methotrexate, when given in combination, patients will have disease evaluations performed after their initial 28 day cycle and then every other cycle of therapy. Disease response will be assessed according to Section 5.4. If there is evidence of efficacy in at least one of the first 9 patients the study will enroll 8 more patients. If 3 or more patients respond of the full 17 (or original 9) then the combination will be considered effective.

3.1 General Schema CHP-948 Study Design



3.1.1

3.1.1 Screening Phase

Potential subjects will be identified through weekly Oncology patient meetings, clinicians and the study coordinator. Physicians may also contact the PI directly if they feel they have an appropriate patient. The subjects will be screened using the protocol inclusion and exclusion criteria. If they are found to be eligible, the CRA will provide the appropriate consent forms and study worksheets to the consenting physician. The attending on service or the primary oncologist will approach the patient/family about the study.

Parental/guardian permission (informed consent) and, if applicable, child assent, will be obtained prior to any study related procedures being performed. Bone marrow samples drawn as SOC for determining relapse will be requested for biology testing once the family consents to the study. Blood samples will be drawn to confirm eligibility based on clinical laboratory parameters.

3.1.2 Treatment Phase

Once eligibility criteria are met the patient will receive the loading dose of oral sirolimus (9mg/m^2) on day 1, followed by daily sirolimus at 3mg/m^2/day. The dose of sirolimus will then be altered to maintain a sirolimus trough level between 8 –13. If the trough is < 8, the daily oral dose will be escalated by 1mg/m^2/day with a repeat trough checked 5-8 days later. If the trough is >13, the daily oral dose will be de-escalated by 1mg/m^2/day. Once weekly oral methotrexate at 20mg/m^2 will be started on day 2 and continued weekly.

To evaluate the primary aim of this study, the efficacy of sirolimus and methotrexate given in combination, patients will have disease evaluations performed after their initial 28 day cycle and then every 28 days. Disease response will be assessed according to Section 5.4. If there is evidence of objective response in at least one of the first 9 patients the study will enroll 8 more patients. If 3 or more patients respond of the full 17 (or original 9) then the combination will be considered effective.

3.1.3 Follow-up Phase

To be eligible for the follow-up phase, subjects must have been enrolled on study and received at least one dose of medication. The follow-up phase will continue for up to 30 days after the last day medication is received or until enrollment on another study, whichever comes first.

3.2 Randomization and Blinding

None

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study

Historically 4-8 patients with relapsed ALL enroll in Phase 1 or 2 trials at CHOP each year. Based upon toxicity and dose levels the study is projected to run 2-3 years. NHL patients will also be eligible and patients can be referred from other

sites for enrollment, possibly decreasing the maximum duration of the study to < 2 years.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will initially open only a CHOP, but may open subsequently at several pediatric oncology centers. The maximum number of evaluable subjects enrolled will be 17.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Males or females ≤ 25 years at the time of study entry.

2) Girls who have experienced menarche must have a negative urine/serum pregnancy test and must use an acceptable method of contraception, including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive, for the duration of the study.

3) Parental/guardian permission (informed consent) and if appropriate, child assent.

4) Histologic Diagnosis

   Patients must have documented recurrent or refractory acute lymphoblastic leukemia (ALL) with ≥ 10 % blasts in the marrow. Patients with recurrent or refractory lymphoblastic lymphoma or peripheral T-cell lymphoma must have radiologic or physical evidence of recurrence measurable by RECIST criteria.

5) Patient must have experienced their second or greater relapse.

6) Patient must have a disease for which there is no known curative therapy.

7) Karnofsky ≥ 50% for patients >10 years of age and Lansky ≥ 50 for children ≤ 10 years of age (Appendix V).

8) Patient must be able to consume oral medication in the form of tablets or solution.

3.4.2 Organ Function Requirements

3.4.2.1 Bone Marrow Function Defined As:

- Patients with ALL and NHL patients with tumor metastatic to bone marrow, who have granulocytopenia, anemia, and/or thrombocytopenia are eligible, but will not be evaluable for hematological toxicity.

3.4.2.2 Adequate Renal Function Defined As:

- Creatinine clearance or radioisotope GFR ≥ 70ml/min/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 (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

3.4.2.3 Adequate Liver Function Defined As:

- Total bilirubin ≤ 1.5 x normal for age, and
- SGPT (ALT) ≤ 5 x normal for age and albumin ≥ 2 g/dL.

3.4.2.4 Adequate Cardiac Function within 6 months of enrollment defined as:

- Shortening fraction of ≥ 28% by echocardiogram, or
- Ejection fraction of ≥ 50% by gated radionuclide study.

3.4.2.5 Adequate Pulmonary Function Defined As:

- Pulse ox > 94%

3.4.3 Exclusion Criteria

1) Pregnancy or Breast-Feeding

Pregnancy tests must be obtained in females of childbearing potential. Pregnant or lactating patients are ineligible for this study due to the unknown human fetal or teratogenic toxicities of sirolimus. Males or females of reproductive age may not participate unless they have agreed to use an effective contraceptive method.

2) Patients With An Uncontrolled Infection

Patients must have any active infection under control. Fungal disease must be stable for at least 2 weeks before enrollment. Patients with bacteremia must have a documented negative blood culture prior to initiating drug.

3) Patients Who Do Not Meet Organ Function Requirements per Section 3.4.2.

4) Patients Currently Receiving Other Investigational Anti-Neoplastic Drugs

5) Patients who have a known allergy to sirolimus, FK506, or other mTOR inhibitors are not eligible.

6) Patients who relapsed after receiving sirolimus GVHD prophylaxis post-HSCT.

7) Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

8) Any investigational anti-neoplastic drug use within 14 days prior to enrollment.

9) Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria will be reported in accordance with IRB Policies and Procedures.

10) Prior Therapy

- Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study. Patients must have recovered from the non-hematologic toxic effects of all prior therapy before entry into this trial. Recovery is defined as a toxicity grade < 2 as defined by the Common Terminology Criteria Version 3 (CTC v3) unless otherwise specified in the Inclusion and Exclusion criteria.

- Myelosuppressive chemotherapy: Must not have been received within 14 days of entry onto this study (28 days if prior nitrosourea). Patients may have received hydroxyurea OR corticosteroids if they have had stable or rising peripheral blast counts for three days.

- Biologic (anti-neoplastic agent): At least 14 days since the completion of therapy with a biologic agent.

- XRT: ≥ 2 wks for local palliative XRT (small port); ≥ 4 weeks must have elapsed if prior craniospinal XRT or if ≥ 50% radiation of pelvis; ≥ 4 wks must have elapsed if other substantial BM radiation.

- Bone Marrow Transplant (BMT)/Stem Cell Transplant (SCT): No evidence of active graft vs. host disease. For allogeneic BMT/SCT, ≥ 3 months must have elapsed.

- Concomitant Medications (see also Section 4.7):

    - Hematopoietic growth factor(s): Must not have received within 1 week of entry onto this study except for erythropoietin.

- Steroids: Patients may have received corticosteroids within two weeks of entry if they have had stable or rising peripheral blast counts for three days. If corticosteroids were administered for life threatening superior vena cava syndrome or spinal cord compression, the patient may enroll when medically stable.

- Hydroxyurea: Patients may have received hydroxyurea if they have had stable or rising peripheral blast counts for three days.

4 STUDY PROCEDURES - TREATMENT PROGRAM

4.1 Pre-Study/Screening Visit

Patients must meet the eligibility criteria as indicated on the eligibility/exclusion criteria worksheet. (see Appendix I). All entry/eligibility studies must be performed within 1 week prior to entry onto the trial with the exception of imaging studies and echocardiograms which are required within 1 month prior to study entry and bone marrows/spinal taps required within 14 days.

4.2 Treatment Plan

Sirolimus Loading Dose: PO 9mg/m^2 on Day 1 only.

Sirolimus Daily Dose: PO 3mg/m^2 on Day 2-28.

Day 5-8 check trough level.

If trough level ≥ 8 ug/L and ≤ 13 ug/L, do not alter daily dose and recheck level weekly.

If < 8 ug/L increase daily dose by 1mg/m^2/day and re-check trough level in 5-8 days.

If > 13 ug/L decrease daily dose by 1mg/m^2/day and re-check trough level in 5-8 days.

Continue to modify daily sirolimus daily dose to keep trough levels ≥ 8 ug/L and ≤ 13 ug/L. See Section 5.5.1 for information on how to obtain a trough level.

Methotrexate: PO 20 mg/m^2/week on Days 2, 9, 16, 23.

4.3 Definition of cycle

One cycle is described as 28 days of therapy. Subsequent cycles will start on day 28 of the prior cycle. There is no limit to the number of cycles a patient may receive as long as the patient has at least stable disease.

4.4 REQUIRED OBSERVATIONS/MATERIAL AND DATA TO BE ACCESSIONED DURING STUDY

STUDIES TO BE OBTAINED	Pre-Study	Cycle 1	Subsequent Cycles	Off Study
Informed Consent/Assent	X			
Demographics/Medical History	X			
Physical Exam (Ht, Wt, BSA, VS)	X	Weekly	Once a cycle	X
Performance Status	X	Once a cycle	Once a cycle	X
CBC, differential, platelets[1]	X	Weekly	Once a cycle	X
Review Eligibility Criteria	X			
TROUGH sirolimus Level[2]		Day 5-8, then weekly	Day 1 of a cycle, or with any addition of a CYP3A4 agonist	
Prior/Concomitant Medications	X	X	X	
Urinalysis	X			
Electrolytes including Ca++, PO_4, Mg++, Cr, ALT, AST, Total bilirubin[3]	X	1-3 X per week	Once a cycle	X
Cholesterol/Triglyceride levels	X	X	Once a cycle	X
Pregnancy Test[4]	X			
Tumor Disease Evaluation	X	D 28	End of every other cycle	X
Bone Marrow Aspirate or Biopsy (Response)[5]	X	D 28	X	X
Spinal Tap[5]	X			
Echocardiogram[6]	X			
Bone Marrow Biology Studies[7]	X	Day 28	X	X
Peripheral Blood Biology Studies[7]	X	D 1, 8, 28	X	X
Oral Drug Diary		Once a cycle	Once a cycle	
Adverse Event/Serious Adverse Event Assessment		X	X	X

OBTAIN OTHER STUDIES AS NEEDED FOR GOOD PATIENT CARE

[1] These are the minimum labs required for the study. CBCs and Chemistries should be obtained as frequently as dictated by good medical care.

[2] See Section 4.10 for timing of sirolimus levels. Trough levels will be checked 5-8 days after any does adjustments.

[3] Tumor lysis labs (electrolytes, BUN, Cr, Ca, PO_4, and uric acid) should be obtained two times in the first week (Section 4.7.2). If there is no evidence of tumor lysis, obtain chemistries as outlined above. On subsequent cycle chemistries should be obtained at least once per cycle but more as dictated by good patient care.

[4] Patients of childbearing potential require a negative pregnancy test prior to starting treatment and per institutional standards.

[5] Response will be evaluated at day 28 of cycle 1 and then at the end of every 28 day cycle until a CR/CRp is obtained. (Section 5.4) If a patient obtains a CR or CRp on the cycle 1, day 28 bone marrow, bone marrows only need to be performed if there is suspicion/evidence of relapse. The on-study bone marrow and LP may be up to 14 days prior to enrollment. Patients with lymphoma and no bone marrow involvement at diagnosis do not need repeat bone marrows.

[6] Echocardiogram needs to be obtained within 6 months of study initiation.

[7] Section 5.6 for timing of biology studies

4.5 Changes to the study protocol

It is acceptable to have a 3 day window for required specimens/tests if the scheduled study day falls on a weekend or holiday or when schedule changes are necessary for patient care.

4.6 Study visits for all cohorts - Cycle 1 and subsequent cycles

4.6.1 Planned follow-up Visits will be scheduled according to the roadmap.

4.6.2 Unscheduled Visits - the oncology outpatient clinic sees all sick patients Monday- Friday from 8am to 4pm. After those hours and on weekends a child is referred to the CHOP Emergency Department and the case is reviewed with the oncologist-on-call.

4.7 Supportive Care/Concomitant Therapy

All prior and concomitant medications used within 7 days prior to the drug initiation visit and through the end of the study will be recorded. Use of hydroxyurea or corticosteroids will be documented up to 30 days prior to drug initiation. The dates of administration, dosage, and reason for use will be included.

Concomitant medications will be recorded for a period of 30 days after patients are no longer on study or until they are enrolled on another study.

4.7.1 General

Medically indicated use of antibiotics, anti-fungals, blood products, antiemetics, and general supportive care should be used as indicated.

4.7.2 Tumor Lysis Syndrome

It is recommended, but not mandated, that all subjects start allopurinol 150mg/m^2 divided TID or urate oxidase (0.1mg/kg IV) prior to the first dose of sirolimus. 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 4.4. 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.

4.7.3 Growth Factor

Routine prophylactic use of sargramostim (GM-CSF), filgrastim (G-CSF), or peg-filagrastim is not permitted. Therapeutic use in patients with serious neutropenic complications such as sepsis syndrome, fungal infection, etc., may be considered at the investigator's discretion. Sirolimus and methotrexate should be held if growth factor is required.

4.7.4 Hypercholesteremia/Hypertriglyceridemia

If cholesterol and /or triglyceride levels are elevated in patients who have completed at least 4 cycles of therapy, and remain in a PR, CRp, or CR, the patient may be started on HMG-CoA inhibitors at the discretion of the treating physician.

4.7.5 Interactions (see Appendix IV)

Medications which may interact with the study drug (CYP3A4 modulators) are not allowed. Ketoconazole, tacrolimus, cyclosporine, Rifampin, and diltiazem may not be used while the patient is taking sirolimus. Fluconazole and voriconazole may be used at standard doses, but a sirolimus trough level must be checked within 5-7 days of initiation

4.7.6 PCP Prophylaxis

Patients should remain on PCP prophylaxis while on study.

4.8 Extramedullary Disease

Patients with CNS and bone marrow involvement of ALL, or CNS involvement of NHL, may receive concomitant weekly intrathecal chemotherapy with IT cytarabine and IT hydrocortisone starting at day 8. If intrathecal chemotherapy was given with the on-study LP or as part of routine relapse therapy when the relapse was discovered the child remains eligible for enrollment. Radiation therapy should not be administered, except for emergent situations or persistent extramedullary disease with resolution of bone marrow disease (until the patient is in a CRp or CR).

4.9 Dose Modifications

The daily dose of sirolimus will be modified to maintain a sirolimus trough level ≥ 8 and ≤ 13.

Sirolimus Daily Dose: PO 3mg/m^2 on Day 2-28.

Day 5-8: Check trough level.

If trough level ≥ 8 ug/L and ≤ 13 ug/L, do not alter daily dose and recheck level weekly.

If < 8 ug/L increase daily dose by 1mg/m^2/day and re-check trough level in 5-8 days.

If > 13 ug/L decrease daily dose by 1mg/m^2/day and re-check trough level in 5-8 days.

Continue to modify daily sirolimus daily dose to keep trough levels ≥ 8 ug/L and ≤ 13 ug/L. See Section 5.5.1 for information on how to obtain a trough level.

Methotrexate: PO 20 mg/m^2/week on Days 2, 9, 16, 23.

4.9.1 Myelosuppression: If a patient with NHL non-metastatic to the marrow, or a patient with acute leukemia who obtains a complete remission (CR) has Grade IV neutropenia, Grade IV anemia, or Grade IV thrombocytopenia, oral sirolimus and methotrexate should be held until count recovery occurs. All drugs should be re-initiated at 50% dosing, and escalated by 25% increments weekly for ANC > 1,000 and platelet count > 100. If cytopenia persists > 14 days a bone marrow aspirate should be performed to evaluate for BM involvement of disease. If the cytopenia resolves and then re-occurs, and the patient has reached a CR, drugs can be held again and dose reduced by another 25%.

4.9.2 Aplastic marrow: If at day 28 of the first cycle a patient has an aplastic marrow (spicules present) with no evidence of leukemic blasts, a second cycle may be initiated. If at the end of 2 cycles the marrow remains aplastic all sirolimus and methotrexate should be held until marrow recovery occurs, or disease become evident. If recovery occurs myelosuppressive therapy (methotrexate, sirolimus) may be re-initiated with a 50% dose reduction.

4.9.3 Organ system toxicity: Drug-related irreversible (within 4 weeks of drug administration) Grade 3 or 4 renal, hepatic, cardiac, central nervous system toxicity that persists > 14 days after cessation of sirolimus and methotrexate will result in the patient being off protocol therapy. Sirolimus and methotrexate should be held at the time the toxicity is identified and if resolved within 2 weeks restarted with a 50% dose reduction. See section 4.9.5 for transaminitis or hyperbilirubinemia.

4.9.4 Stomatitis: A sirolimus trough should be obtained when Grade II or greater stomatitis is identified, and drug dosage altered accordingly. Patients with Grade III or greater stomatitis that does not improve in 7 days without intervention, or requires IV hydration or IV pain medication, should have sirolimus and methotrexate held until symptoms improve. Patient may re-initiate drugs with a 50% dose reduction of both sirolimus and methotrexate when the stomatitis resolves to Grade <2. Evaluate for presence of herpes simplex if indicated.

4.9.5 Liver Dysfunction: For increase in hepatic transaminases (SGPT/ALT or SGOT/AST) to greater than 5x ULN consistent with Grade 3 toxicity, obtain Total bilirubin.

Continue full dose therapy unless either of the following occurs:

1) Total bilirubin > 2.0 mg/dL
2) SGPT/ALT or SGOT/AST > 20x ULN (consistent with Grade 4 toxicity) on two determinations at least one week apart.

If either of these occurs, hold MTX and monitor labs as above, weekly. Restart at full dose therapy when the transaminase is less than 5x ULN, and/or Total bilirubin is normal.

4.10 Rescue Medication Administration

Routine prophylactic use of sargramostim (GM-CSF), filgrastim (G-CSF), or peg-filagrastim is not permitted. Therapeutic use in patients with serious neutropenic complications such as sepsis syndrome, fungal infection, etc., may be considered at the investigator's discretion. Sirolimus and methotrexate should be held if growth factor is required.

4.11 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules, AEs, or due to progression of disease. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject is evaluable. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

4.11.1 Early Termination Study Visit

A CBC with differential will be documented at the time of study withdrawl/termination.

5 STUDY ENDPOINTS AND EVALUATIONS

5.1 Primary Endpoints

- Efficacy of combination as determined by having at least 3 CR's in the first 17 evaluable patients enrolled (17% CR rate).
- Safety and tolerability of sirolimus in combination with methotrexate

5.2 Secondary Endpoints

- Sirolimus median dose required to keep trough levels > 8 and ≤ 13.
- Biology (To evaluate the effect of sirolimus on intracellular targets, including p70/S6 kinase (a marker of mTOR inhibition), phosphoAKT, P27kip1, and STAT5 in peripheral blood mononuclear cells, peripheral blood lymphoblasts, and bone marrow lymphoblasts.)

5.3 Screening, Baseline and On-study Evaluations

5.3.1 Physical Examination and current medical history will include:

- History of Present Illness: duration, signs, symptoms
- Constitutional: fever, chills, fatigue, wt. change (↑ or ↓) appetite change, sleep

- ENT: mucositis, dental , swallowing
- Respiratory: cough, pneumonia Cardiovascular: chest pain, SOB
- GI: constipation, diarrhea, abdominal pain, nausea, vomiting
- Musculoskeletal: pain, h/o fracture
- Heme/Lymph: bruising, petechiae, bleeding
- Other: dehydration, electrolyte abnormality. Please explain.
- Infection: source/type
- Performance Status (Karnofsky, Lansky)

5.3.2 Demographic Information collected includes:

- Age at enrollment on study
- Race
- Gender
- Ethnicity
- Diagnosis
- Previous Treatment Protocols (chemotherapy and radiation)
- Baseline toxicity present when enrolled on study.

5.3.3 The following vital signs and anthropometric measurements will be performed as per Nursing Standards at The Children's Hospital of Philadelphia:

- Blood Pressure
- Respiratory Rate
- Heart Rate
- Temperature
- Weight and Length/Height Measurements

5.3.4 Laboratory Evaluations obtained (Please see section 4.4)

5.4 Efficacy Evaluations of disease status – done on Day 28

5.4.1 Response criteria (Leukemia):

- Complete Response (CR) - M1 bone marrow (<5% blasts) with adequate bone marrow cellularity, no evidence of circulating blasts or extramedullary disease and normalization of peripheral blood counts (neutrophil count =1,000/µL and platelets =100,000/ µL).

- Complete Response in the absence of total platelet recovery (CRp) - M1 bone marrow (<5% blasts) with adequate bone marrow cellularity, no evidence of circulating blasts or extramedullary disease and

normalization of peripheral blood counts except for platelets (neutrophil count =1,000/µL, platelets < 100,000uL).

- Partial Response (PR) - M2 bone marrow (5% but <25% blasts), with no evidence of circulating blasts or extramedullary disease and normalization of peripheral blood counts (neutrophil count =1,000/µL and platelets =100,000/µL). A response of PR may not be assigned to a patient that has < 25% blasts (M3) marrow at diagnosis.

- Stable Disease (SD) - Patient meeting neither the requirements for a PR or progressive disease.

- Progressive Disease – An increase of at least 25% in the absolute number of leukemic cells in peripheral blood or bone marrow, the development of extramedullary disease, or other evidence of increased tumor burden.

5.4.2 Response Criteria (for NHL patients)

This study will use the (RECIST) Response Evaluation Criteria in Solid Tumors from the NCI. Patients may be enrolled with evaluable disease but not measurable disease. These patients will not be evaluated for response. Initial disease response evaluation is at day 28 of cycle 1. If a patient has obtained a PR or SD they should continue to be imaged at the end of every 28 day cycle. Once a CR is obtained, imaging of the primary will be every other cycle.

- Measurable Disease - The presence of at least one lesion that can be accurately measured in at least one dimension with the longest diameter at least 20 mm. With spiral CT scan, lesions must be at least 10 mm. The investigator will identify up to 10 measurable lesions to be followed for response.

- Serial measurements of lesions are to be done with CT or MRI. The same method of assessment is to characterize each identified and reported lesion at baseline and during follow-up.

- Quantification of Disease Burden - The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the disease measurement.

- Complete Response (CR) - Disappearance of all target lesions. If immunocytology is available, no disease must be detected by that methodology.

- Partial Response (PR) - At least a 30% decrease in the disease measurement, taking as reference the disease measurement done to confirm measurable disease at study entry.

- Progressive Disease (PD) - At least a 20% increase in the disease measurement, taking as reference the smallest disease measurement recorded since the start of treatment; or the appearance of one or more new lesions.

- Stable Disease (SD) - Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest disease measurement since the treatment started.

5.4.3 Response Assessment

Serial measurements of lesions are to be done with CT or MRI on Day 28. The same method of assessment is to characterize each identified and reported lesion at baseline and during follow-up.

5.4.3.1 Quantification of Disease Burden -The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the disease measurement.

5.4.3.2 Complete Response (CR) -Disappearance of all target lesions. If immunocytology is available, no disease must be detected by that methodology.

5.4.3.3 Partial Response (PR) - At least a 30% decrease in the disease measurement, taking as reference the disease measurement done to confirm measurable disease at study entry.

5.4.3.4 Progressive Disease (PD) - At least a 20% increase in the disease measurement, taking as reference the smallest disease measurement recorded since the start of treatment; or the appearance of one or more new lesions.

5.4.3.5 Stable Disease (SD) - Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest disease measurement since the treatment started.

5.4.3.6 Response Assessment - Each patient will be classified according to their “best response” for the purposes of analysis of treatment effect. Best response is determined from the sequence of the objective statutes described above.

5.4.3.7 Best Response - Two objective status determinations of CR before progression are required for best response of CR. Two determinations of PR or better before progression, but not qualifying for a CR, are required for a best response of PR. Two determinations of stable/no response or better before progression, but not qualifying as CR or PR are required for a best response of stable/no response; if the first objective status is unknown, only one such determination is required. Patients with an objective status of progression on or before the second evaluations (the first evaluation is the

first radiographic evaluation after study drug has been administered) will have a best response of increasing disease. Best response is unknown if the patient does not qualify for a best response of increasing disease and if all objective statuses after the first determination and before progression are unknown.

Use of the definition is illustrated in Table 1 with several sequences of objective statuses and the corresponding best response. The best response is the same if these sequences are preceded by the objective statuses of unknown or stable or if unknowns separate the first objective status from the second.

Table 1. Sequences of objective statuses with corresponding best response.

1st Status	2nd Status	3rd Status	Best Response
Progression			Increasing Disease
Stable, PR, CR	Progression		Increasing Disease
Unk	Progression		Increasing Disease
Stable	Stable	Progression	Stable
Stable, Unk	PR, CR	Progression	Stable
Stable, Unk	Unknown	Progression	Unknown
PR	PR	Progression	PR
PR	CR	Progression	PR
PR, CR	Unknown	Progression	Unknown
CR	CR	Progression	CR
Unknown	Stable	Progression	Stable

5.5 Pharmacokinetic Evaluation

5.5.1 Description of Assay

Plasma sirolimus levels will be determined by commercially available assay. Trough levels should be obtained 20-24 hours from the prior dose. Patients should be advised NOT to take the sirolimus in the morning prior to the clinic appointment when trough levels are going to be obtained.

5.5.2 Sampling Schedule

Sirolimus levels should be obtained prior to the dose on days 5-8 and then weekly if target trough level is met. If the sirolimus dose level is modified a repeat trough

should be obtained in 5-8 days. If the drug is held for toxicity a level should be sent at the time the drug is held. In subsequent cycles levels should be obtained weekly and modified as described in section 4.9. If drugs that are metabolized through CYP 4A3 are added to the patient's regimen a trough level should be obtained within 5-7 days.

5.5.3 Sample Collection and Handling Instructions

3-4 cc in a purple top (EDTA) tube to be sent to central dispatch for shipment to the commercial assay laboratory. Record the exact time that the sample is drawn along with the exact time that the drug was administered. This should be a TROUGH level: patients should be advised NOT to take the sirolimus prior to the clinic appointment.

5.6 Biology Studies

5.6.1 Peripheral Blood Mononuclear Cells/ Peripheral Blood Lymphoblasts

Peripheral blood mononuclear cells will be collected on Days 1, 8, and 28. If a patient is removed from study due to progressive disease peripheral blood should also be sent on the day that they are withdrawn.

5.6.2 Bone Marrow

Bone marrow will be collected prior to study and on day 28. The day 28 marrow may be replaced by peripheral blood if peripheral leukemic blast count is >5,000ul. If a patient with NHL has a negative bone marrow at enrollment they do not require repeat bone marrows.

5.6.3 Collection of Bone Marrow or Peripheral Blood:

Bone marrow aspiration:

- Collect 5 mL of bone marrow in preservative free heparin (100 units heparin/1 mL of bone marrow) by aspiration.

Peripheral blood collection:

- Collect peripheral blood (15 mL in children >10 kg weight, 10 mL in children ≤10 kg weight) in 1-3 green top (sodium heparin) tubes.

<u>Shipment Of Peripheral Blood or Bone Marrow:</u>

- Place bone marrow or peripheral blood in polypropylene screw top tube(s).
- Label tube with patient's registration number, the study ID (CHP X), and date and time it was drawn.
- Place tube(s) in container.
- Place the container with the conical tube in styrofoam box.
- Package sample as appropriate for biologic material.
- Ship the sample on the same day it was obtained with Federal Express overnight priority delivery to:

Dr. Stephen Grupp's lab
The Children's Hospital of Philadelphia
CTRB, Room 3006
3501 Civic Center Blvd.
Philadelphia, PA 19104

Attention: David Teachey, MD
Phone: Yueh Chang (215)-590-6118
Phone: David Teachey, MD (267) 426-5802, pager 15386

- **Samples must be received within 24 hours of obtaining the sample.**
- **Do not ship samples for delivery on a weekend or holiday**

5.6.4 Documenting specimen delivery

All specimens (blood and bone marrow) will be delivered and labeled with the patient ID number upon arrival in the lab. Any patient identifying information will be removed upon arrival. The research assistant will deliver the specimens to Dr. Grupp's lab and all specimens will be signed for including the following information:

- type of specimen
- amount (cc and number of tubes)
- date and time specimen obtained
- person delivering
- person accepting
- date delivered
- time delivered

6 STATISTICAL CONSIDERATIONS

To evaluate the primary aim of this study, the efficacy of sirolimus and methotrexate when given in combination, patients will have disease evaluations performed after their initial 28 day cycle and then every cycle of therapy as indicated by peripheral counts.

The following Simon's two stage design will be used:

Cumulative Number of Responses Decision:

Stage 1: Enter 9 patients

responses	
0	Terminate the trial: agent ineffective
1 CR/CRp or 2 PRs	Inconclusive result, continue trial (proceed to stage 2)

Stage 2: Enter 8 additional patients

responses	
2 or less	Terminate the trial: agent ineffective
3 or more	Terminate the trial: agent effective

We will consider sirolimus/methotrexate not of sufficient interest for further evaluation if the true response rate is 5% and of sufficient activity/interest if the true response rate is 25%. The optimal two-stage design to test the null hypothesis that P<=0.050 versus the alternative that P>=0.250 has an expected sample size of 11.96 and a probability of early termination of 0.630. If the drug is actually not effective, there is a 0.047 probability of concluding that it is. If the drug is actually effective, there is a 0.188 probability of concluding that it is not. After testing the drug on 9 patients in the first stage, the trial will be terminated if 0 respond. If there has been no responses when patient 9 enrolls, further enrollment will be suspended until that patient is fully evaluable. The second tier will not enroll until the medical monitor and study committee meet and certify responses and toxicity. If the trial goes on to the second stage, a total of 17 patients will be studied. If the total number responding is less than or equal to 2, the drug combination is rejected.

6.1 Sample Size and Study Duration

Total number of evaluable patients enrolled will be 17. Historically 4-8 patients with relapsed ALL enroll in Phase 1 or 2 trials at CHOP each year. Based upon toxicity and dose levels the study is projected to run 2-2.5 years. NHL patients will also be eligible and patients can be referred from other sites for enrollment, possibly decreasing the maximum duration of the study to < 2 years.

6.2 Statistical Analysis

6.2.1 Clinical Trial Analysis

Patient characteristics such as age, gender, ethnicity, disease, and disease status, will be tabulated with descriptive statistics. All severe adverse events will be tabulated. The overall and disease specific percentage of patients responding will be calculated with 95% confidence intervals. Kaplan-Meier analysis will be used to describe the time to disease progression for patients receiving sirolimus.

6.2.2 Biology Study Analysis

The presence of p70/S6 kinase, phospho-AKT, p27kip1 and STAT5 will be assayed in leukemic blasts at day 0, day 8, (if peripheral blasts remain present) and day 28 from the bone marrow. DHFR, cyclin D1 and Rb will also be evaluated. They will also be evaluated at time of progression. These signaling intermediates will also be assessed in peripheral blood mononuclear cells. Expression levels of these molecules will be quantified by gel densitometry and described with summary statistics. Levels of expression will be compared with paired t-tests.

6.3 Definitions

6.3.1 Evaluable For Toxicity

Any patient who receives at least one dose of sirolimus will be evaluable for Adverse Effects.

6.4 Inclusion Of Women And Minorities

The study is open to all participants regardless of gender or ethnicity. Review of accrual to past COG 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 toxicity profile or response rate with gender or ethnicity.

6.5 Statistical Methods

6.5.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

6.5.2 Efficacy Analysis - Clinical Trial Analysis

The primary analysis will be based on an intention to treat approach and will include all subjects who complete 1 cycle of therapy.

Patient characteristics such as age, gender, ethnicity, disease, and disease status, will be tabulated with descriptive statistics. All severe adverse events will be tabulated. The overall and disease specific percentage of patients responding will be calculated with 95% confidence intervals. Kaplan-Meier analysis will be used to describe the time to disease progression for patients receiving sirolimus.

6.5.3 Pharmacokinetic Analysis

Mean and median serum trough levels will be determined for each patient on study. Correlation between trough levels and toxicity will be analyzed. Trough levels will also be correlated to response data in a descriptive manner

6.5.4 Biology Study / Pharmacodynamic Analysis

The presence of ribosomal protein s6, AKT, p27kip1 and STAT5 will be assayed in leukemic blasts at day 0, day 8 (if peripheral blasts remain present) and day 28 bone marrow and peripheral blood. DHFR, cyclin D1 and Rb will also be evaluated. They will also be evaluated at time of progression. These signaling intermediates will also be assessed in peripheral blood mononuclear cells. Changes in protein levels will be quantified by gel densitometry and described with summary statistics. Relative changes in protein expression will be compared using the paired student t-test.

6.5.5 Safety Analysis

Example: All subjects entered into the study at Visit 1 will be included in the safety analysis. The frequencies of AEs by type, body system, severity and relationship to study drug will be summarized. SAEs (if any) will be described in detail.

AE incidence will be summarized along with the corresponding exact binomial 95% two-sided confidence intervals.

7 STUDY MEDICATION

7.1 Description - Source and Pharmacology: Sirolimus (Rapamune®, rapamycin)

Approved roadmap abbreviation: SIR NSC#226080

SIROLIMUS (AY-22989, rapamycin, Rapamune®) NSC# 226080 (01/17/08)

Source and Pharmacology: Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus.* Sirolimus is a potent immunosuppressive agent which prolongs the survival of the host and transplanted grafts in animal transplant models of kidney, heart, skin, islet, small bowel, pancreatico-duodenal, and bone marrow. In rodent models of autoimmune disease, sirolimus suppresses immune-mediated events associated with systemic lupus erythematosus, collagen-induced arthritis, autoimmune type I diabetes, autoimmune myocarditis, experimental allergic encephalomyelitis, graft-versus-host disease, and autoimmune uveoretinitis. Sirolimus inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin {Altman, #65}-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Sirolimus also inhibits antibody production. In cells, sirolimus binds to the immunophilin, FK Binding Protein-12 (FKBP-12), to generate an immunosuppressive complex. The sirolimus: FKBP-12 complex has no effect on calcineurin activity. This complex binds to and inhibits the activation of the mammalian Target Of Rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle. In some studies, the immunosuppressive effect of sirolimus lasts up to 6 months after discontinuation of therapy. The plasma protein binding of sirolimus is approximately 92% mainly to albumin, alpha-1-acid glycoprotein and lipoproteins and has a high level of association with erythrocytes. Following administration of sirolimus oral solution, sirolimus is rapidly absorbed, with a mean time-to-peak concentration of approximately 1 hour (range 1-3 hours). The systemic availability of sirolimus was estimated to be approximately 14% after the administration of sirolimus oral solution. The mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution; however, clinical equivalence has been demonstrated at the 2-mg dose level. Sirolimus is extensively metabolized by O-demethylation and/or hydroxylation to at least seven major metabolites. The parent drug contributes to more than 90% of the immunosuppressive activity. The main

route of elimination is through the feces (91%). The mean t 1/2 increased from 79 ± 12 hours in subjects with normal hepatic function to 113 ± 41 hours in patients with impaired hepatic function. Males have a 12% lower clearance of sirolimus than females after oral solution administration. No differences were demonstrated between black and non-blacks. After administration of the oral solution and tablets with a high fat meal, the maximum concentration was reduced, and the time to maximum concentration was increased. The total exposure to drug (AUC) was also increased. Sirolimus is a substrate for both cytochrome P450 IIIA4 (CYP3A4) and P-glycoprotein (P-gp). Sirolimus is extensively metabolized by the CYP3A4 isozyme in the intestinal wall and liver and undergoes counter-transport from enterocytes of the small intestine into the gut lumen by the P-gp drug efflux pump. Sirolimus is potentially recycled between enterocytes and the gut lumen to allow continued metabolism by CYP3A4. Therefore, absorption and subsequent elimination of systemically absorbed sirolimus may be influenced by drugs that affect these proteins. Drugs that stimulate or inhibit p-450 enzymes will alter clearance of sirolimus and close attention to potential drug interactions is crucial.

7.1.1 SIROLIMUS:
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	Headache (L), hypertension (L), nausea, immunosuppression (L), diarrhea, constipation, fever	Chest pain, insomnia, dyspepsia, vomiting, dyspnea	Hypotension, asthma, increased cough, flu like syndrome, tachycardia, anorexia, sensitivity reactions
Prompt Within 2-3 weeks, prior to the next course	Tremor (L), renal dysfunction, elevated creatinine/BUN, anemia, asthenia, pain (abdominal, back, pain), hyperlipidemia, hypercholesteremia, hypertrigylceridemia, hyperglycemia, peripheral edema, weight gain, arthralgia	Elevated LFTs, UTI, URIs, mild thrombocytopenia, leukopenia, hyper/hypokalemia (L), hypophosphatemia, rash, hives, pruritis, hyperuricemia, delayed wound healing, hypomagnesaemia (L)	Gastritis, esophagitis, flatulence, CNS abnormalities: (confusion (L), somnolence (L), depression (L), anxiety, anxiousness, paresthesias, emotional labiality, hypo/hypertonia, dizziness, neuropathy, hypesthesia, nervousness), infections (bacterial, fungal, viral-sepsis, cellulitis, herpes simplex & zoster, EBV, mycobacterial, sinusitis, pharyngitis, abscess, pneumonia, bronchitis, peritonitis), pleural effusions,

			pleural edema, hypoxia thrombosis, thrombophlebitis, myalgia, delayed wound healing,
Delayed Any time later during therapy, excluding the above conditions	Acne		Skin ulcer, hirsutism (hypertrichosis) (L), gingival hyperplasia, abnormal vision, ear pain, cataracts, otitis, tinnitus, hemorrhage, ileus, chronic renal dysfunction, renal tubular necrosis, post transplant diabetes mellitus (L), CHF, ascites, thrombocytopenic purpura (hemolyticuremic syndrome), arthrosis, bone necrosis, osteoporosis
Late Any time after completion of treatment			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing	Sirolimus was embryo/feto-toxic in rats at dosages of 0.1mg/kg and above (approximately 0.2 to 0.5 the clinical doses adjusted for body surface area). Embryo/feto toxicity was manifested as mortality and reduced fetal weights (with associated delays in skeletal ossification). Sirolimus is excreted in trace amounts in milk of lactating rats. It is not known whether sirolimus is excreted in human milk.		

7.1.2 Formulation and Stability:

Sirolimus is available as an oral solution containing 1 mg/mL sirolimus. Sirolimus is also available as a white, triangular-shaped tablet containing 1-mg sirolimus, and as a yellow to beige triangular-shaped tablet containing 2-mg sirolimus. The inactive ingredients in sirolimus oral solution are Phosal 50 PG ® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. Sirolimus oral solution contains 1.5%-2.5% ethanol.

The inactive ingredients in sirolimus tablets include sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, and other ingredients. The 2 mg dosage strength also contains iron oxide yellow 10 and iron oxide brown 70. Sirolimus oral solution should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days for the bottles). Amber syringes and caps are provided for dosing and the

product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately. Sirolimus oral solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product. Sirolimus tablets should be stored at 20°-25°C (68°-77°F). Use cartons to protect blister cards and strips from light. Dispense in a tight, light-resistant container as defined in the USP.

7.1.3 **Guidelines for Administration:**

Sirolimus Administration, Monitoring and Dose Adjustments –
Sirolimus is available in pill or liquid form. Because the liquid has a bitter taste, pills have generally been better tolerated and are preferred. If administration of the liquid is necessary, the drug can be mixed with water or orange juice or given through an NG/NJ tube (do not give with grapefruit juice). If enteral tubes are used they must be primed with sirolimus prior to administration of the medication. Levels of sirolimus should be drawn at least twice per week while hospitalized, then weekly or monthly thereafter unless a change in medication (e.g. use of itraconazole) or liver function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels of sirolimus must be assayed by HPLC or HPLC-MS. If ELISA or other immunoassays are used, laboratories must have performed standardization titers compared to HPLC methods and dosing targeting and reporting must be adjusted to conform to the HPLC standard.

Administer at a consistent time of day and at consistent intervals with regard to meals. Sirolimus may be given with food as long as it is given the same way each time; however, administration with food significantly alters the rate and extent of absorption. The amber oral dose syringe should be used to withdraw the prescribed amount of sirolimus oral solution from the bottle. In order to improve palatability, it is recommended that the correct amount of sirolimus from the syringe be emptied only into a glass or plastic container holding at least two (2) ounces (1/4 cup, 60 mL) of water or orange juice. **No other liquids, including grapefruit juice, should be used for dilution.** Stir vigorously and drink or administer at once. Refill the container with an additional volume (recommended minimum of four [40] ounces (1/2 cup, 120 mL) of water or orange juice, stir vigorously, and drink or administer at once to assure delivery of all of the medication. Small children may not be able to consume the recommended volumes of water or orange juice suggested for dilution and may need lesser volumes.

See Treatment and Dose Modifications sections of the protocol.

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

Concomitant Medications/Drug Interactions: Sirolimus is a CYP 3A4 inhibitor and a P-glycoprotein substrate. Because of this, patients on medications that are

CYP 3A4, 5, or 7 substrates or inhibitors may have an increase in sirolimus level (see table below). Conversely, patients who start taking CYP 3A4, 5, or 7 inducers may have a decrease in sirolimus levels. **Any patient on any of the medications listed (See Appendix V) needs to have careful monitoring of sirolimus to keep the levels in the therapeutic range.**

7.2 METHOTREXATE (MTX, amethopterin, Trexall®) NSC #000740 (102006)

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 0.1µmol/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 >30mg/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 12mg/m², the lumbar concentration of MTX is ~ 100 x's 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.

7.2.1 Toxicity:

	Common Happens to 22-100 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	Transaminase elevations	Nausea, vomiting, anorexia,	Anaphylaxis, chills, fever, dizziness, Malaise, drowsiness, blurred vision,

days of receiving drug			acral erythema, urticaria, pruritis, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, Seizures[1], photosensitivity
Prompt: Within 2-3 weeks, prior to the next course		Myelosuppression, stomatitis, Gingivitis, photosensitivity, Fatigue	Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity[1] (headache, drowsiness, aphasia, pareisis, blurred vision, transient blindness, dysarthria, hemiparesis, decreased reflexes) Diarrhea, conjunctivitis
Delayed: Any time later during therapy		Learning disability1 (L)	Pneumonitis, pulmonary fibrosis(L), Hepatic fibrosis (L), osteonecrosis (L), leukoencephalopathy[1] (L), pericarditis, Pericardial effusions, hyperpigmentation of the nails
Late: Any time after completion of treatment			Progressive CNS deterioration[1]
Unknown Frequency and Timing:	Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations		

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

7.2.2 Formulation & Stability

Methotrexate for oral use is available as 2.5mg, 5.0mg, 7.5mg, 10mg and 15 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: anhydrous lactose, crospovidone, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, pregelatinized starch, sodium carbonate monohydrate, talc and titanium dioxide and various dyes. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

Methotrexate for Injection is available as a lyophilized powder for injection in 20 mg and 1 gm vials. The powder for injection contains approximately 0.14 mEq sodium in the 20 mg vial; 7 mEq sodium in the 1 g vial. Methotrexate for Injection is also available as a 25mg/mL solution in 2,4,8,10,20 and 40mL preservative free vials and 2 and 10mL vials with preservative. The 2, 4, 8, 10, 20, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, 4.3, and 8.6 mEq sodium per vial,

respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative and must not be used for intrathecal or high dose therapy.

Sterile methotrexate powder or solution is stable at 20 to 25 Cº (68 to 77 Fº); excursions permitted to 15 to 30 Cº (59 to 86 Fº). Protect from light

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

Oral administration: Food delays absorption and reduces peak concentration. MTX for oral use should preferentially be given on an empty stomach, 1 hour before or 2 hours after a meal and at the same time each day. Oral methotrexate solution may be substituted for the oral tablet dosage form.
Supplier: Commercially available from various manufacturers. See package insert for further information

7.3 Drug Accountability

Patients, or their guardian, will be provided a prescription of the daily dose of sirolimus. They will receive the initial loading dose in the oncology clinic or as an inpatient. A prescription for the assigned methotrexate dose will also be provided to the patient/family. A daily drug diary will be provided to the families to document all oral, at home, doses of study medications.

8.0 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

8.2 Adverse Event Reporting

The Investigator is responsible for recording and reporting unanticipated problems related to research that occur during and after study treatment. The plan for Adverse Event reporting should be consistent with the CHOP IRB Guidelines. For example: “All on-site SAEs (CHOP or related sites) will be reported to the IRB in accordance with CHOP IRB policies. AEs that are not serious will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.”

8.2.1 General Guidelines For All Adverse Events

Adverse event collection and reporting is a routine part of every clinical trial. The first step is to identify the event using the Common Toxicity Criteria (CTC) V3. ALL serious adverse events (SAEs) will be reported to the IRB.

A copy of the Common Terminology Criteria (CTC) version V3 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTC version 3.0. The severity of the event should then be graded using the CTC criteria. Next, determine if the adverse event

is related to the medical treatment or procedure (attribution). If so, determine whether the adverse event is expected or unexpected. With this information and the adverse event reporting section in each protocol, the investigator can determine whether an adverse event should be reported to the IRB as an expedited report or a routine report.

8.2.2 Persistent Adverse Events

An adverse event that persists from one course (cycle) to another should only be reported once unless the grade becomes more severe in a subsequent course. An adverse event, which resolves and then re-occurs during a different course (cycle) must be reported each course (cycle) it re-occurs.

A patient experiences Grade 3 thrombocytopenia during cycle one. During cycle two the adverse event persists but the severity remains unchanged. During cycle three the adverse event persists but increases in severity to Grade 4. The following should be submitted as expedited reports:

Cycle One – Grade 3 Thrombocytopenia

Cycle Two – No Report

Cycle Three – Grade 4 Thrombocytopenia

8.2.3 Baseline Adverse Events

An adverse event should NOT be reported if a patient is entered on a study with a pre-existing condition (e.g., elevated laboratory value). If the adverse event increases in severity, the investigator should re-assess the event to determine if the event should be reported. No modification in grading should be made to account for abnormalities noted at baseline. For example:

A patient enters a trial with an AST equivalent to Grade 1. If the AST remains unchanged at the end of cycle one, the adverse event should NOT be reported. If the AST increases to a Grade 3 level, the adverse event should be re-assessed and reported if it fulfills the other adverse event reporting criteria. The AST would be reported at Grade 3 with no adjustment for the baseline AST equivalent to Grade 1.

A patient enters a study with diarrhea equivalent to Grade 2. The diarrhea resolves during the first cycle of therapy. If, during a subsequent cycle the patient experienced Grade 2 diarrhea, the adverse event should be re-assessed and reported if it fulfills adverse event reporting guidelines.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory

finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs

The Investigator is responsible for promptly notifying the IRB of all study on-site SAEs and other unanticipated problems related to research using the CHOP Internal SAE reporting form and in accordance with the following timeline.

Type of Internal Adverse Event	Initial Notification (Phone, Email, Fax)	Written Report

Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs Unexpected and Related	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other SAEs	N/A	Summary of SAEs Reported at Time of Continuing Review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. All SAE will be followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be in accordance with regulatory requirements.

8.7 Medical Emergencies

Any child on trial will be evaluated emergently as needed in the outpatient oncology clinic or emergency department as indicated.

9.0 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods - None

9.2 Data Collection and Management-

- Confidentiality will be maintained by providing a de-identified patient ID number. This number will be used in the database. All specimens from The Children's Hospital of Philadelphia will be sent with patient labels. Once the assays are run, the information will be reported using the de-identified patient ID number. The statistical analysis will only contain de-identified ID numbers.

- A master list containing PHI and subject ID will be kept in the study binder separate from data forms that have only a study ID number. The master list will be on a separate computer in the study coordinator's office which will be locked.

- All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA. Investigator and other site

personnel will not use such data and records for any purpose other than conducting the study. All records will be kept in a locked filing cabinet in the office of the Research Coordinator.

- All files will be password protected on a computer that will be password protected as well.

1. Security. A copy of the password-protected file will be on the research coordinator's computer in a locked office along with the original file which will be stored in one of the Hospital's secure servers.

2. Anonymization, de-identification or destruction. The identifiers for all patients will be destroyed after publication. The other data will be retained for three years. The laboratory maintains a file drawer specifically for such archives, each folder labeled "Destroy by a certain date," with the earliest dates at the front."

9.3 Regulatory and Ethical Considerations

9.3.1 Data and Safety Monitoring Plan

The Principal Investigator will monitor the study regularly and review all evaluations of patients' eligibility, evaluability, and dose limiting toxicities in the study database. If of the first 6 patients enrolled, DLTs of the combination are noted in at least 2, the study will be temporarily closed and either modified or terminated as the dose combination is considered too toxic.

At least every 6 months the PI will meet with 2 of the other co-chairs of the study committee and the medical monitor, Frank Balis, MD, to review all potential DLTs and assign proper attribution. They will also determine official response based upon the criteria in Section 5.4.
The study PI and medical monitor will also independently review all serious, unexpected, (possibly) related SAEs and all other unanticipated problems involving risks to research subjects or others.

After 9 patients have enrolled and had response based criteria the Review Committee will meet to decide progression to Stage 2 or Study termination if the combination is deemed ineffective.

All SAE's that are unexpected and related will be reported to the IRB as outlined in Section 8.5 by the PI and the study coordinator. All other significant events will be summarized at the time of continuing review.

9.3.2 Risk Assessment -

Medication:

- Sirolimus – see Section 7.1.1 above for a list of medication toxicities
- Methotrexate - see Section 7.2 above for a list of medication toxicities

Procedures:

- Bone Marrow Aspirate- minor increase above minimal risk, often being performed as standard of care.
- Peripheral blood draw (through Broviac, port, peripheral IV)- minimal risk, often being performed as standard of care.

Risks are a minor increase above minimal or greater than minimal and have a prospect for direct benefit. The major risk to the patient enrolled on trial is the individual and combined drug toxicities which are being defined in the study. The risk of the study related procedures are minimal and are often standard of care for patients with refractory and relapsed ALL/NHL.

9.3.3 Potential Benefits of Trial Participation –
The potential benefit of the combination treatment with sirolimus and methotrexate is that it may cause patients cancer to stop growing or to decrease production for a period of time. It may lessen the symptoms, such as pain, that are caused by the disease. We know that methotrexate is effective in treating ALL and NHL in newly diagnosed patients and in relapsed patients. Because there is no information about this combination's effect on cancer in humans, we do not know if there will be any direct benefit from participating in this study. Information learned from this study may help future patients with ALL/NHL

9.3.4 Risk-Benefit Assessment

Patients enrolling on this trail have been refractory to multiple first and second line drug combinations to treat ALL/NHL. If untreated, the patient will die of disease. This drug combination involves greater than minimal risk to participants, but the risks are reasonable when balanced with the prospect for benefit and the alternative outcome.

9.4 Recruitment Strategy

Eligible subjects will be identified during weekly meetings where potential candidates are discussed. The primary oncologist for the patient can request information about the study from the Principal Investigator to determine if the study is appropriate and the patient is eligible. An eligibility checklist will be sent to the attending physician to screen for eligibility. If the patient is eligible, their primary doctor will review the study with the parents (patient if appropriate) during a clinic visit or if they are inpatient, a meeting will be set up to see if they are interested in participating. Patients will come from The Children's Hospital of Philadelphia and other hospitals who have eligible patients. The subjects may be the investigator's patients or from other care providers.

9.5 Informed Consent/Assent

The primary oncologist or member of the study team will approach the family to discuss the protocol and obtain consent and assent.

9.6 Payment to Subjects/Families

There will be no payment to subjects or families.

10 PUBLICATION

It is planned that the results of this study will be published in the appropriate cancer journal.

Appendix I: Eligibility Criteria Form

APPENDIX I: Pre-screening - Eligibility/Exclusion Criteria Worksheet

Phase 2 Trial of Sirolimus with Methotrexate in Relapsed/Refractory Lymphoblastic Leukemia and Lymphoma

Patient ID:_____Patient Initials:_____

1. Is the patient age 25 or younger?	□Yes	□ No	
2. ALL: Histologic diagnosis with >10% blasts in the marrow?	□Yes	□ No	
NHL: Radiologic evidence of Relapse?	□Yes	□ No	
3. Is this the patient's second or greater relapse?	□Yes	□ No	
4. Lansky ≥ 50% if ≤ age 10?	□Yes	□ No	
Karnofsky ≥ 50% if ≥ age 10?	□Yes	□ No	
5. Pregnancy Test Negative?	□Yes	□ No	
6. Creatinine clearance or radioisotope GFR ≥ 70ml/min/m^2 OR	□Yes	□ No	
a serum creatinine based on age /gender as identified in Protocol section 3.4.2.2?	□Yes	□ No	
7. Pulse ox >94%?	□Yes	□ No	
8 Uncontrolled infection?	□Yes	□ No	
If yes:			
Patients with fungal disease: stable for 14 days?	□Yes	□ No	□ N/A
Patients with bacteremia: negative blood culture?	□Yes	□ No	□ N/A
9. Total Bilirubin <1.5 x normal for age?	□Yes	□ No	
ALT < 5 x normal for age?	□Yes	□ No	
Albumin ≥ 2g/dL?	□Yes	□ No	
10. Shortening fraction by echo ≥ 28% OR	□Yes	□ No	
Ejection fraction ≥ 50% by gated radionuclide study within 6 months?	□ Yes	□ No	
11. Patient is not taking other investigational anti-neoplastic drugs?	□Yes	□ No	
12. Patient does not have known allergies to sirolimus, FK-506 or mTOR inhibitors.	□Yes	□ No	
13. Has no existing non-hematologic toxicities > grade 2?	□Yes	□ No	
14. Patient received no myelosuppressive chemo within 14 days? (2 if prior nitrosourea.)	□Yes	□ No	
15. The PI has been contacted if the patient has had Hydroxyurea OR Corticosteroids within 14 days of enrollment?	□Yes	□ No	
16. Patient has not taken any biologic agents within 14 days?	□Yes	□ No	
17. ≥14 days have elapsed since local palliative XRT (small port)	□Yes	□ No	
≥28 days since prior craniospinal XRT or 50% radiation of pelvis?	□Yes	□ No	
≥28 days if other substantial BM radiation?	□Yes	□ No	
18. For BMT/SCT - no evidence of active GVHD.	□Yes	□ No	
For Allogeneic BMT/SCT, ≥ 3 months must have elapsed.	□Yes	□ No	
19. No Hematopoietic growth factors within 7 days of entry? (except erythropoietin.)	□Yes	□ No	
20. Does the patient have Extramedullary Disease?	□Yes	□ No	□ N/A
22. Does the patient have bone marrow Involvement?	□Yes	□ No	□ N/A
22. Signed informed consent?	□Yes	□ No	

Clinician Name _____

Clinician Signature_____ Date_____

PI signature_____ Date_____

Appendix II: Roadmap for Therapy

CHP-948: A PHASE 2 TRIAL OF SIROLIMUS WITH MTX IN RELAPSED/REFRACTORY LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA

Last Name _____ First Name_____ MRN: _____ PH # _____

Cycle # _____ Wt __ __ __.__ kg Ht __ __ __.__cm BSA __.__ __ m2

PATIENT LABEL

Sirolimus - oral

Day 1: Loading Dose - 9 mg/m²

Days 2-28: Daily Dose - 3 mg/m²

Check trough level 5-8 days after starting daily dose

Trough Level	< 8ug/L	≥ 8 and ≤ 13ug/L	>13 ug/L
Dose	increase daily dose by 1 mg/m²/day	no change	decrease daily dose by 1 mg/m²/day
Trough	recheck in 5-8 days	recheck weekly	recheck in 5-8 days

Methotrexate (MTX) - oral

Days 2,9,16,23

20 mg/m²

OBSERVATIONS - Description

Set 1: History, PE(Ht,Wt,VS)
Set 2: Pregnancy Test/Performance Scale
Set 3: CBC, differential, platelets
Set 4: Tumor lysis labs, electrolytes (Sect 4.2)
Set 5: ALT, AST, Total Bilirubin
Set 6: Cholesterol/Triglycerides
Set 7: Sirolimus Trough Levels
Set 8: Biology Studies (Sect 5.6)
Set 9: Tumor Disease Evaluation & BM

DATE			SIROLIMUS dose				MTX dose	OBSERVATIONS
Due	Given	DAY	Actual Dose Given	Trough Level	Adjusted Total Daily Dose/m2			
		1	_____ mg LOAD		See above			1,2,3,4,5,6,8 (blood),9
		2	____mg DAILY				___mg	
		3	___mg					4
		4	___mg					
		5	___mg					4
		6	___mg					
		7	___mg					
		8	___mg					1,3,4,5,7,8 (blood)
		9	___mg				___mg	
		10	___mg					
		11	___mg					
		12	___mg					4
		13	___mg					
		14	___mg					
		15	___mg					1,3,4,5,7
		16	___mg				___mg	
		17	___mg					
		18	___mg					
		19	___mg					4
		20	___mg					
		21	___mg					
		22	___mg					1,3,4,5,7
		23	___mg				___mg	
		24	___mg					
		25	___mg					4
		26	___mg					
		27	___mg					
		28	___mg					1,3,4,8 (blood&BM),9

Appendix III- Medication Diary

CHP 948: PATIENT DIARY FOR MEDICATION ADMINISTRATION - BRING THIS FORM TO YOUR CLINIC VISITS

Patient ID: _____ Cycle # _____ Start Date: __/__/_____ End Date: __/__/_____ Institution: _____

Record date and time of each dose of Sirolimus and Methotrexate given. Comment if there are any changes.

Do not take Sirolimus on a day that you are having a trough level done in clinic. You may take your dose after the clinic visit.

SIROLIMUS (oral) Dose _____	METHOTREXATE (oral) Dose ____
• Take on empty stomach (1 hour before or 2 hours after meals/tube feeds) • Take at the same time each day and do not take with dairy products	• Take with water or orange juice, no grapefruit juice • Take in the morning before breakfast (same time each day)

Day	Date	Time (Circle am or pm)		Medication	Changes	Comments/Issues
Day 1			am	Sirolimus		
Day 2			am	Sirolimus		
			am / pm	Methotrexate		
Day 3			am	Sirolimus		
Day 4			am	Sirolimus		
Day 5			am	Sirolimus		
Day 6			am	Sirolimus		
Day 7			am	Sirolimus		
Day 8			am	Sirolimus		
Day 9			am	Sirolimus		
			am / pm	Methotrexate		
Day 10			am	Sirolimus		
Day 11			am	Sirolimus		
Day 12			am	Sirolimus		
Day 13			am	Sirolimus		
Day 14			am	Sirolimus		
Day 15			am	Sirolimus		
Day 16			am	Sirolimus		
			am / pm	Methotrexate		
Day 17			am	Sirolimus		
Day 18			am	Sirolimus		
Day 19			am	Sirolimus		
Day 20			am	Sirolimus		
Day 21			am	Sirolimus		
Day 22			am	Sirolimus		
Day 23			am	Sirolimus		
			am / pm	Methotrexate		
Day 24			am	Sirolimus		
Day 25			am	Sirolimus		
Day 26			am	Sirolimus		
Day 27			am	Sirolimus		
Day 28			am	Sirolimus		

Questions or Concerns: Please call Nancy Sacks, Study Coordinator at 267-426-5613.

Appendix IV - relevant drug interactions

Table of Clinically Relevant Drug Interactions for CYP 3A4, 5, or 7

CYP 3A4,5,7 SUBSTRATES	CYP 3A4,5,7 INHIBITORS	CYP3A4,5,7 INDUCERS
Macrolide antibiotics:	**HIV Antivirals:**	**HIV Antivirals:**
clarithromycin	delaviridine	efavirenz
erythromycin	indinavir	nevirapine
NOT azithromycin	nelfinavir	**Other:**
telithromycin	ritonavir	barbiturates
Anti-arrhythmics:	**Other:**	carbamazepine
quinidine	amiodarone	glucocorticoids
Benzodiazepines:	aprepitant	modafinil
alprazolam	NOT azithromycin	phenobarbital
diazepam	chloramphenicol	phenytoin
midazolam	cimetidine	rifampin
triazolam	clarithromycin	St. John's wort
Immune Modulators:	diethyl- dithiocarbamate	troglitazone
cyclosporine	diltiazem	oxcarbazepine
tacrolimus (FK506)	erythromycin	pioglitazone
HIV Protease Inhibitors:	fluconazole	rifabutin
indinavir	fluvoxamine	
ritonavir	gestodene	
saquinavir	grapefruit juice	
Antihistamines:	itraconazole	
astemizole	ketoconazole	
chlorpheniramine	mifepristone	
Calcium Channel Blockers:	nefazodone	
amlodipine, diltiazem	norfloxacin	
felodipine, nifedipine	norfluoxetine	
nisoldipine, nitrendipine	mibefradil	
verapamil	star fruit	
HMG CoA Reductase Inhibitors:	verapamil	
atorvastatin, cerivastatin	voriconazole	
lovastatin, NOT pravastatin		
simvastatin		
Other:		
aripiprazole		
buspirone		
cisapride		
imatinib		
haloperidol (in part)		
methadone		
pimozide		
quinine		
NOT rosuvastatin		
sirolimus		
sildenafil		
tamoxifen		
trazodone		
vincristine		

Adapted from table prepared by Division of Pharmacology School of Medicine at Indiana University. New drug interactions may be identified after this table was printed; please check periodically for updates at http://medicine.iupui.edu/flockhart/

Appendix V: 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.

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