Status Page

PROTOCOL **09-421**

BIDMC
closed to accrual

DFCI
BWH
Open to accrual

DATE POSTED: 05/10/16

No new subjects may be enrolled in the cohort/arms/site(s) as described above.
Any questions should be directed to the study's Principal Investigator

Status Page

PROTOCOL **09-421**

Phase II
Closed to accrual

Phase I
Open to accrual

Closure Effective Date: 12/20/13

No new subjects may be enrolled in the site(s) as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

Date Submitted: ***[03/10/16]***
Date Posted: ***[04/25/2016]***

Alert Page

DF/HCC Protocol #: **[09-421]**

Dose Escalation Table

Effective Date	Dose Level/T-LGL and CLL/SLL	Dose (insert units)
Closed	1	12.5 mg, Once Daily
Closed	2	25 mg, Once Daily
Closed	3	50 mg, Once Daily
11/10/14	**4**	**100 mg Once Daily**
Not open yet	5	150 mg Once Daily

Note: The row for the current dose level is **highlighted and bolded**.

A Phase I/II Study of Pyrimethamine, a STAT3 Inhibitor, for the Treatment of Relapsed Chronic Lymphocytic Leukemia / Small Lymphocytic Lymphoma and T-Large Granular Lymphocytic Leukemia

DF/HCC Biomedical Protocol Template: Version 9. October 18, 2016.

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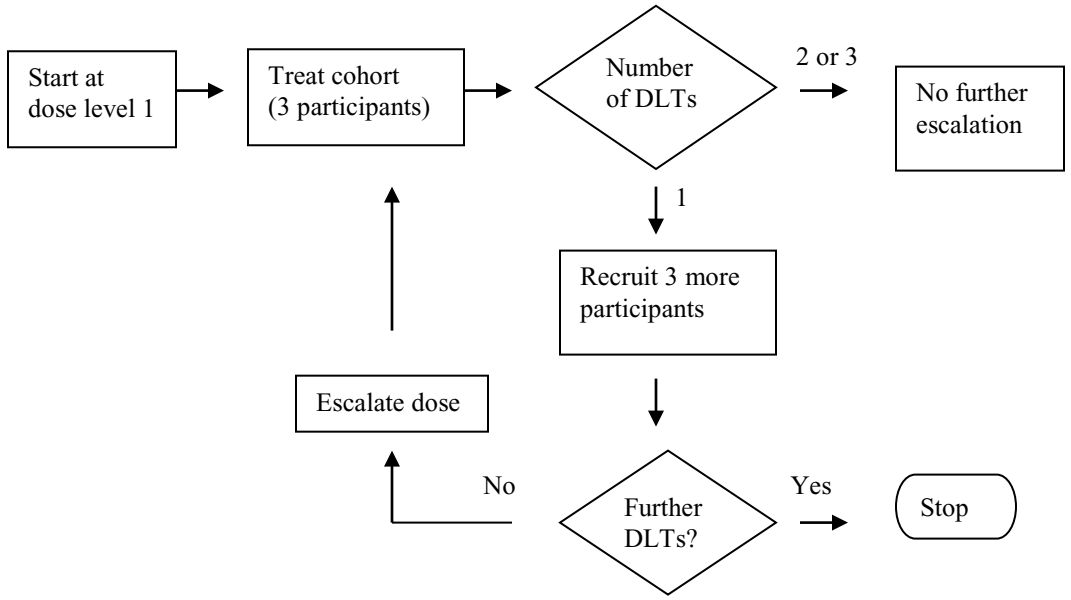
SCHEMA

Phase I Component



Subsequent Phase II Trial

Single daily oral 50 mg dose: The MTD established in the Phase I portion was 50 mg.

One cycle = 28 days

↓

Evaluation for toxicity & response

Response of SD or better may remain on study indefinitely if toxicity is acceptable

Progressive Disease will come off study

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Study Design

This study includes a brief Phase I dose escalation to assess the tolerability of pyrimethamine in relapsed Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, CLL/SLL, and T-Large Granular Lymphocytic Leukemia, T-LGL, and to correlate dose with pharmacodynamic efficacy. It then moves rapidly to a Phase II study to assess efficacy, using a Simon two stage design. Due to PK and PD results that suggest that a higher dose between 100 and 150 mg may be required for optimal efficacy, and the absence of clear drug-related toxicity thus far, the protocol is being amended to continue the phase 1 dose escalation to a higher dose.

1.2 Primary Objectives

Phase I: To determine the MTD and recommended Phase 2 dose of pyrimethamine in relapsed CLL/SLL and T-LGL

Phase II: To determine the ORR of pyrimethamine in relapsed CLL/SLL and T-LGL

1.3 Secondary Objectives

- To assess the toxicity profile of pyrimethamine in relapsed CLL/SLL and T-LGL, both acutely and over prolonged daily dosing.
- To determine pyrimethamine levels in vivo with prolonged dosing.
- To determine the progression-free survival following pyrimethamine for the treatment of relapsed CLL/SLL and T-LGL.
- To determine whether pyrimethamine inhibits STAT3 in vivo by assessing downregulation of STAT3 dependent gene expression in CLL cells, and T-LGL and/or peripheral blood mononuclear cells.
- To determine whether known prognostic factors in CLL/SLL and T-LGL correlate with response to pyrimethamine.
- To assess the impact of CT scans on response evaluation in relapsed CLL/SLL and T-LGL.

2. BACKGROUND

2.1 Investigational Agent: Pyrimethamine

Pyrimethamine (5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine, Daraprim) is an oral folic acid antagonist used as an antiparasitic compound. It is a reversible weak inhibitor of dihydrofolate reductase. Due to a differential requirement between host and parasite for nucleic acid precursors involved in growth, pyrimethamine is highly selective against plasmodia and *Toxoplasma gondii*.

Pyrimethamine is well absorbed with a plasma half-life of approximately 96 hours. Pyrimethamine is 87% bound to human plasma proteins. At the maximum dose tested in this trial, namely 50 mg/day, mean plasma pyrimethamine concentrations were 1,893+/-1,182 ng/ml[1]. As a folate antagonist, caution is recommended in patients with possible folate deficiency, such as individuals with malabsorption syndrome, alcoholism, or pregnancy, and those receiving therapy such as phenytoin or lorazepam, which may increase the risk of hepatotoxicity.

The 50 mg/day dose maximum proposed for this trial is the recommended dose for the treatment of toxoplasmosis, and is routinely used for at least 1-3 weeks depending on treatment response. At this dose, anorexia and vomiting may occur. Vomiting may be minimized by giving the medication with meals; it usually disappears promptly upon reduction of dosage. Doses used in toxoplasmosis may produce bone marrow suppression, which can be reversed with discontinuing the drug and with addition of leucovorin. Side effects include megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, hematuria, and disorders of cardiac rhythm. Hypersensitivity reactions, occasionally severe (such as Stevens-Johnson syndrome), have been reported but primarily when pyrimethamine is administered concomitantly with a sulfonamide.

Due to the possibility of myelosuppression and given the patient population in this study, we include a brief Phase I dose escalation to assess the tolerability of pyrimethamine in relapsed CLL/SLL, and to correlate dose with pharmacodynamic efficacy. The Phase 1 starting dose of 12.5 mg. is the lowest convenient dose available and significantly lower than the usual dose used for infectious diseases. We then move rapidly to a Phase II study to assess efficacy, using a Simon two stage design.

2.2 Study Diseases: Chronic Lymphocytic Leukemia and T Large Granular Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL / SLL) is the most common form of leukemia in the United States with more than 13,000 new cases per year[2]. The disease incidence increases with age and is more common in men than women. Although classified as a leukemia, the clinical behavior and treatment of CLL is more similar to many low-grade lymphomas. Patients are observed until they are deemed to require therapy based on worsening symptoms or cytopenias, increasing lymphocytosis, lymphadenopathy or splenomegaly, or transformation of disease[3]. Therapy has been palliative, with no established therapy shown to improve survival.

The last ten years have resulted in significant therapeutic advances, with the advent of chemoimmunotherapy combinations with complete remission rates approaching 50% and progression-free survivals of 3-5 years[4-6]. However 10-20% of patients still fail to respond or relapse early, and all patients still relapse eventually. For these patients, especially those who are

fludarabine refractory with bulky adenopathy, or those with poor risk cytogenetics, there remains an urgent need for new more effective therapies that are less toxic. The field has therefore turned to targeted drugs, designed to directly address the mechanism of carcinogenesis, in an effort to improve efficacy and reduce toxicity.

T-LGL is an uncommon low-grade lymphoproliferative disorder that can occur as an isolated entity or in the context of rheumatologic or neoplastic disease. Many patients remain asymptomatic and do not require treatment. However, some patients develop anemia (either autoimmune or hypoproliferative), neutropenia, infection, or disease related symptoms such as fatigue. Oral methotrexate and oral cyclophosphamide have been the most commonly utilized agents with response rates ranging from 38-64%[7]. These agents are not curative and can have significant toxicity including myelosuppression, mucositis, and liver function abnormalities. Other agents that have been investigated with varying success include cyclosporine and alemtuzumab[8,9] However, cyclosporine can cause renal insufficiency and is immunosuppressive while alemtuzumab is profoundly myelosuppressive and immunosuppressive and can be challenging to obtain commercially due to marketing restrictions.

A large body of literature has emerged demonstrating the importance of STAT3 and STAT5b mutations in T-LGL. Most of these mutations are activating somatic mutations in the SH2 domain of STAT3 though other STAT3 mutations have been described[10,11]. Therefore we propose amending the existing protocol to include patients with T-LGL. We are not requiring a demonstrable STAT3 mutation since the presence of these mutations is not clearly known to be predictive of response to STAT3 inhibitors.

2.3 Rationale

2.3.1 Biology of the STAT family of Transcription Factors

The function of lymphoid cells is regulated by cytokines which control processes such as survival, self-renewal, proliferation, and differentiation. Although cytokines activate a number of signaling pathways, increasing evidence suggests that STATs, for signal transducers and activators of transcription, play a key role in this process. STATs are present in the cytoplasm of cells under basal conditions. When stimulated by a growth factor or cytokine, STATs become phosphorylated on a unique tyrosine residue which triggers their dimerization. STAT dimers then translocate to the nucleus where they bind to specific nine base pair sequences in the regulatory region of target genes, thereby activating transcription (Figure 1). Given that STATs control genes that regulate critical biological functions including cell proliferation and survival, physiological STAT activation is rapid and transient. Roughly one hour after STAT activation, the STATs have been dephosphorylated and have returned to the cytoplasm. Although classically STATs are activated by tyrosine phosphorylation, certain STAT family members, including STAT3, have a highly conserved serine residue towards the carboxyl terminus that is also phosphorylated in a highly regulated manner. Increasing evidence indicates that phosphorylation of this residue, serine 727, can have activating effects independent of tyrosine phosphorylation[12,13].

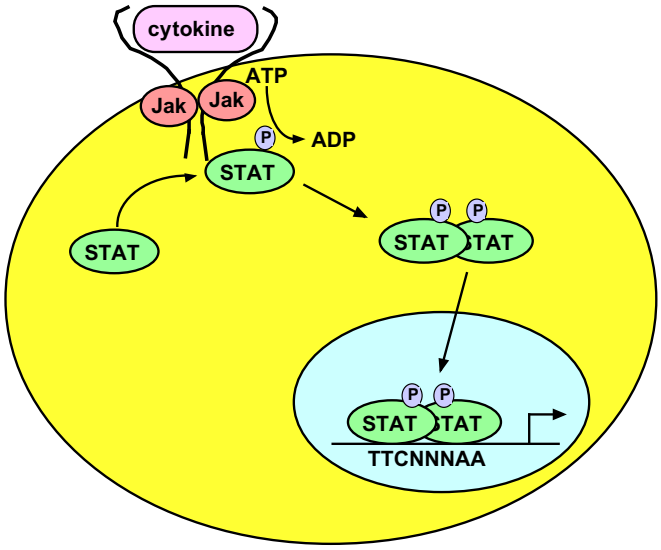


Figure 1. Activation of STATs in response to cytokines or other signals leads to the regulation of genes which play a key role in cellular proliferation and survival.

Given the importance of STATs in mediating the physiological regulation of these genes, it is not surprising that inappropriate activation of STATs occurs commonly in hematologic cancers. In fact some abnormality in STAT signaling has been reported in every form of leukemia and lymphoma. We had found that STAT3 is phosphorylated on serine 727 in CLL cells isolated from essentially all patients with this disease[14]. Serine phosphorylated STAT3 can be found in the nucleus, and can drive expression of genes regulating survival and proliferation[15]. Furthermore, natural products with therapeutic activity toward CLL can decrease the serine phosphorylation of STAT3[16]. Genes whose expression are activated by STAT3 control critical cellular functions such as cell survival (e.g., Mcl-1 and Bcl-xl), which is a major factor in both the pathogenesis of CLL, and its resistance to curative cytotoxic therapy[17]. While continued STAT3 function is critical for tumor cell survival, STAT3 can be inhibited in normal cells with little consequence[18]. Taken together, these findings suggest that STAT3 is an excellent target for the treatment of CLL and T-LGL.

2.3.2 Identification of STAT3 inhibitors. Given that inappropriate phosphorylation of STAT3 is observed nearly universally in CLL and appears to contribute directly to the pathobiology of CLL cells, there would be great utility in the development of small molecules that could specifically and effectively inhibit this pathway. Since the goal was to identify drugs that can be translated rapidly into human clinical trials, the Prestwick Collection, a library containing 1120 compounds most of which have already been tested in humans and are either FDA-approved or known to be safe, was screened with a STAT3 inhibition assay in the laboratory of Dr. David Frank, Director of the Frank Laboratory, a division of the Louis B. Mayer Research Laboratories at DFCI. See Section 8, Correlative/Special Studies.

The compound that led to the greatest specific inhibition of STAT3 activity in the initial screen was pyrimethamine, a compound that is FDA approved for treating parasitic infections such as toxoplasmosis and malaria. It is orally bioavailable and has relatively few significant side effects. Given its slow elimination, it is generally dosed once daily. Pyrimethamine shows half-maximal activity (EC_{50}) at approximately 1.5 μM, well within the plasma concentrations found with clinical use. Pyrimethamine has no effect on STAT3 phosphorylation or nuclear translocation, but it appears to inhibit STAT3 localization to its cognate genomic DNA sequences, thereby inhibiting its function. Although pyrimethamine is known as an inhibitor of dihyrofolate reductase (DHFR), albeit a weak one, the potent DHFR inhibitor methotrexate did not inhibit STAT3 activity in this assay. Therefore, STAT3 inhibition by pyrimethamine is probably distinct from its effect on DHFR. The effect of pyrimethamine was examined in MDA-MB-231 cells, which are characterized by constitutive STAT3 activation. Using quantitative RT-

PCR normalized to GAPDH, we found that 1 μM pyrimethamine, a concentration achievable in vivo, decreased expression of Bcl-xl and cyclin D1 by approximately 60%. Pyrimethamine showed no toxicity in non-transformed hematopoietic cells, epithelial cells, or fibroblasts.

2.3.3 Pyrimethamine shows therapeutic effects in CLL cells in vitro. In model systems, pyrimethamine effectively inhibits STAT3-dependent gene transcription at low micromolar concentrations that can be achieved clinically in humans (Figure 2). By contrast, pyrimethamine has no effect on gene expression driven by NF-κB or the closely related transcription factor STAT5. Pyrimethamine (5 μM) significantly decreased expression of a panel of STAT3-dependent target genes, including BCL3, BCL6, cyclin D1, and SMAD7, while showing no effect on expression of "housekeeping" genes including tubulin and GAPDH, or NF-κB-dependent genes including A20 and TLR4. Dr. Frank's lab next assessed the effect of pyrimethamine on the viability of primary CLL cells in culture. As early as 48 hours after treatment, pyrimethamine caused a significant dose-dependent decrease in survival of CLL cells, beginning at concentrations as low as 1 μM, and it does so through induction of apoptosis as measured by annexin V staining. Thus, pyrimethamine, a well tolerated FDA approved drug, inhibits STAT3 and induces apoptosis of primary CLL cells in vitro.

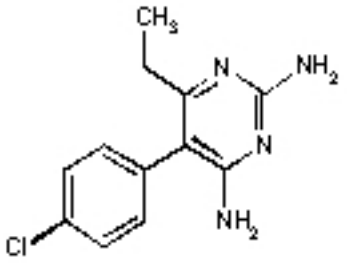


Figure 2. The anti-parasitic compound pyrimethamine was the most active and specific STAT3 inhibitor among 1120 compounds screened in the Prestwick Collection.

2.3.4 Study Rationale

Based on these data, we propose a clinical trial of the STAT3 inhibitor pyrimethamine in relapsed CLL/SLL and T-LGL. The drug is FDA approved and has been well-tolerated in a wide variety of patients, suggesting that its toxicity profile is favorable. The in vitro data suggest that pyrimethamine may be highly effective against CLL/SLL in vivo, warranting a clinical test.

2.3.5 Results from Initial Phase 1 Study

In the first phase of this study, patients were accrued to the three planned dose levels, sixteen in all. Three patients each were enrolled on cohorts 1 and 2 with no DLTs and no significant drug-related toxicities. Cohort 3 enrolled 10 patients, also with no DLTs observed. The reason 10 patients were enrolled is that 4 were removed from study prior to one month of therapy and were therefore not evaluable for DLT. Patients 7, 11, and 13 were all removed for early progressive disease and not evaluable for DLT. Patient 8 was removed due to pneumonia that was not considered drug-related as this patient had had many prior serious pneumonias and the pneumonia began at the same time or even prior to study initiation. Thus, only patients 9, 10,

and 12 were all evaluable and had no evidence of DLT. The cohort was then expanded to six, where no patients experienced DLTs. The few significant toxicities at least possibly related to study drug in these six patients all occurred after cycle 1, which was the DLT evaluation period. These toxicities did include one instance of asymptomatic rapidly reversible grade 3 transaminitis (which occurred also in the setting of initiation of doxycycline and a change in type of hormone replacement therapy); transient grade 4 thrombocytopenia in a patient with baseline grade 3 thrombocytopenia and a history of prior stem cell transplant; and transient grade 4 thrombocytopenia concomitant with infection. None of these would have met DLT criteria even had they occurred in cycle 1. We therefore moved ahead and opened the phase 2 portion at what we thought was the recommended phase 2 dose of 50 mg daily.

Subsequent PK and PD data suggest that the 50 mg dose is just reaching the lower end of the efficacy range in blood in a subset of the patients. Given that the drug was well tolerated and patients with toxoplasmosis normally receive significantly higher doses, we have decided to go back to phase 1 and do further dose escalation, in hopes of achieving higher drug levels which may then enhance our PD results and hopefully by extension efficacy. Best response at the lower doses was prolonged stable disease (7 months in a very heavily pretreated patient)

2.4 Correlative Studies Background

To optimally utilize pyrimethamine as a therapy for CLL, it will be essential to determine if pyrimethamine is, in fact, inhibiting STAT3 function in CLL cells. If pyrimethamine is clinically effective, then it will be important to determine the lowest dose necessary to achieve STAT3 inhibition, to minimize the chance for non-specific toxicities. If pyrimethamine does not show activity in these patients, then it will be essential to know whether STAT3 was successfully inhibited.

In order to address these questions, at the time of each clinic visit, heparinized blood samples will be drawn for several assays. First, pharmacokinetics will be performed on plasma to assess steady-state levels of pyrimethamine over time, and drug accumulation over time. Second, two pharmacodynamic assays will be performed. The first will employ quantitative flow cytometry to assess total STAT3 levels as well as phosphorylation of STAT3 in vivo in CLL cells over time. The second will use PCR to assess the expression of a panel of ten STAT3 target genes that Dr. Frank's lab has found to be most informative of pyrimethamine-induced STAT3 inhibition in CLL cells treated in vitro, as well as four genes they have found to be invariant which will be used for normalization. Taken together these assays should enable us to determine whether adequate pyrimethamine levels were present and whether pyrimethamine successfully inhibited STAT3 phosphorylation and activation of target genes.

Preliminary results of PK and PD analyses from the first three dose cohorts, up to 50 mg daily, suggest that the achieved blood levels of pyrimethamine thus far may be lower than required for most effective STAT3 inhibition. For that reason we are adding dose cohorts to the Phase 1 study.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

Subjects with CLL/SLL:

3.1.1 Subjects must be diagnosed with CLL / SLL (chronic lymphocytic leukemia / small lymphocytic lymphoma) based on the standard histologic and immunophenotypic criteria described in the WHO classification of lymphoid malignancies, including immunophenotypic confirmation that the tumor cells co-express B cell antigens CD19 / 20 and CD5. Mantle cell lymphoma should be excluded based on positive staining of the tumor cells for CD23, or the absence of staining of the tumor cells for cyclin D1 or the absence of t(11;14). This diagnosis should be confirmed at a Dana-Farber Harvard Cancer Center institution (DFCI, BWH, MGH, BIDMC) within approximately one month after the subject is registered. Any question on histology confirmation should be brought to the attention of the Principal Investigator.

3.1.2 Participants must have measurable disease, defined as lymphocytosis ≥ 5,000 / ul, or at least one palpable or CT measurable lesion > approximately 1.5 cm, or bone marrow involvement > approximately 30%.

3.1.3 Subjects must have relapsed after at least one prior purine analogue-containing regimen (fludarabine, cladribine or pentostatin), OR at least two non-purine analogue containing regimens.

Subjects with T-LGL:

T-LGL judged by the investigator to require therapy based upon:

1) Severe neutropenia (absolute neutrophil count <500/microL)
2) Moderate neutropenia (absolute neutrophil count <1000/microL) with recurrent infections
3) Symptomatic or transfusion dependent anemia
4) Severe thrombocytopenia (<50,000/microL)
5) Hepatic infiltration resulting in abnormal liver function tests
6) Symptomatic splenomegaly

Patients with previously untreated or relapsed/refractory disease will be eligible
All Subjects:

3.1.4 Age ≥18 years.

3.1.5 Life expectancy of greater than 3 months.

3.1.6 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix B).

3.1.7 Participants must have normal organ function as defined below:

- total bilirubin $\leq$ 1.5X institutional ULN unless due to indirect hyperbilirubinemia related to Gilbert's syndrome or hemolysis, or to disease infiltration
- AST (SGOT)/ALT (SGPT) $\leq$ 5 X institutional upper limit of normal
- creatinine $\leq$2X institutional ULN

3.1.8 Subjects must require treatment based on IWCLL 2008 criteria (Appendix A).

3.1.9 The effects of pyrimethamine on the developing human fetus are unknown. For this reason and because pyrimethamine is teratogenic in animals, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Participants who have had chemotherapy or radiotherapy within 3 weeks prior to entering the study or those who have not recovered from clinically significant adverse events due to agents administered more than 3 weeks earlier. The Principal Investigator or treating investigator determine if any abnormal laboratory values or toxicities are due to prior agents administered vs. disease and if they are clinically significant.

3.2.2 Participants may not be receiving any other study agents.

3.2.3 Known CNS involvement with CLL or T-LGL.

3.2.4 History of allergic reactions or sensitivity to pyrimethamine.

3.2.5 Patients taking folic acid are eligible if the folic acid is discontinued prior to pyrimethamine administration and not taken for the duration of time enrolled on this study.

3.2.6 Prior allogeneic SCT is an exclusion only if the subject has active graft vs host disease or requires immunosuppression other than a constant stable dose of glucocorticoids (the latter is permitted).

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection not controlled by appropriate antibacterial, antiviral or antifungal therapy, symptomatic congestive heart failure, unstable angina pectoris, clinically significant cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Pregnant women are excluded from this study because pyrimethamine is a/an class C agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with pyrimethamine, breastfeeding should be discontinued if the mother is treated with pyrimethamine. These potential risks may also apply to other agents used in this study.

3.2.9 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with pyrimethamine. In addition, these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

The investigators will work hard to enroll women and minorities on this clinical trial. CLL is approximately two-fold more common in men than women but we will aim for representation of women at least equivalent to the balance among those diagnosed with the disease. The Inclusion / Exclusion criteria are broad and should not have a negative impact on accrual of underrepresented populations.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment within two weeks. Issues that would cause treatment delays should be discussed with the Principal Investigator. Minor delays due to physician clinic schedule, holidays, vacations, etc. are permitted. If a participant does not receive protocol therapy following registration as described herein, the participant's protocol status should be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist unless granted an eligibility deviation from the Regulatory Sponsor and the IRB.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for pyrimethamine are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Subjects will self-administer this oral drug once daily, in the morning, with food. Dose is to be held until lab work results are reported on days that subjects are scheduled for clinic (i.e, Cycle 1 Day 1, 8 and 15, Cycle 2 Day 1, etc.). Therapy will be continuous, with one cycle arbitrarily defined as 28 days (+/- 3 days). All subjects will initially be treated for one 28 day cycle, followed by response and toxicity evaluation. On the day of a clinic visit, subjects will delay taking their dose until after their laboratory tests have been drawn at clinic. The subject may delay eating or eat again after the dose. This will be noted on the diary.

At screening all subjects will be assessed by blood draw for folate deficiency prior to initiating study therapy. Those found to be deficient will be repleted with folic acid, 1 mg. per day for at least 7 days, until level reaches normal range. If deficient at baseline, folate level should be redrawn approximately every month while on study drug, or at required visits, whichever is less. Once within normal range, subjects should discontinue supplemental folate administration. Baseline assessments will not need to be repeated if they fall out of screening window due to folate repletion.

The initial component of this study will be a Phase I dose escalation to assess the safety of pyrimethamine in this patient population. The first three dose levels below were completed with expansion, but the PK and PD data suggested inadequate blood drug levels. For that reason and because the safety data thus far look good (see below), cohorts 4 and 5 have been added as per the table.

Table 1: Dose-Escalation Schedule

Dose-Escalation Schedule 1 cycle = 28 days (+/- 3 days)		
	Dose Level	**Dose of Pyrimethamine**
Starting Dose →	**1**	**12.5 mg, Once Daily***
	2	**25 mg, Once Daily**
	3	**50 mg, Once Daily**
	4	**100 mg Once Daily**
	5	**150 mg Once Daily**

Following completion of the additional cohorts of the Phase 1 study, the PK, PD and safety data will be assessed to determine the recommended Phase 2 dose. Any subjects who are continuing on study from the Phase I portion, who are deriving clinical benefit without significant toxicity and who are currently on a lower dose than the recommended Phase 2 dose, may be considered for dose escalation to the newly determined Phase 2 dose, at the discretion of the treating physician and the Principal Investigator.

*The 25 mg tablet is scored, and will be split in half either by the research nurse or pharmacist.

5.1 Pre-treatment Criteria

5.1.1 Cycle 1, Day 1

There should be no clinically significant change in the status of the patient between screening and C1D1. If there is, the laboratory values of the eligibility criteria should be rerun, and the patient approved for study start by the treating physicians or PI. As per Section 9, Study Calendar, CBCD and chemistries should be drawn on Day 1 of each cycle.

5.1.2 Subsequent Cycles

All toxicities for which pyrimethamine was held must have resolved to grade 1 or less prior to re-initiation of study therapy. For a detailed discussion of the management of hematologic toxicity **please see section 6.3.1**

5.2 Agent Administration

5.2.1 Pyrimethamine

- Administration – Subjects will self-administer Pyrimethamine once daily, in the morning, with food.

- Dosing – The daily dose will be determined during the Phase I study by the cohort to which the study is enrolling. (See Table 1: Dose-Escalation Table, Section 5.0) Following the determination of the Phase 2 dose, all subjects will be enrolled to that dose level.

- Hydration – No hydration is required. *

- Oral Doses – The daily dose should be administered with food. Any doses vomited within one hour of administration should be retaken. Any doses missed can be taken later on the same day they were missed, with food. If a missed dose is not discovered until the following day, the dose should not be made up. There are no foods or liquids that are contraindicated.

*Investigators should monitor patients for tumor lysis with labs twice per week in the first cycle and at least weekly thereafter (in the first cycle), and subjects will be encouraged to maintain oral hydration. Intravenous hydration or additional treatment for TLS should commence if any laboratory evidence of such is demonstrated. At a minimum, hyperuricemia, hydration, and electrolyte disturbances will be monitored closely and addressed promptly.

5.3 Definition of Dose-Limiting Toxicity

Dose limiting toxicity (DLT) will be defined as any non-hematologic toxicity of grade 3 or greater severity (excluding asymptomatic grade 3 laboratory abnormalities that are not life-threatening and respond to treatment; grade 3 fatigue; grade 3 nausea, vomiting or diarrhea occurring without optimal prophylaxis). Infectious toxicities will only be considered dose-limiting if grade 4 or greater. Any grade 4 non-hematological toxicity, as well as any irreversible grade 2 cardiac, renal or neurologic toxicities, will be considered dose-limiting. Grading of non-hematologic toxicities will be according to CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to toxicities experienced during the first cycle of treatment only.

Hematologic toxicity will be graded according to the 2008 IWCLL criteria (see table in section 6.3.1 under dose modifications)[19]. Hematologic toxicity will only be considered dose-limiting as follows:

Grade 4 neutropenia persisting for at least 7 days after discontinuation of study drug in Cycle 1 only. Neutropenia will not considered a DLT for subjects with T-LGL.

Grade 4 thrombocytopenia persisting for at least 7 days after discontinuation of drug in Cycle 1 only.

Abnormal hematologic values are not noted as inclusion or exclusion criteria. Subjects with starting ANC $\leq$ 1000 (or greater than 1000 but requiring growth factor support) or starting platelet count $\leq$20,000 will not be evaluable for hematologic toxicity or hematologic DLT, as per consensus recommendations for clinical trials in CLL and T-LGL. Determination of whether to hold pyrimethamine for hematologic toxicities will be determined in these patients on a case by case basis by the treating physician and the Principal Investigator. As in Section 5.4 following, growth factor support and/or platelet transfusion are allowed at the investigator's discretion.

Dose-limiting toxicities will be determined during the 1st 28-day cycle only. There are no DLT parameters post Cycle 1.

Management and dose modifications associated with the above adverse events are outlined in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

Dose escalation will proceed within each cohort according to the following scheme.

Number of Participants with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 participants at the next dose level.
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional participants will be entered at the next lowest dose level if only 3 participants were treated previously at that dose.
1 out of 3	Enter at least 3 more participants at this dose level. • If 0 of these 3 participants experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional participants will be entered at the next lowest dose level if only 3 participants were treated previously at that dose.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended Phase II dose. At least 6 participants must be entered at the recommended Phase II dose.

5.4 General Concomitant Medication and Supportive Care Guidelines

Patients will receive the following additional supportive care medications:

- Allopurinol 300 mg po QD day 1-10 after initiation of study therapy. This would be considered standard of care, unless the subject has an allergy. Subjects with T-LGL are not required to receive allopurinol.

- Anti-emetics may be prescribed as needed; no specific premedication is required or suggested.

- Subjects who have been taking trimethoprim-sulfamethoxazole for PCP prophylaxis should be assessed for their real risk of PCP. Due to an increased risk of hypersensitivity reactions and/or myelosuppression when given together with

pyrimethamine, this medication should be discontinued if possible or changed to atovaquone or other equivalent PCP prophylaxis during the time on study therapy.

- Subjects receiving acyclovir or equivalent prophylaxis should continue these medications. Consideration should be given to initiating acyclovir prophylaxis at 400 mg po TID (or equivalent) for those patients not yet receiving it.

- Lorazepam given concurrently with pyrimethamine has been associated with an increased risk of transaminitis. If substitution with another benzodiazepine or other medication is possible, then that is preferred; otherwise close monitoring is appropriate.

- All patients requiring transfusions should receive irradiated, leukopore filtered blood products.

- Subjects may receive neupogen, pegfilgrastim, erythropoietin or related growth factors at the discretion of the treating physician. Subjects in whom study therapy has been held due to myelosuppression may be given a short course of leucovorin 10 mg po QD, at the discretion of the treating physician in consultation with the Principal Investigator.

- Because there is a potential for interaction of pyrimethamine with other concomitantly administered drugs, especially sulfonamides or myelosuppressive drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

5.5 Duration of Therapy

Initial response assessment will occur after 28 days of study therapy. Those subjects with stable or responding disease may continue on study indefinitely if their treating physician and the Principal Investigator feel that they are deriving clinical benefit. Subjects with significant adverse events or progressive disease should be removed from study.

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of significant adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Participant decides to withdraw from the study, or

- General or specific changes in the participant's condition or compliance render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.6 Duration of Follow Up

Participants will be followed until initiation of their next therapy or until death, whichever occurs first. Their general medical condition and survival status will be followed for the duration of their life, even after additional therapies. Subjects not presenting in clinic on a regular basis may be called by the study staff when possible. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.5 apply. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator (or Protocol Chair), Jennifer R Brown MD PhD at 617-632-4894.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations, and as per Table 2, Section 6.3. Toxicity assessments for non-hematologic toxicities will be done using CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocol Development/electronic_applications/ctc.htm. Please see below for appropriate evaluation of hematologic toxicities, which will be according to the IW-CLL 2008 criteria.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Event Lists(s) for Pyrimethamine

Hypersensitivity reactions, occasionally severe (such as Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme, and anaphylaxis), and hyperphenylalaninemia, can occur particularly when **pyrimethamine** is administered concomitantly with a sulfonamide. Anorexia and vomiting may occur. Vomiting may be minimized by giving the medication with meals; it usually disappears promptly upon reduction of dosage. Higher doses may produce megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, hematuria, and disorders of cardiac rhythm. Hematologic effects, however, may also occur at low doses in certain individuals. Pulmonary eosinophilia has been reported rarely.

6.2 Toxicity Management

Following the ingestion of 300 mg or more of **pyrimethamine**, gastrointestinal and/or central nervous system signs may be present, including convulsions. The initial symptoms are usually gastrointestinal and may include abdominal pain, nausea, severe and repeated vomiting, possibly including hematemesis. Central nervous system toxicity may be manifest by initial excitability, generalized and prolonged convulsions which may be followed by respiratory depression, circulatory collapse, and death within a few hours. Neurological symptoms appear rapidly (30 minutes to 2 hours after drug ingestion), suggesting that in gross overdosage pyrimethamine has a direct toxic effect on the central nervous system. The fatal dose is variable, with the smallest reported fatal single dose being 375 mg. There are, however, reports of pediatric patients who have recovered after taking 375 to 625 mg.

There is no specific antidote to acute pyrimethamine poisoning. In the event of overdosage, symptomatic and supportive measures should be employed. Gastric lavage is recommended and is effective if carried out very soon after drug ingestion. Parenteral diazepam may be used to control convulsions. Folinic acid should also be administered within 2 hours of drug ingestion to be most effective in counteracting the effects on the hematopoietic system. Due to the long half-life of pyrimethamine, daily monitoring of peripheral blood counts is recommended for up to several weeks after the overdose until normal hematologic values are restored.

6.3 **Dose Modifications/Delays**

As noted above, an individual patient who experiences DLT will have therapy held until resolution to grade 1 or less, and may then resume therapy at one dose level below their

previous level at the discretion of the investigator and treating physician. If the toxicity persists at >grade 1 for more than 4 weeks, the subject must be removed from the study.

In the Phase 2 study, all grade 3 (excluding asymptomatic laboratory abnormalities, or grade 3 nausea, vomiting or diarrhea readily controlled with appropriate supportive care) or grade 4 non-hematologic toxicities will result in at least temporary discontinuation from study drug. When the toxicity resolves to grade 1 or less, the treating physician and the Principal Investigator may make a decision about the appropriateness of continuing the patient on study. Toxicity grading is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) non-hematologic toxicities. Any non-hematologic toxicity (excluding asymptomatic laboratory abnormalities, or grade 3 nausea, vomiting or diarrhea readily controlled with appropriate supportive care) that persists at > grade 1 for more than four weeks will result in permanent discontinuation from the study.

Table 2: Dose-Modification

Dose-Modification		
	Dose Level	**Reduce Dose To:**
Starting Dose	**150 mg**	**100 mg**
	100 mg	**50 mg**
	50 mg	**25 mg**
	25 mg	**12.5**
	12.5	**Hold**

Subjects removed from the study for toxicity will be followed until initiation of next therapy or death.

6.3.1 Hematologic Toxicity

Hematologic toxicity will be graded according to IWCLL 2008 criteria[19], as enumerated in the table below.

ANC / μl	**Toxicity Grade**	**Decrease in Plts from Pre-Tx Value (Use this correction if starting plt count < 100,000)**
>= 2,000	0	0-10%
>= 1,500 - 2000	1	11-24%
>= 1,000 – 1,500	2	25-49%

>= 500 – 1,000	3	50-74%
< 500	4	>=75%

Subjects will not be evaluable for hematologic toxicity, hematologic dose-limiting toxicity or holding of therapy based on low ANC if the starting ANC < 1000 or the starting ANC is greater than 1000 with the assistance of growth factors. The ANC may be supported by growth factors throughout study therapy for this group of patients. In particular, LGL patients with pre-existing neutropenia will not be evaluable for toxicity related to neutropenia and may continue study drug at their assigned dose regardless of neutrophil count.

Subjects with baseline platelets < 20,000 will not be evaluable for platelet toxicity. Subjects with baseline platelets ≥100,000 will be evaluated per CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE); i.e, do not use the correction in the table above for assessing platelet toxicity when baseline platelets ≥100,000. When baseline and Cycle 1 Day 1 ANC and/or platelets differ, the lower of the two values should be used for assessing DLT, dose modifications and hematologic toxicity.

For subjects with initial ANC =/>1000, neutropenia grading will follow the table above and development of grade 4 neutropenia will result in temporary discontinuation of study drug. Consideration may be given to administration of folic acid, low-dose leucovorin or growth factors to these patients. Once recovery occurs subjects may be restarted at their previous dose. Those whose grade 4 ANC persists for at least 7 days despite these supportive care measures will be considered to have suffered dose limiting toxicity if this happens in the first cycle, only. If these subjects experiencing DLT are continued on study after resolution, they should undergo a one dose level reduction in dose. Although NOT considered a DLT in cycle 2 and beyond, if a subject experiences grade 4 ANC that persists for at least 7 days despite the supportive care measures listed above, the subject may undergo a one dose level reduction in dose at investigator discretion

Thrombocytopenia will be graded based on the table above, using adjusted parameters for all subjects with a starting platelet count < 100,000. For those subjects with a starting platelet count =/> 100,000, development of thrombocytopenia will be graded based on CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). Any subject with a starting platelet count < 20,000 will not be evaluable for hematologic toxicity, hematologic dose-limiting toxicity or holding of therapy based on thrombocytopenia. For any subject with a starting platelet count > 20,000, development of a platelet count < 20,000 is considered grade 4 thrombocytopenia and will result in holding of study drug. Consideration may be given to administration of folic acid or low-dose leucovorin. Persistence of grade 4 thrombocytopenia for > 7 days despite these supportive care measures will be considered a DLT if this happens in the first cycle. If these subjects experiencing DLT are continued on study after resolution within one month, a dose reduction by one dose

level per the dose escalation table is required. If grade 4 thrombocytopenia persists for > 7 days in cycle 2 or beyond, consideration may be given to a one dose level reduction, at the discretion of the Principal Investigator and treating investigator.

6.3.2 Rash

For grade 3 or greater skin rash, study drug should be discontinued. If the rash resolves (back to grade 1 or less, within 4 weeks) with supportive care and is not IgE mediated or accompanied by other signs of allergic hypersensitivity, patients may resume study therapy at one lower dose level, or if already at the lowest dose level, may resume at that dose with addition of prophylactic antihistamine. Consideration may be given to use of a prophylactic antihistamine, particularly diphenhydramine, even in patients resuming at a reduced dose.

6.3.3 Anorexia/Vomiting:

For grades 1-4 anorexia/vomiting, study drug should be continued with initiation of antiemetic therapy. If drug needs to be held until patient can tolerate drug with supportive care, patient may resume at same dose level. If antiemetics do not bring relief, then the dose should be reduced one level. Patients with grade 3 or 4 anorexia/vomiting that do not respond to supportive care should be discontinued from study drug, but will remain on study for follow-up procedures until initiation of another therapy.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Pyrimethamine

7.1.1 Description

The chemical name of pyrimethamine is 5-(4-chlorophenyl)- 6-ethyl-2,4-pyrimidinediamine. It acts as a folate antagonist and a STAT3 inhibitor.

Pyrimethamine is well absorbed orally with peak levels occurring between 2 to 6 hours following administration. It is eliminated slowly and has a plasma half-life of approximately 96 hours. Pyrimethamine is 87% bound to human plasma proteins.

7.1.2 Form

Pyrimethamine is supplied as 25 mg tablets. Depending on dose level, 0.5, 1 or 2 tablets will be taken by mouth once per day while on study. Do not crush or chew. *The 25 mg tablet is scored, and will be split in half either by the research nurse or pharmacist.

7.1.3 Storage and Stability

Pyrimethamine is stored at room temperature and protected from light.

7.1.4 Availability

The agent is commercially available, but will be provided free of charge to patients enrolled on this protocol.

7.1.5 Ordering

The agent will be ordered by the Investigational Pharmacy who will maintain adequate stocks for this protocol.

7.1.6 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the CTEP home page at http://ctep.cancer.gov for the Procedures for Drug Accountability and Storage or to obtain a copy of the DARF.)

7.1.7 Destruction and Return

Study drug for daily oral administration will be supplied by the DFCI pharmacy as 25 mg tablets in bottles. Drug accountability will be performed as per current DF/HCC SOP All unused study drug should be returned by the patient to the Investigator or research nurse/PA.

At the end of the study, unused supplies of pyrimethamine should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacokinetic Studies

Pharmacokinetics will be performed to assess pyrimethamine accumulation over time, given the long half-life of the drug. 1 heparin-anticoagulated green top tube will be drawn prior to therapy, prior to dose on **one** of days 2-5 (optional) and prior to dose on each subsequent clinic visit (weekly during cycle 1, every other week during cycle 2, and monthly thereafter until discontinuation from study drug. Pharmacokinetics will also be drawn at time of off study visit and once again 2-4 weeks later if the patient has

not started additional therapy. These samples will be collected at all of the above timepoints and transferred for processing and freezing.

PK samples are drawn, processed and stored as follows:

The Frank Laboratory – a division of
Louis B. Mayer Research Laboratories
David Frank, MD, Laboratory Director
Mayer 5-522B
Dana-Farber Cancer Institute
44 Binney St.
Boston, MA 02115

The frozen samples will be processed in batches by the DF/HCC Core Pharmacokinetics laboratory run by Dr. Jeff Supko:

Clinical Pharmacology Lab
Jeffrey Supko, MD, Director
Gray / Jackson
Massachusetts General Hospital
55 Fruit St.
Boston, MA 02114

8.2 Pharmacodynamic Studies

Pharmacodynamic studies will be run in Dr. Frank's lab.

Two 6 cc heparin-anticoagulated green top tubes (or equivalent) will be collected and transferred to Dr. David Frank's lab on Mayer 5 on the following days/times:
-Screening
-Pre and two hours post drug on day 1 of cycle 1
-One day among Days 2-5 therapy (optional)
-Day 8, 15, 22 cycle 1
-Day 1 and 15 cycle 2
-Day 1 of subsequent cycles up to cycle 6
-Off study visit
-2-4 weeks after off study visit (if subsequent therapy has not begun)
-3 month follow up appointment (if subsequent therapy has not begun)

As outlined above, prior to initiating therapy, and at the time of each subsequent clinic visit while on study, patients will have approximately 10 ml of blood drawn into a heparinized tube. Mononuclear cells will be isolated by Ficoll-based density separation, and the proportion of CLL cells will be determined by flow cytometry. If greater than 90% of the isolated cells are CLL cells (CD5+CD19+CD20+), these will be used for subsequent analyses. If less than 90% display a CLL phenotype, then we will use antibody-based

magnetic cell separation to increase the purity to this level. It will be necessary to isolate a minimum of 5 x 10^6 cells for the required analyses.

Cells will be analyzed in two ways in parallel, as follows.

8.2.1 Laboratory Correlative Studies: Determination of STAT3 Inhibition In Vivo

8.2.1.1 STAT3 Phosphorylation

An aliquot of 1 x 10^6 cells will be used for a quantitative flow cytometric assay to measure total STAT3 and serine phosphorylation of STAT3. Cells will be fixed, permeabilized, and stained with the specific antibodies, employing a technique we have used successfully on clinical CLL specimens in the past [20]. This analysis will indicate the concordance of normalized expression of STAT3 target genes (as described below) with the level and phosphorylation of STAT3. In addition, it will allow a longitudinal analysis of changes in the quantity and phosphorylation state of STAT3 in CLL cells of patients on pyrimethamine. If pyrimethamine is decreasing the survival of CLL cells in these patients, then it will be expected that the total number of CLL cells will decline over time. Furthermore, if CLL cells persist, it will allow a determination of whether a change in the proportion of CLL cells with activated STAT3 is occurring over time.

8.2.2.1 STAT3 Target Gene Expression

We will also analyze expression of a panel of 10 STAT3 target genes that we have found to be most informative of pyrimethamine-induced STAT3 inhibition in CLL cells treated in vitro, as well as four genes we have found to be invariant which will be used for normalization. STAT3 target gene expression will be quantitated for individual genes, and a weighted normalized score will also be generated [12]. This analysis will provide a correlation of STAT3-dependent gene expression with STAT3 phosphorylation by the flow cytometric assay, a determination of the effect of pyrimethamine dosing on STAT3-dependent gene expression, and a longitudinal analysis of STAT3 dependent gene expression with pyrimethamine treatment in individual patients. Furthermore, in conjunction with the pharmacokinetic analyses described above, these studies will provide correlations between plasma concentrations of pyrimethamine and effects on STAT3 phosphorylation and STAT3-dependent gene expression.

8.3 Determination of CLL and T-LGL Biologic Prognostic Features

For CLL patients, peripheral blood will be collected in two 6 cc green top tubes and one 6 cc red top tube prior to therapy on all DFCI patients, along with saliva under the auspices of protocols 01-206 or 99-224 (if the patient has a low white blood cell count, then four 6 cc green top tubes will be collected instead). These samples can be

drawn at screening or Day 1 (prior to therapy). The CLL Research Consortium Tissue Bank, our collaborator on Protocol 99-224, will determine IgVH mutational status and ZAP70 expression on all samples from DFCI subjects. This will be determined from three 6 cc green top tubes. **BIDMC subjects are unable to be registered to 99-224, but will have three 6 cc green top tubes drawn as part of this protocol, and they will be banked here for prognostic factor measurements later.*

In addition, and for all T-LGL patients, two 6 cc green tops & one 6 cc red top will be used for banking of peripheral blood mononuclear cells in the JFB 426 Brown lab, at screening and at day 15 of therapy (if the patient has a low white blood cell count, then four 6 cc green top tubes will be collected instead). For those patients seen at BIDMC, these green-topped tubes and red-topped tube will be collected as part of this protocol and transferred to JFB426 Brown lab for banking.

The clinical flow cytometry laboratory will determine CD38 expression. The DFPCC/BWH clinical cytogenetics laboratory will perform interphase FISH cytogenetics.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 month prior to start of protocol therapy. CBCD and chemistry blood will be redrawn on Cycle 1 Day 1. Scans must be done ≤4 weeks prior to the start of therapy. In the event that the participant's condition is significantly deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

All assessments must be performed prior to administration of any study medication. Failure to perform any assessment prior to administration of study drug will constitute a protocol violation. The Principal Investigator will determine if the event is a minor or major violation, per DF/HCC SOPs. Informed Consent must take place prior to any study specific procedures. All study assessments and medications should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

Study Calendar Follows

Test/Procedures	**Pre-Study**	**Weekly in 1st 28 day**	**Every 2 wks in 2nd cycle**	**Every 3-6 months at investigator discretion and**

		cycle (TLS blood X2 1st week of C1)	**and then monthly**	**at discontinuation of study treatment**
Informed Consent	X			
History/PE/AE evaluation/ Perf. status	X	X	X	X
Weight/VS	X	X	X	X
Screen & Replete Folate (replete as necessary)	X		X[10]	
Concomitant Meds	X	X	X	
CBC with Diff*	X	X	X	X
CMP[1,*]	X	X	X	X
Tumor Lysis Monitoring[1A]	X	X		
CAP CT**	X		X[2]	X[2]
Tumor Measurement by physical exam[9]	X		X[9]	X[9]
Bone Marrow Biopsy	X			X[7]
FISH[8]	X			
Patient Diary	X[4]	X[4]	X[4]	X[4]
Pharmacokinetic Studies	X[5a]	X[5a]	X[5a]	X[5a]
Pharmacodynamic Studies	X[5b]	X[5b]	X[5b]	X[5b]
CD4 count (T cell subsets)	X		X[6]	X[6]
IgG, IgA, IgM	X		X[6]	X[6]
Beta-2-microglobulin	X		X[6]	X[6]
Flow cytometry for CD38[8]	X			
Samples for CLL prognostic factors/tissue banking[3]	X[3]	X[3]		
IgVH Testing	X			

1 Comprehensive Metabolic Panel: Creatinine, glucose, total bilirubin, alkaline phosphatase, and SGOT/SGPT, LDH. Serum beta-HCG within seven days prior to initiation of therapy for women of child-bearing potential.

1A Investigators should monitor patients for tumor lysis with calcium, magnesium, phosphate, uric acid and LDH, in addition to a full comprehensive metabolic panel, twice during the first week, weekly for the rest of cycle 1, and then subsequently at times of other lab draws for the rest of the study. Treatment for TLS should commence should evidence of such

be demonstrated. At a minimum, hyperuricemia, hydration, and electrolyte disturbances should be monitored closely and addressed promptly.

2 CT scans (chest/abdomen/pelvis) to be performed at screening in all patients and again at the end of the 1st, 3rd and 6th months of therapy; subsequent scans shall be at the discretion of the treating physician if needed for adequate response evaluation.

3 Please see Section 8.3 ″Determination of CLL Biologic Prognostic Factors″ for details on sample collection.

4 Diary dispensed with study drug and retrieved at scheduled visits.

5a One heparin-anticoagulated green top tube (pharmacokinetic studies) to be collected during screening, on cycle 1 day 1 prior to drug; prior to drug on one day among days 2, 3, 4 or 5, (optional); and prior to dose (trough) at each subsequent clinic visit as per Section 8.1, above. A heparin-anticoagulated green top tube will also be collected at time of off study visit and once again 2-4 weeks later if the patient has not started additional therapy. All samples are to be delivered to the David Frank lab on Mayer 5. This includes BIDMC patients.

5b Two 6 cc heparinized blood for cell isolation (pharmacodynamic studies) is to be collected during screening, on cycle 1 day 1 prior to drug and approximately two hours after drug administration, prior to dose (trough) on days 2, 3 4 or 5 of therapy, pre drug on cycle 1 days 8, 15,and 22, cycle 2 days 1 and 15, and day 1 of subsequent cycles monthly up to cycle 6. Pharmacodynamic studies will also be collected at time of off study visit, 2-4 weeks later if the patient has not started additional therapy, and lastly at the 3 month follow up appointment (if subsequent therapy has not started). These are to be delivered to the David Frank lab on Mayer 5. This includes BIDMC patients.

6 These laboratory tests will be done at baseline, at the end of cycles 1, 3, 6, end of treatment, and every 3-6 months in follow-up.

7 Bone marrow biopsy will be done at screening (unless done within previous 6 months and the Principal Investigator feels it does not need to be repeated, or if prior marrow was positive and the patient has not had CLL treatment since) and thereafter only to confirm CR. Confirmation of complete response by bone marrow biopsy should occur approximately 3 months after the clinical criteria for CR are first met. Bone marrow biopsy is not required at off study.

8 To be performed on bone marrow if aspirate obtained; otherwise on peripheral blood prior to therapy initiation.

9 Up to 6 palpable nodes should be followed as targets, with bi-dimensions noted by the clinician whenever possible. To be performed at screening in all patients and again at the end of the 1st, 3rd and 6th months of therapy, and in follow up if needed for adequate response evaluation.

10 If deficient at baseline, folate level should be redrawn approximately every month while on study drug, or at required visits, whichever is less.

* Hematology and chemistry blood tests should be resulted and patient assessed for toxicity prior to taking dose of pyrimethamine.

** Subjects with T-LGL do not require radiology

10. MEASUREMENT OF EFFECT

Responses will be formally assessed initially at the conclusion of the 1st 28 day cycle, then at the conclusion of three cycles (three months), and then every three months thereafter, until disease progression, start of another treatment, or death. Response will be assessed in two ways: (1) By NCI-WG 1996 criteria[3] (as updated in the IWCLL 2008 criteria Section 10.2)[19] without the use of CT scans. In this case lymph nodes will be assessed based on physical exam measurements; (2) With the incorporation of CT findings as suggested for clinical trials in IWCLL 2008. Thus CT scans will be performed on all patients at the end of the 1st month, 3rd month and 6th month, if still on study we will evaluate the impact of the CT scans (1) if used only to confirm CR; (2) if used to assess lymphadenopathy regardless of PE findings. After the 6 month evaluation, further CT scans will generally not be done, but may be done at the discretion of the treating physician.

Following the initial screening bone marrow biopsy, additional bone marrow biopsies will be performed only to confirm complete remission.

T-LGL Response Criteria[21]:

Response to treatment should be evaluated by history, physical examination, and complete blood count with differential:

- Complete response — A hematologic complete response is defined as the complete normalization of blood counts (ie, hemoglobin >12 g/dL; platelets >150,000/microL; absolute neutrophil count >1500/microL; absolute lymphocyte count <4000/microL) in the setting of a circulating LGL count of less than 500/microL.
- Partial response — A hematologic partial response is defined as an improvement in blood counts that does not meet criteria for complete remission (eg, an absolute neutrophil count >500/microL or decreasing transfusion requirements).
- Treatment failure — Treatment failure is defined as an inability to achieve an at least partial response after four months of therapy.
- Progressive disease — Progressive disease is defined as a worsening of cytopenias, hepatomegaly, or splenomegaly

10.1 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression or death, whichever occurs earlier.

10.2 Antitumor Effect – Hematologic Tumors

Parameter	CR*	PR*	PD*
Group A			
Lymphadenopathy^	None > 1.5 cm	Decrease ≥50%	Increase ≥50%
Hepatomegaly	None	Decrease ≥50%	Increase ≥50%
Splenomegaly	None	Decrease ≥50%	Increase ≥50%
Blood lymphocytes	<4000/µL	Decrease ≥50% from baseline	Increase ≥50% over baseline
Marrow#	Normocellular, < 30% lymphocytes, no B-lymphoid nodules.	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from

			normal.
	Hypocellular marrow defines CRi		
Constitutional Symptoms@	None	Any	Any
Group B			
Platelet count	> 100 000/μL	> 100 000/μL or increase ≥50% over baseline	Decrease of ≥50% from baseline secondary to CLL
Hemoglobin	> 11.0 g/dL (untransfused and w/o erythropoietin)	> 11 g/dL or increase ≥50% over baseline	Decrease of > 2 g/dL from baseline secondary to CLL
Neutrophils#	> 1500/μL	> 1500/μL or > 50% improvement over baseline	Any

* CR (complete remission): all of the criteria have to be met, and patients have to lack disease-related constitutional symptoms
* PR (partial remission): at least two of the criteria of group A plus one of the criteria of group B have to be met. PR to be held at least two months and confirmed by restaging.
* SD is absence of progressive disease (PD) and failure to achieve at least a PR; PD: at least one of the above criteria of group A or group B has to be met.

^Sum of the products of multiple lymph nodes (as evaluated by CT scans in clinical trials, or by physical examination in general practice).
#These parameters are irrelevant for some response categories.
@Constitutional symptoms: Unintentional weight loss ≥10% within the previous 6 months, significant fatigue (ECOG PS 2 or worse, Fevers >100.5F or 38.0 C for 2 or more weeks without other evidence of infection, night sweats >1 month without evidence of infection

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1General

Adverse event collection and reporting is a routine part of every clinical trial. This study will use the descriptions and grading scales found in the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) that is available at http://ctep.cancer.gov/reporting//ctc.html and the IWCLL criteria for hematologic malignancies as outlined in Section 6.3.1.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication. Participants who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

11.2 Definitions

11.2.1 Adverse Event (AE)

An adverse event is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

11.2.2 Serious Adverse Event (SAE)

A serious adverse event is an undesirable sign, symptom, or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.2.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.2.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of common expected adverse events associated with the study agent(s).

11.2.3.2. Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.2.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.3 Recording Adverse Events

Adverse event information will be obtained at each contact with the participant. All adverse events will be recorded on the appropriate study-specific case report forms (CRFs).

11.4 Reporting Adverse Events

Each adverse event will be assessed to determine if it meets the criteria for serious adverse event. If a serious adverse event occurs, expedited reporting will follow local policies, and federal guidelines and regulations as appropriate.

It is the responsibility of the participating investigator to notify the Principal Investigator (or Protocol Chair), IRB, and others of all serious adverse events as required in the protocol.

The Principal Investigator (or Protocol Chair) will provide information with respect to adverse events and safe use of the study treatment (e.g., safety reports, Action Letters) to all participating investigators as soon as the information becomes available.

11.5 Sponsor Notification by Investigator

11.5.1 Serious Adverse Event Reporting Requirements

All events meeting the criteria for Serious Adverse Event (see Section 11.2.2) that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported as serious adverse events. Grade 4 hematogic toxicites are excluded from SAE reporting after the first cycle. Grade 4 ALC reporting is excluded from SAE reporting during all cycles.

The participating investigator must report each serious adverse event, regardless of attribution, to the Principal Investigator (or Protocol Chair) within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone and facsimile to:

Jennifer R Brown MD PhD Tel 617-632-4894 Fax 617-582-7909

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.5.2 Non-Serious Adverse Event Reporting Requirements

Non-serious adverse events will be reported to the Principal Investigator (or Protocol Chair) on the toxicity Case Report Forms.

11.6 Institutional Review Board (IRB) Notification by Investigator

The participating investigator will report all adverse events and serious adverse events to the Principal Investigator (or Protocol Chair) and to the IRB according to the local IRB's policies and procedures in reporting adverse events.

In the event of a multi-center study, the Principal Investigator (or Protocol Chair) will report adverse events and serious adverse events from all participating sites to the DFCI IRB according to the IRB's policies and procedures in reporting adverse events.

11.7 Food and Drug Administration (FDA) Notification by Sponsor-Investigator

The Sponsor-Investigator will report to the FDA any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.8 Hospital Risk Management Notification by Investigator

The participating investigator will report to the Principal Investigator (or Protocol Chair) and to local Risk Management any subject safety reports or sentinel events that require reporting according to institutional policy.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT

On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- ICH Consolidated Good Clinical Practice: Guidelines (E6) www.fda.gov/cder/guidance/iche6.htm
- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

- Title 21 Part 11 – Electronic Records; Electronic Signatures
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
- Title 21 Part 50 – Protection of Human Subjects
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
- Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
- Title 21 Part 56 – Institutional Review Boards
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
- Title 21 Part 312 – Investigational New Drug Application
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- Institutional research policies and procedures
  www.dfhcc.harvard.edu/clinical-research-support/clinical-research-operations-cro/policies-and-procedures

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14. STATISTICAL CONSIDERATIONS

14.1 Primary Objectives

14.1.1 Primary Objectives for Phase I Trial

A standard 3+3 dose-escalation design is used in the Phase I portion for assessing the safety and determining the MTD and recommended dose for the Phase II portion of this study of pyrimethamine in relapsed CLL/SLL and T-LGL patients.

14.1.2 Primary Objectives for Phase II Trial

After MTD is established in Phase I, 20 additional patients will be enrolled in the Phase II study and treated with pyrimethamine at the MTD. The primary objective for this portion of the study is to determine the overall response rate of pyrimethamine in these patients, including both CLL/SLL and T-LGL.

14.2 Sample Size and Patient Accrual

14.2.1 Phase I

To assess the MTD of pyrimethamine, a standard 3+3 dose-escalation design is used, with 5 planned pyrimethamine dose levels (Section 5); the observation period is one cycle (28 days). 3 patients are enrolled in the study initially, if no DLT is observed in the first 3 patients at a given dose level of pyrimethamine, escalation will proceed. If 2 or 3 DLTs are observed in the first 3 patients, escalation stops. If 1 DLT is observed, an additional 3 patients will be enrolled at the same dose level. If no DLT is observed in the additional 3 patients, escalation proceeds. If any DLTs are observed among the 3 additional patients, escalation stops.

Based on this dose escalation scheme, the probability of escalating to the next dose level for various true underlying rates of DLT is given in Table 1.

Table 1 Probability of Escalation for Various True Underlying DLT Rates for Pyrimethamine

True DLT Rate	1.0%	10.0%	20.0%	30.0%	40.0%	50.0%
Probability of Escalation	0.99	0.91	0.71	0.49	0.31	0.17

If the true underlying proportion of DLT is 30% at the current dose during the treatment period, there is a 49% chance of escalating to the next dose. We anticipate that a minimum of 2 to a maximum of 12 additional patients will be enrolled in the Phase I portion of the study.

14.2.2 Phase II

The Phase II portion is a single arm study which consists of determining the overall response rate of pyrimethamine in relapsed CLL/SLL and T-LGL patients. The overall response rate is not expected to differ between these two diseases and therefore one overall response rate will be reported. A 35% overall response rate will be considered promising. The first response evaluation will be assessed after the first cycle of treatment then at the end of three cycles.

Six Phase I patients treated at the MTD of pyrimethamine will be enrolled in the Phase II part of the study. A Simon's two stage optimal design is used to compute the sample size. A total number of 26 patients is needed in order to detect a 35% response rate, assuming the ORR for the null hypothesis is 10%, with 90% power and 10% type I error. The study will stop early if only 1 or 0 responses out of the first 10 patients is observed, and the drug will not be declared promising if less than 5 responses are seen in the total 26 patients. The probability of stopping at the first stage if the true ORR is less than 35% is 0.736.

14.3 Primary Analysis

The overall response rate and a 90% confidence interval will also be calculated, using the method of Atkinson and Brown for a two stage study. Descriptive statistics for patients' baseline characteristics will be summarized using descriptive statistics (median, interquartile range, proportions).

14.4 Secondary Objectives

- To assess the toxicity profile of pyrimethamine in relapsed CLL/SLL and T-LGL, both acutely and over prolonged daily dosing.
- To determine pyrimethamine levels in vivo with prolonged dosing.
- To determine the progression free and overall survival following pyrimethamine for the treatment of relapsed CLL/SLL and T-LGL.
- To determine whether pyrimethamine inhibits STAT3 in vivo by assessing downregulation of STAT2 dependent gene expression in CLL cells and/or peripheral blood.
- To determine whether known prognostic factors in CLL/SLL correlate with reponse to pyrimethamine.
- To assess the impact of CT scans on response evaluation in relapsed CLL/SLL and T-LGL.

14.5 Secondary Analysis

Kaplan Meier method will be used to estimate the median of overall and progression free survival. The number and percent of patients who experienced toxicities on pyrimethamine after at least one dose of treatment will also be summarized. The paired t-test or Wilcoxon's signed rank test, depending on the symmetry of the data, will be used in univariate analyses for continuous variables of gene expression data, based on the ratio of post-therapy to baseline expression. Spearman's correlation coefficients will be used

for assessing relationships between continuous variables for some known prognostic factors and Fisher's exact test will be used for finding associations between categorical variables.

14.6 Reporting and Exclusions

14.6.1 Evaluation of toxicity. All participants who take at least one dose of pyrimethamine on study will be evaluable for toxicity from the time of their first treatment.

14.6.2 Evaluation of response. All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, 8) CRi or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

All of the participants who met the eligibility criteria should be included in the main analysis of the response rate. Participants in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. If a bone marrow is not assessed at restaging it will be noted on the appropriate CRF, and an otherwise complete response would become a PR.

15. PUBLICATION PLAN

The results will be made public within approximately 24 months of the end of data collection. The initial report may be a meeting abstract followed by a manuscript for a peer-reviewed journal. This study will be registered at clinicaltrials.gov after IRB approval and activation.

16. REFERENCES

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Appendix A IWCLL 2008 Indications for Therapy for CLL

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
2. Massive (ie, at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3. Massive nodes (ie, at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts obtained at intervals of 2 weeks over an observation period of 2 to 3 months. In patients with initial blood lymphocyte counts of less than 30×10^9/L (30 000/μL), LDT should not be used as a single parameter to define a treatment indication. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy (see section 10.2).
6. Constitutional symptoms, defined as any one or more of the following disease-related symptoms or signs:
    a. Unintentional weight loss of 10% or more within the previous 6 months;
    b. Significant fatigue (ie, ECOG PS 2 or worse; inability to work or perform usual activities);
    c. Fevers higher than 100.5°F or 38.0°C for 2 or more weeks without other evidence of infection; or
    d. Night sweats for more than 1 month without evidence of infection.

Appendix B

Performance Status Criteria

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

Appendix C

Study Diary

Pyrimethamine Study Drug Diary

Study Participant
Self-Administration
Study Drug Diary
Dana-Farber/Harvard Cancer Center

Participant Identifier: _____
Protocol # :
Your MD _____ Phone _____
Your RN _____ Phone _____

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09 - 6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken
Example: Allopurinol	300 mg	Jan 1 to 10	Per MD

STUDY DRUG INSTRUCTIONS:

Study Drug: Pyrimethamine
How Much: Your dose is
How Often: You will take Pyrimethamine once daily
When: You should take your dose orally, in the morning, with food.

HOLD DOSE ON DAYS THAT YOU REPORT TO CLINIC UNTIL YOUR DOCTOR OR NURSE TELLS YOU THAT YOUR LAB RESULTS ARE COMPLETED

SPECIAL INSTRUCTIONS:

* Please remember to take Allopurinal as prescribed, 300 mg once per day, on days 1 through 10
* Please keep track of the Allopurinol on this diary, as in example to the left.
* Drug must be kept in original container.
* There are no restricted foods or beverages
* Any doses missed can be taken later on the same day they were missed, with food.
* If a missed dose is not discovered until the following day, the dose should not be made up.
* Any doses vomited within one hour of administration should be retaken
* Please remember to bring any unused study drug, all empty containers, and diary to the next clinic visit.

Study Participant Initials _____ Date _____

FOR STUDY TEAM USE ONLY	
Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

DOSING LOG

Pyrimethamine

Cycle: ___ For each AM dose take:

Please indicate the date, time, amount taken and any comments.

	Date	Dose	Time	Comments	Food
Ex:	6/1/2009	X mg	9:00 AM	vomited PM pill	yes
Day 1					
Day 2					
Day 3					
Day 4					
Day 5					
Day 6					
Day 7					
Day 8					
Day 9					
Day 10					
Day 11					
Day 12					
Day 13					
Day 14					
Day 15					
Day 16					
Day 17					
Day 18					
Day 19					
Day 20					
Day 21					
Day 22					
Day 23					
Day 24					
Day 25					
Day 26					
Day 27					
Day 28					

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity

Dana-Farber Cancer Institute
Nursing Protocol Education Sheet

Protocol Number:	**09-421**
Protocol Name:	**A phase I/II study of Pyrimethamine, a STAT3 inhibitor, for the treatment of relapsed Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma and T-LGL**
DFCI Site PI:	Jennifer Brown, MD, PhD
DFCI ResearchNurses:	Karen Francoeur, RN; Victoria Patterson, RN; Kathleen McDermott, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	*Pyrimethamine is an oral folic acid antagonist used as an antiparasitic agent. See section 2.1 for description of this agent, precautions in people with possible folate deficiency and for potential toxicities, like myelosuppression, vomiting, anorexia, etc…* Current dose as per A.P. Each cycle = 28 days.
Dose Calcul-ation	Doses of *Pyrimethamine* are fixed in mg The Recommended Phase II dose is found as noted on Alert Page
Study Drug and Administration	**Pyrimethamine-** See TREATMENT PLAN – sec. 5 • Self-administered, once daily in the AM with food. • Instruct patient to not take drug at home on clinic visit days (e.g. Cycle 1 Day 1,8, and 15, C2D1, etc…). Dose is not to be taken until labs results are reviewed on those days. • Instruct patients on Drug Diary use. • Vomited doses not made up unless vomited within 1 hr of intake. Missed, e.g. forgotten doses can be made up on same day but not later • Section 5.1 Pre treatment criteria • Section 5.2 Agent Administration, to include twice weekly labs in Cycle 1 for TLS monitoring.
Dose Modifications and Toxicity	Definition of DLT- sect. 5.3 • Anticipated toxicities – sect. 6.1; management per Setion 6.2 • Dose Modifications/Delays – see sect. 6.3. Note guidelines for Heme toxicity in sect. 5.3, for Rash in 6.3.2, and for Anorexia/Vomiting in sect. 6.3.3
Concomitant Meds	Review sect. 5.4 for all Supportive care guidelines and conc. Meds allowed, prohibited or to used with caution • Allopurinol days 1-10 daily unless pt. has allergy to it. • Antiemetics as needed but Lorazepam is to be used with caution due to risk of ↑LFT's • Use of Bactrim discouraged; Acyclovir prophylactic is recommended.
Required Data (ECG, V/S, etc…)	• Note folic acid blood draws at screening and periodically – see sect. 5 and 9 (**Study Calendar**) • PD blood draws per Section 8.2 and Study Calendar • PKs per Section 8.2 and Study Calendar

Charting Tips	Please be sure to DOCUMENT time of drug administration, PD blood draws, any patient teaching.