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Phase I/II Trial Evaluating the Safety and Efficacy of Ruxolitinib in Patients with Smoldering or Chronic Adult T-Cell Leukemia (ATL)

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PRÉCIS

Background:

- Adult T-cell leukemia is a lymphoproliferative disorder characterized by the presence of CD4/CD25 expressing T cells (IL-2R alpha expressing) in the peripheral blood, in lymphoid and other tissues.
- In smoldering and chronic ATL the HTLV-1 encoded protein, Tax constitutively activates interleukin-2 (IL-2), IL-9 and IL-15 autocrine/paracrine systems that in turn activate the Janus kinase (JAK)-1/3/STAT5 pathways.
- Ruxolitinib a therapeutic agent inhibits cytokine mediated JAK1/2 activation and *ex vivo* proliferation of malignant T cells from patients with ATL.
- Ruxolitinib is a potent orally bioavailable JAK1/2 inhibitor not licensed for the treatment of ATL.

Primary Objective:

- To determine the maximum tolerated dose and clinical response rate for ruxolitinib given at doses of 30, 40 or 50 mg orally twice daily in patients with smoldering, chronic and biologically indolent acute or lymphomatous subtype of ATL

Eligibility:

- Patients ≥ 18 years old with pathologically confirmed adult T-cell leukemia: smoldering or chronic, or previously treated lymphomatous or acute subtypes with clinically indolent behavior indicated by lack of significant symptoms and treatment free interval of greater than 6 months.
- Patients must have measurable or evaluable disease. Patients with > 10% of their PBMCs having the characteristic abnormal (i.e., $CD3^{dim}$, $CD4^{+}$ $CD25^{+}$ expressing) FACS profile for circulating ATL cells will be considered to have measurable disease.
- Patients with symptomatic leukemic meningitis, bony or GI tract involvement, serum calcium or LDH > 1.5 X the upper limit of normal will be excluded. However, patients that have both ATL and another HTLV-1 associated disease such as tropical spastic paraparesis (HAM/TSP) will be included.
- No prior treatment with another JAK inhibitor; patients previously treated in this protocol at the lower dose are eligible to restart treatment at the higher dose levels.

Design:

- This is a pilot open-label, trial with off label-use of oral ruxolitinib that will enroll 27 to 33 patients with smoldering or chronic or clinically indolent ATL. Groups of 3 to 6 newly enrolled or reenrolled patients will begin treatment at a dose of 30 mg orally given twice daily. If this dose is tolerated without exceeding the criteria for dose limiting toxicity (DLT) during the first cycle of treatment, the tolerability of treatment at 40 mg and then 50 mg twice daily will be evaluated.

TABLE OF CONTENTS

PRÉCIS 2
TABLE OF CONTENTS 3
STATEMENT OF COMPLIANCE 5
1 INTRODUCTION 5
1.1 Study Objectives 5
1.2 Background and Rationale 5
1.3 Inhibition of Cytokine Stimulation in the Treatment of ATL and Rationale for ruxolitinib 8
1.4 Preclinical Experiments with JAK inhibitors 9
1.5 Clinical Experience with Ruxolitinib 14
1.6 Rationale for Patient Selection, Change in the Treatment Schedule and Target Response Rate in the Initial Phase II Trial 16
1.7 Rationale for Multiple Assessments of STAT5 and phosphoSTAT5 19
2 ELIGIBILITY ASSESSMENT AND ENROLLMENT 20
2.1 Eligibility Criteria 20
2.2 Screening Evaluation 21
2.3 Participant Registration and Status Update Procedures 23
2.4 Baseline Evaluation 24
3 STUDY IMPLEMENTATION 25
3.1 Study Design 25
3.2 Drug Administration 28
3.3 Dose Modifications 28
3.4 Clinical Monitoring 28
3.5 Study Calendar 31
3.6 Concurrent Therapy 34
3.7 Cost and Compensation 34
3.8 Criteria for Removal from the Protocol Treatment and Off-Study Criteria 34
4 CONCOMITANT MEDICATIONS/MEASURES 35
5 BIOSPECIMEN COLLECTION 35
5.1 Correlative Studies for Research 35
5.2 Sample Storage, Tracking and Disposition 36
6 DATA COLLECTION AND EVALUATION 38

6.1 Data Collection....38
6.2 Response Criteria....39
6.3 Toxicity Criteria....41
7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING....41
7.1 Definitions....41
7.2 OHSRP Office of Compliance and Training / IRB Reporting....41
7.3 NCI Clinical Director Reporting....42
7.4 NIH Required Data and Safety Monitoring Plan....42
8 STATISTICAL CONSIDERATIONS....42
9 COLLABORATIVE AGREEMENTS....43
10 HUMAN SUBJECTS PROTECTIONS....43
10.1 Rationale for Subject Selection....43
10.2 Participation of Children....43
10.3 Participation of Subjects Unable to Give Consent....44
10.4 Evaluation of Benefits and Risks/Discomforts....44
10.5 Consent and Assent Process and Documentation....45
11 REGULATORY AND OPERATIONAL CONSIDERATIONS....46
11.1 Study Discontinuation and Closure....46
11.2 Quality Assurance and Quality Control....46
11.3 Conflict of Interest Policy....46
11.4 Confidentiality and Privacy....47
12 PHARMACEUTICAL INFORMATION....47
12.1 Ruxolitinib (Jakafi, ICNB018424)....48
13 REFERENCES....50
14 APPENDICES....53
APPENDIX A: PERFORMANCE STATUS CRITERIA....54
APPENDIX B: DEFINITIONS OF ATL SUBSETS....55
APPENDIX C: STRONG INHIBITORS of P450 ISOFORM CYP 3A4....56

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the maximum tolerated dose and clinical response rate for ruxolitinib given at doses of 30, 40 or 50 mg orally twice daily in patients with smoldering, chronic and biologically indolent acute or lymphomatous subtype of ATL

1.1.2 Secondary Objective

- To define the safety profile, time to progression when ruxolitinib is administered at doses of 30, 40 or 50 mg orally twice daily in patients with smoldering, chronic and biologically indolent acute or lymphomatous subtype of ATL

1.2 BACKGROUND AND RATIONALE

1.2.1 Epidemiology of ATL

ATL is an aggressive T-cell lymphoproliferative disorder characterized by the presence of malignant CD4/CD25-expressing T cells in the peripheral blood and in lymphoid and other tissues [1-6]. The disease exhibits a striking clustering of cases in certain geographic regions, notably southwestern Japan, the Caribbean basin, northeastern South America, central Africa, and the southeastern United States. Epidemiologic studies demonstrate a clear association of the disease with the presence of a retrovirus, human T-cell lymphotropic virus type 1 (HTLV-1)[7, 8]. The vast majority of HTLV-1-infected individuals are asymptomatic carriers, but infected individuals are nevertheless capable of transmitting the virus. An infected individual appears to have an approximately 0.1 percent per year risk of developing frank ATL; this is equivalent to a cumulative lifetime risk of 2 to 5 percent [2, 3].

1.2.2 Biology of HTLV-1 Associated ATL

ATL cells exhibit characteristic morphological features (flower-like cells with deeply indented nuclei, derived from mature helper T-cells with the surface phenotype CD3+dim, CD4+, CD7-, CD8-, CD25+) [9]. ATL cells usually contain chromosomal abnormalities. Although no individual

cytogenetic change is characteristic of the disease; the degree of aneuploidy correlates with clinical aggressiveness. The molecular mechanism by which HTLV-1 transforms T cells is not fully defined, but the HTLV-1 genome is not reported to contain an oncogene. The site of integration for the provirus for an individual patient is identical for all the transformed cells for the particular individual, but the integration site differs from person to person and the mechanism of transformation is not insertional mutagenesis. HTLV-1 is a complex retrovirus that encodes regulatory (Tax and Rex) genes and accessory p12, p13, p30 and HBZ genes to control viral replication and proliferation of infected cells [10-13]. The HTLV-1 bZIP factor (HBZ) gene is transcribed from the minus strand of provirus using 3' the long terminal repeat [14]. The contribution of HBZ to the pathophysiology of ATL is being seen as increasingly important as its role in the immune suppression and transformation into leukemia or lymphoma is described [15, 16]. Another relatively novel feature of the HTLV-1 genome is the presence of a region called pX, which encodes nonvirion proteins including 42-kDa Tax and 27-kDa Rex [10, 11]. Tax is a trans-activating transcription factor that interacts with members of the HTLV-1 long terminal repeat (LTR) activating transcription factor/cAMP responsive element (CRE) and stimulates the expression of a number of viral genes [12, 13]. Deletion of the sequences within these two genes renders an infectious viral clone noninfectious. Tax induces the transcription of various cellular genes including those encoding IL-2 receptor alpha chain (IL-2Rα), IL-2, IL-3, IL-9, IL-15, IL-15 receptor alpha chain (IL-15Rα), TNF, GM-CSF, TGF-β, PTHrP, vimentin, and c-fos [17-21]. HTLV-1 infection of T cells *in vivo* and *in vitro* is also associated with constitutive IL-2R alpha gene expression and facilitates IL-2 expression inducing an autocrine IL-2/IL-2R loop in infected T-cells in the early stages of ATL [18]. It has been proposed that in the early phases of ATL the HTLV-1 induced leukemogenesis result from Tax driven stimulation of these genes involved in cellular proliferation [16, 17] eventually resulting in T-lymphocyte immortalization [10, 16]. Investigators in the Lymphoid Malignancies Branch have also described another Tax driven autocrine stimulatory loop involving IL-15 and its private receptor, IL-15R alpha and analogous paracrine stimulatory loop with IL-9 production by ATL cells and IL-9R alpha expression on monocytes [19-21]. The presence of these non-IL-2 driven autocrine or paracrine loops point toward potential escape mechanisms developing in Lymphoid Malignancies Branch patients treated with the anti-IL-2Rα monoclonal antibody (daclizumab, anti-Tac) that has been a core component of many of our clinical trials. Still the efficacy of Daclizumab suggests that targeting the common IL-2, IL-9, and IL-15 γc cytokine receptor subunit and its associated signal transduction elements JAK1, (Janus kinase) JAK3 and STAT5 might be a more viable treatment strategy [22]. More direct inhibition of this signal transduction pathway with clinically effective JAK1 or JAK3 inhibitors that are now available could have greater efficacy than could be achieved by antibody-mediated inhibition of the single IL-2/IL-2R cytokine system[22-25].

1.2.3 Clinical Features of ATL

Frank ATL often has its onset 20-30 years following a blood transfusion from an infected individual or perinatal infection from the mother infected with HTLV-1 [3, 4, 8]. The principal clinical features of full-blown acute ATL include frequent skin involvement, moderate lymphadenopathy with relative sparing of the mediastinum, CNS and lung involvement, and hepatosplenomegaly. The peripheral blood may show a high leukemic cell count without much anemia and with only modest involvement of the bone marrow. The occurrence of hypercalcemia, unusual in other types of leukemia and lymphoma, is characteristic of ATL. Patients with ATL manifest a profound degree of immunosuppression and often develop opportunistic infections including Pneumocystis carinii and Cryptococcus meningitis. The clinical aggressiveness of ATL varies from patient to

patient over a very broad spectrum. Attempts to define the different subtypes of ATL eventually led to the now widely accepted Shimoyama classification system that subdivides the disease into four categories (**Table 1**) [5].

Table 1: Shimoyama classification ATL

Subtype of ATL	Clinical features	Lymphocyte count	Associated Biochemical abnormalities
Smoldering	Skin and pulmonary lesions No lymphadenopathy (LNs) or organ involvement	≥ 5% abnormal T cells but normal absolute lymphocyte count (ALC) < 4 x 10^9/L	Calcium, lactic dehydrogenase (LDH) < 1.5 x upper limit of normal (ULN)
Chronic	Skin and lung involvement, no CNS, bone, GI tract involvement, no ascites or pleural effusions	ALC > 4 x 10^9/L T cells > 3 x 10^9/L	Elevated LDH but < 2 x ULN Normal Calcium
Lymphoma	Biopsy proven LN	Normal ALC, ≤ 1% abnormal circulating T-cells	
Acute	Tumor lesions	leukemia	Not required for diagnosis

1.2.4 Treatment of ATL

1.2.4.1 Cytotoxic Chemotherapy for ATL

The standard combination chemotherapy regimens known to be active against the more common aggressive non-Hodgkin's lymphomas or acute lymphoblastic leukemia have shown increasingly disappointing activity in the treatment of ATL patients [5, 26, 27]. Over the past few years the majority of patients with the more aggressive categories of ATL, acute and lymphoma type; have been treated with combination chemotherapy including CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) and VEPA (vincristine, cyclophosphamide, prednisolone, and adriamycin). The Japanese Lymphoma Study Group protocol also assessed a combination treatment regimen with nine drugs (doxorubicin, cyclophosphamide, vincristine, prednisone, vindesine, methotrexate, etoposide, procarbazine, and bleomycin), or a comparable protocol with these drugs plus cisplatin [26, 27]. A total of 854 patients with HTLV-1-antibody-positive ATL newly diagnosed from 1983-1987 were analyzed for prognostic factors and survival following combined chemotherapy by the Japanese Lymphoma Study Group [4]. The median survival time (MST) was 10 months and projected 2- and 4-year survival rates of all patients were 28 percent, and 12 percent, respectively. Impaired performance status, high lactic dehydrogenase values, age > 40 years, greater number of lesions, and hypercalcemia were associated with shortened survival. Survival data for treated patients in the different clinical subtypes, most often receiving combination chemotherapy, showed MST of 6.2 months for acute type, 10.2 months for lymphoma type, and 24.3 months for chronic type. This correlated with projected 4-year survival rates of 5% for acute subtype, 5.7% for lymphomatous subtype, 26.9% for chronic subtype and 58% for smoldering subtype. The members of the Lymphoma Study Group concluded after reviewing 854 cases of treated ATL patients that "The various combination chemotherapies so far developed have not increased significantly the survival of patients with

ATL." As a consequence of this generally held opinion, investigational therapies with monoclonal antibodies, biological response modifiers, anti-retroviral drugs and recently molecular therapeutics have become the most common treatments administered to ATL patients.

1.2.4.2 Azidothymidine and interferon alpha (AZT/IFNα) for ATL

In part due to the generally poor results for conventional combination chemotherapy in the treatment of ATL patients, attempts to target the underlying HTLV-1 driven pathophysiology with less toxic agents led to combination therapy with the antiviral agent AZT and cytokine IFNα, a synthetic version the anti-viral protein. In a study reported by Gill [28], 6 partial and 5 complete remissions (PRs and CRs) were observed in 11 of 19 ATL patients treated with this combination. Despite the response rate, the median survival duration of the whole group was only 3 months and 13 months in the 11 responding patients [28]. The recent meta-analysis by Bazarbachi reported that first line therapy with AZT plus IFNα produced a 5-year overall survival rate of 46% in 75 patients [29] . While this is an encouraging result unlike most patients referred the NCI, the Bazarbachi group does not treat patients who have received previous cytotoxic treatment such as CHOP. It must be emphasized that patients treated with chemotherapy followed by AZT and IFNα had a much poorer response rate[29]. Furthermore, in contrast, only 2 of 11 (18%) heavily pretreated ATL patients receiving AZT/IFNα from our group showed a response; in both cases a partial response (PR). There is some information regarding cellular or molecular changes that occurs in previously treated ATL patients that could account for this diminished responsiveness. Pancewicz [30] reported that previously treated patients with mutated p53 did not respond to AZT/IFNα treatment and that relapse in individual metastases was associated with a tumor clone carrying the mutated inactive p53. Patients with elevated IRF4 and cRel are also described as being resistant to antiviral treatment [31]. These results are all consistent with the notion that ATL patients with biologically aggressive disease (needing multiple rounds of treatment) are not appropriate patients for the AZT/IFNα combination.

1.3 INHIBITION OF CYTOKINE STIMULATION IN THE TREATMENT OF ATL AND RATIONALE FOR RUXOLITINIB

1.3.1 Anti-IL-2R alpha-Directed Monoclonal Antibodies for ATL

The Lymphoid Malignancies Branch has evaluated treatment with antibodies targeting the IL-2R alpha [32] in a number of lymphoid malignancies including ATL. Targeting the IL-2Rα interrupts the binding of IL-2 to the receptor inhibiting the cellular activation which we now understand transmitted through the JAK1, JAK3/STAT5 pathways. Initially, the efficacy of the murine monoclonal anti-Tac antibody was assessed in 19 patients with acute, chronic and lymphomatous ATL (NCI Protocol 83-C-0023) resulting in 2 CRs, 4 PRs and one mixed response [33]. The emergence of human anti-mouse antibodies (HAMA) limited the ability to administer the treatment repeatedly. Following the conclusion of that trial, the efficacy of unlabeled Hu-anti-Tac (NCI Protocol 00-C-0030) administered at high doses (4, 6 and 8 mg/kg given every 2nd and 3rd week) to ATL patients was examined. While 2 of 14 phase I patients responded to treatment, there were no additional responses among nine patients in the phase II portion of the trial. Of note, none of the patients enrolled in the trial had the less aggressive chronic or smoldering disease (unpublished observations); all of the patients had lymphomatous or acute ATL. Previous protocols 90-C-0043, 93-C-0066 and 96-C-0147 assessed the efficacy and toxicity of the anti-Tac mAb conjugated with Yttrium 90 (^{90}Y) and later with the addition of the chelater Ca-DTPA to

reduce toxicity by accelerating the urinary excretion of ^{90}Y. Seven of the 16 evaluable patients so treated achieved a PR and two a CR [33]. Although 45% of patients in 96-C-0147 responded to treatment during the phase I portion of this trial only one response was seen among nine patients treated with the phase II dose of 25mCi of ^{90}Y.

1.3.2 Role of JAK in Cytokine Driven Transactivation and the Pathogenesis of ATL

The HTLV-1 encoded Tax protein that constitutively activates the IL-2, IL-9 and IL-15 signaling pathways, transmitting an activation signal through intermediaries JAK1 and JAK3 to STAT5 proteins that ultimately bind to gamma activation sites in the promoters of inducible cytokines in the cell nucleus. The JAK tyrosine kinases function as common relay elements in the Tax initiated proliferative signal (**Figure 1**) and point out the potential therapeutic impact for JAK1 and JAK3 inhibition in the treatment of ATL [17, 22-25].

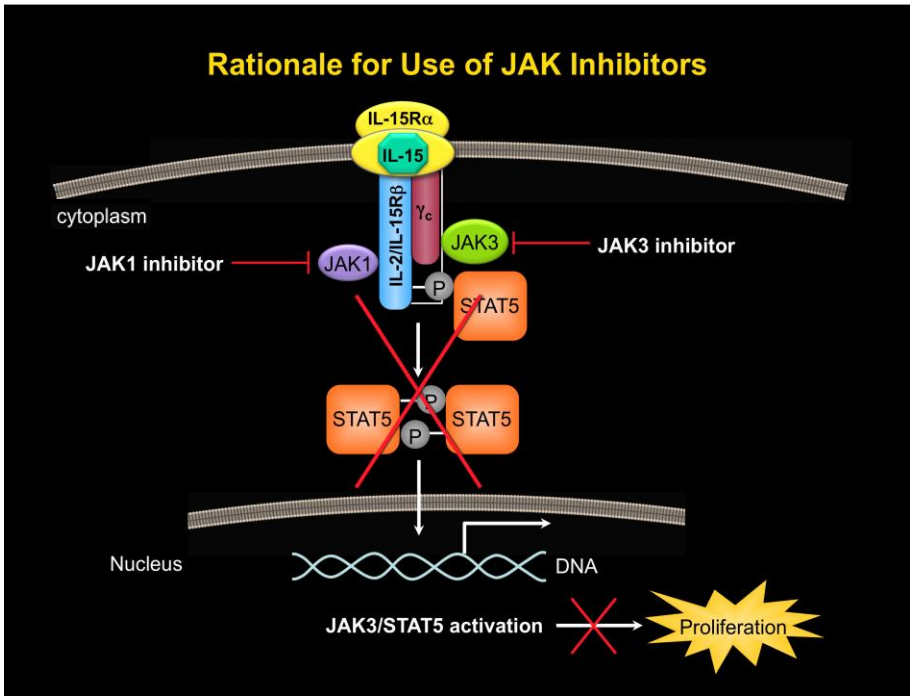


Figure 1: Rationale for use of JAK1 inhibitor ruxolitinib. The interaction of IL-2 or IL-15 with its heterotrimeric alpha-beta gamma receptor initiates signaling that involves JAK1 associated with IL-2/IL-15R beta and JAK3 associated with the common gamma chain. Activated (phosphorylated) JAK1 and JAK3 then lead to the phosphorylation and transfer of STAT5 to the nucleus. Inhibitors of either JAK1 or JAK3 inhibit this cytokine mediated-signaling pathway.

We previously utilized the antibody to the IL-2 receptor alpha developed in our laboratory (daclizumab) in the treatment of patients with ATL with meaningful efficacy [32]. However, the presence of two autocrine and one paracrine cytokine stimulatory loop presented limitations for this therapeutic strategy that involved a single monoclonal antibody directed toward IL-2 receptor alpha.

1.4 PRECLINICAL EXPERIMENTS WITH JAK INHIBITORS

1.4.1 In vitro treatment of PBMCs and Cell lines with JAK inhibitors

To begin the examination of JAK inhibition in the treatment of ATL, our laboratories performed experiments to evaluate the activity of the JAK3 and JAK2 inhibitor, CP-690,550 (Tofacitinib) in

the cytokine dependent *ex vivo* proliferation that is characteristic of the peripheral blood mononuclear cells (PBMCs) from patients with smoldering or chronic subtypes of ATL [34]. CP-690,550 at 50 nM concentration inhibited the 6-day *ex vivo* spontaneous proliferation of PBMCs from ATL patients by a mean of 66% (**Figure 2**).

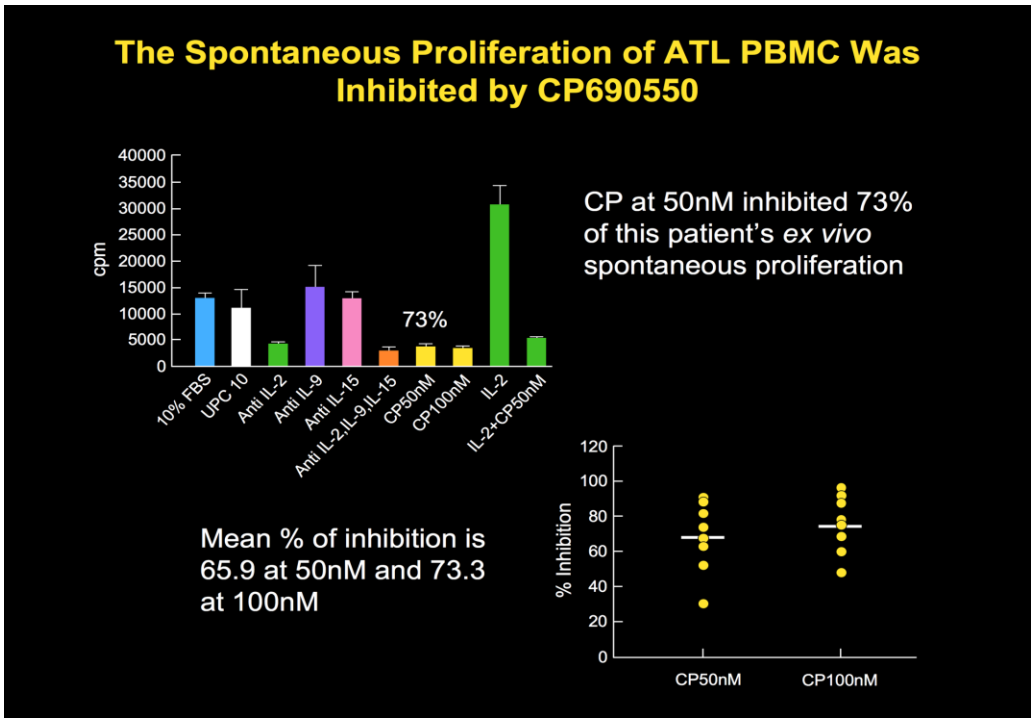


Figure 2: ***Ex vivo*** **peripheral blood mononuclear cells from patients with ATL were cultured in RPMI 1640 median supplemented with 10% fetal bovine serum for 6 days in the presence or absence of monoclonal antibodies or the JAK inhibitor CP-690,550 (Tofacitinib). To assay proliferation the cells were pulsed for 6 hours with μCi of ^{3}H-thymidine harvested and counted with a β-plate counter. The upper left side image demonstrates that the *ex vivo* PBMCs of this single patient with ATL manifested spontaneous proliferation (CPM 13,000) when cultured in FBS alone. This proliferation was inhibited by the addition of an antibody to IL-2 or the combination of anti-IL-2/IL-9 and IL-15 antibodies. Comparable inhibition was achieved with 50 and 100 nM of CP-690,550. The results of studies of 8 patients with chronic or smoldering ATL are shown in the lower right side with a mean percent inhibition of 65.9% at 50 nM CP-690,550.**

Increasing the concentration of CP-690,550 to 100nM an achievable *in vivo* level showed only marginally increased inhibition (73%) of these ATL cells. The suppression of proliferation by 690,550 even at 50 nM was significantly greater than anti-IL-9 or anti-IL-15 monoclonal antibody treatment. The JAK3 inhibitor was slightly more effective in suppression of proliferation the patient's cells than anti-IL-2 antibody and less effective than the combination of all three antibodies. The ability of CP-690,550 to inhibit the JAK-STAT activation cascade was tested in splenocytes obtained from the IL-15 transgenic CD8 T-cell leukemic mice that have an autocrine IL-15/IL-15Rα loop analogous to the Tax driven process seen in HTLV-1 associated ATL. As

can be seen in **Figure 3**, treatment with CP-690,550 substantially inhibited the phosphorylation of JAK3 and prevented the phosphorylation of STAT-5.

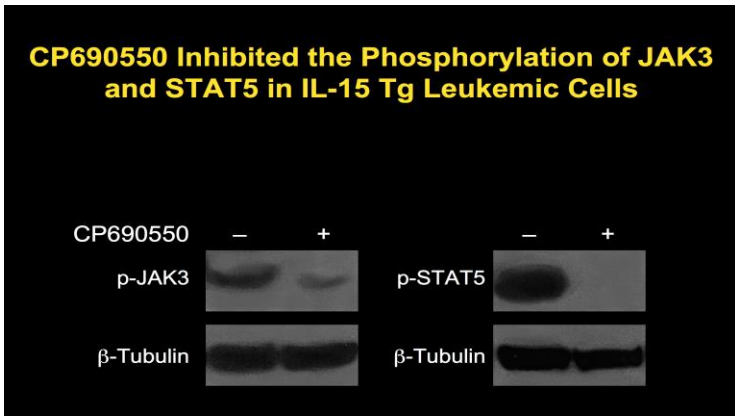


Figure 3: CP-690,550 (Tofacitinib) inhibited the phosphorylation status of JAK3/STAT5 in splenocytes from IL-15 transgenic CD8 T-cell leukemia bearing mice that manifest an autocrine IL-15/IL-15R alpha CD8 leukemic T-cell proliferation.

Similarly, Ruxolitinib, AZD1480 (JAK1, 2 inhibitors) and Tofacitinib (JAK 2, 3 inhibitor) have also been shown to effectively inhibit the IL-2 and IL-15 stimulation of the cytokine dependent NK92 cell line (Craig Thomas unpublished observations). (**Figure 4**)

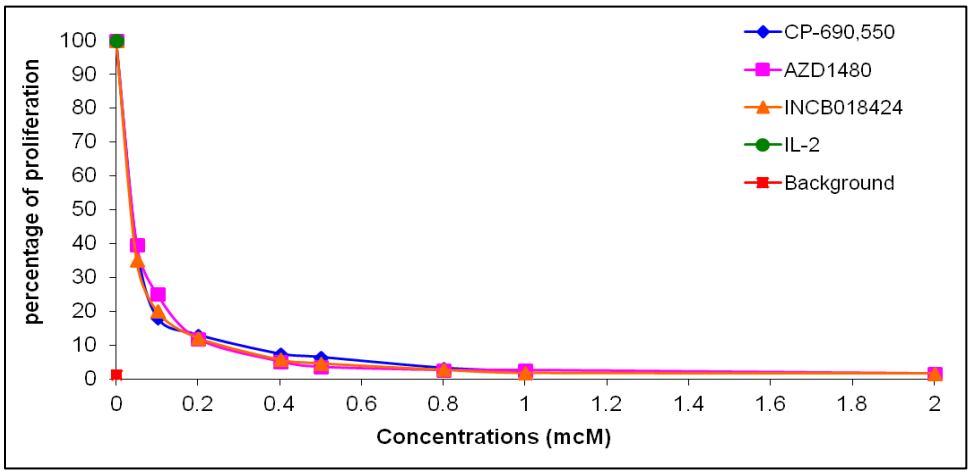


Figure 4: The proliferation of the cytokine-dependent NK-92 cell line mediated by IL-2, assessed by ^{3}H-thymidine addition during the last 6 hours of a 48-hour culture were inhibited by serially-increasing concentrations of the JAK3/JAK2 inhibitor CP-690,550 (Tofacitinib), the JAK1/2 inhibitors AZD1480, and ruxolitinib (INCB018424).

As seen in **Figure 5**, ruxolitinib could profoundly inhibit proliferation in cell line of an ATL patient at doses that have no effect on control cell lines (e.g. CEM).

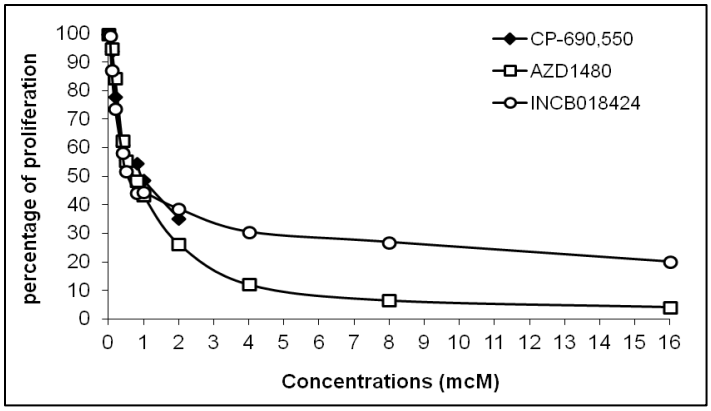


Figure 5: The proliferation of the peripheral blood mononuclear cells containing leukemic cells from an ATL patient cell line were inhibited by serially-increasing concentrations of the JAK3/2 inhibitor CP-690,550 (Tofacitinib) or the JAK1/2 inhibitors AZD1480 and INCB018424 (ruxolitinib).^{3}H-thymmidine was added during the last 6 hours of the cultures.

Figure 1 shows preliminary results with Ruxolitinib inhibiting the spontaneous proliferation of PBMCs *ex vivo* from patients with smoldering or chronic ATL. This particular patient seems to have a largely IL-2 driven clone with some IL-9 stimulation based on the results with the individual anti-cytokine antibodies. Of interest are the results in the last 2 columns to the right showing that ruxolitinib at concentrations of 1 and 10 μM inhibited these cells better than the combination of anti-IL-2, anti-IL-15 and anti-IL-9 antibodies.

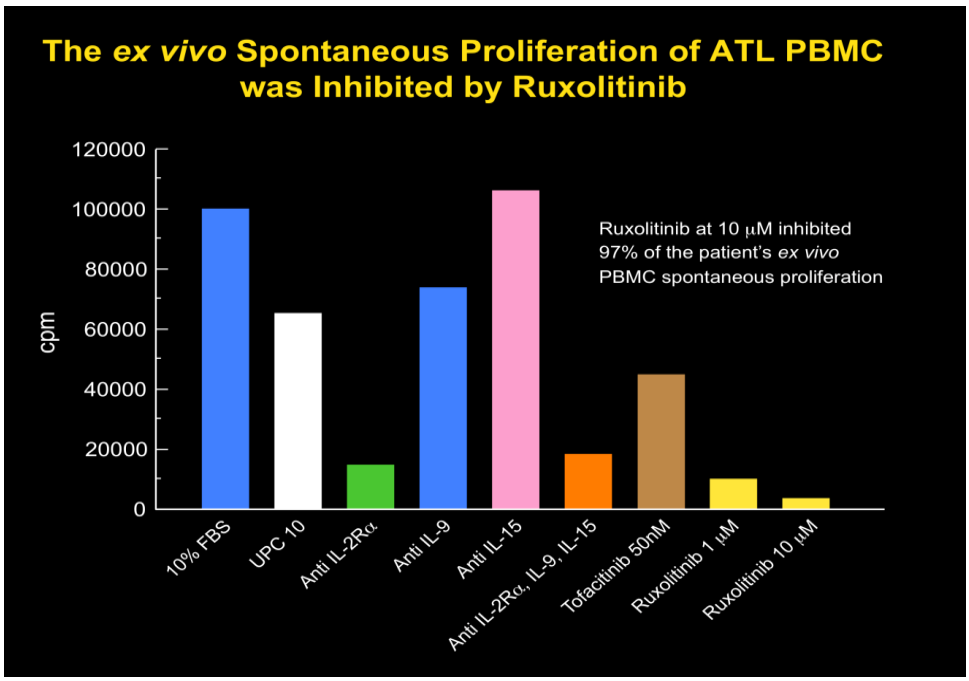


Figure 6: *Ex vivo* peripheral blood mononuclear cells from patients with ATL were cultured in RPMI 1640 median supplemented with 10% fetal bovine serum for 6 days in the presence or absence of monoclonal antibodies or the JAK1/2 inhibitor ruxolitinib (yellow). To assay proliferation the cells were pulsed for 6 hours with μCi of ^{3}H-thymidine harvested and counted with a β-plate counter.

Additionally, we have seen that ruxolitinib inhibited the proliferation of CD8 T-cell lines derived from patients with refractory celiac disease which has been shown to have an IL-15 autocrine loop as well as the survival of CD8 T-cells (studied immediately *ex vivo*) from intestinal biopsies of such patients.

1.4.2 Molecular Interference Screening with JAK loss of function cell lines

To perform a molecular interference screening analysis of target tumor cell lines including ATL, Lymphoid Malignancies Branch laboratory investigators led by Dr. Louis Staudt have created a library of retroviral vectors for inducible expression of small hairpin RNAs (shRNAs) [35]. A critical feature of this system is the inclusion of a 60-base pair molecular barcode in each vector which allows the abundance of each vector to be monitored in a population of transduced cells. This extensive shRNA library contains 11,274 vectors that targets 3,033 human genes and can be used to assess therapeutic potential of molecular therapeutics. Using this inducible shRNA library, a loss-of-function genetic screen, half of the cultures were induced to express shRNA and half served as the control to assess the consequence of depleting essential genes in the intended treatment population. Relative abundance of shRNAs is monitored by their unique barcodes using microarray and the relative amount of shRNA indicates how much "interference" was required to suppress the target gene. When this screen was applied to three distinct ATL cell lines, JAK1 was shown to be critical in the proliferation and survival of the six of the seven cell lines studied (**Figure 7**) adding support to the notion that JAK signaling is critical in ATL and an appropriate therapeutic target.

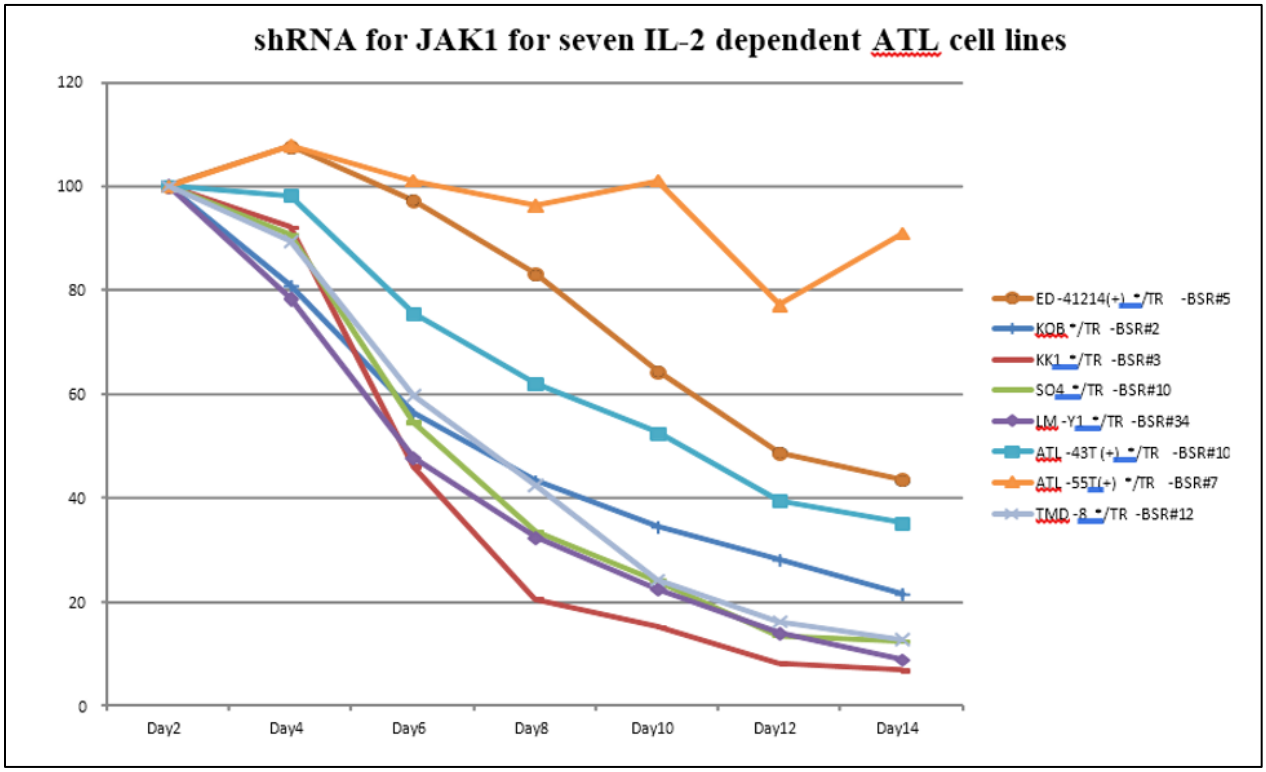


Figure 7: Using an inducible shRNA for JAK1 a loss of function screen applied to seven distinct ATL cell lines JAK1 was shown to be critical in the proliferation and survival of six of seven lines studied.

1.4.3 In Vitro Matrix screening assessment

Preliminary results from matrix high-throughput screening for cellular signaling analyses directed at known targets and pathways showed that a JAK1/2 inhibitor inhibited the proliferation and survival of the 5 ATL cell lines tested with LD50s of 1.6 uM, 5.1 uM, 8.1 uM, 18.7 uM and 25.6 uM. Taken as a whole, these preclinical studies have demonstrated the importance of the JAK1 and 3 signaling elements in ATL lymphocytes, the ability of current clinical agents to inhibit pathophysiologic IL-2, IL-9 and IL-15 driven stimulation in ATL and strongly support a clinical trial evaluating the JAK1 inhibitor ruxolitinib for the treatment of patients with smoldering or chronic ATL.

1.5 CLINICAL EXPERIENCE WITH RUXOLITINIB

1.5.1 Pharmacokinetics (PK) and Pharmacodynamics (PD) of Ruxolitinib

The Janus-associated kinases 1 and 2 mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. Absorption, distribution, metabolism and excretion (ADME) and pharmacokinetic studies performed to support the licensing application for ruxolitinib showed rapid and near complete absorption (95%) after administration with maximum plasma concentration (C_{max}) of 1.47 μM achieved within 1 to 2 hours post dose. The mean C_{max} and total exposure (AUC) of ruxolitinib increased proportionately over the (single) dose range of 50 to 200 mg. The apparent volume of distribution of ruxolitinib at steady state is 53 to 65 L with high protein binding (97%) that is mostly to albumin. Ruxolitinib administration resulted in maximum inhibition of STATs phosphorylation 2 hours after dosing with return to near baseline by 10 hours in both healthy subjects and myelofibrosis patients. CYP3A4 is the major enzyme responsible for the metabolism of ruxolitinib. Following a single dose of [^{14}C]-labeled ruxolitinib in healthy adult subjects, elimination was predominantly through metabolism with 74% of radioactivity excreted in urine and 22% via feces [36].

1.5.2 Ruxolitinib Clinical Trial Results

Several Phase 1, 2 studies and two randomized Phase 3 studies (studies 1 and 2) were conducted in patients with myelofibrosis (MF) (either primary myelofibrosis, post-polycythemia Vera myelofibrosis [PV] or post-essential thrombocytopenia-myelofibrosis [ET]) [36, 37]. Patients with a platelet count > 200 x 10^9/L were started on ruxolitinib orally 20 mg twice daily. In the 155 MF patients receiving ruxolitinib there was a 35% reduction in spleen volume in 65 (41.9%), as compared to one (0.7%) in the 154 patients in the placebo group. 148 patients receiving ruxolitinib achieved ≥ 50% improvement from baseline in the total symptoms score, in contrast to 8 of 152 patients 5.3% in the placebo-control group [36, 37]. The safety data base in solid tumors and hematologic malignancies consisted of 787 patients treated with ruxolitinib in 6 studies evaluating patients with MF (N=679), prostate cancer (N=22), multiple myeloma (N=13), ET and PV (N=73), of whom 617 patients received ruxolitinib. Hematologic events were the most frequently reported adverse events (AEs) primarily thrombocytopenia and anemia. The majority of these AEs are grade 1-2 and seldom led to drug discontinuation (<1% of patients). Of the 155 patients who received ruxolitinib in study one 10 (6.5%) had grade 3 thrombocytopenia and one (0.6%) had grade 4 thrombocytopenia as contrasted with one and 0 patients in the placebo group. Ten patients had grade 3 and 6 patients had grade 4 anemia compared to 5 and 0 in the placebo group. Biochemistry abnormalities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol were also reported and again the majority of these increases were grade 1 or 2 with no grade 4 observed. In this study there were 9 deaths in the ruxolitinib group and 11 in the placebo group. The safety data base which is primarily from patients with an underlying

hematopoietic production defect indicates a well-tolerated drug that did cause additional decreases in blood elements.

1.5.3 Summary of Adverse Events

Table 2: Adverse Reactions Occurring in Patients on the Phase III MF Trial

Adverse Reaction*	Ruxolitinib (N=155)			Placebo (N=151)		
	All Grades (%)	Grade 3 (%)	Grade 4 (%)	All Grades (%)	Grade 3 (%)	Grade 4 (%)
Bruising	23.2	0.6	0	14.6	0	0
Dizziness	18.1	0.6	0	7.3	0	0
Headache	14.8	0	0	5.3	0	0
Urinary Tract Infections	9.0	0	0	5.3	0.7	0.7
Weight Gain	7.1	0.6	0	1.3	0.7	0
Flatulence	5.2	0	0	0.7	0	0
Herpes Zoster	1.9	0	0	0.7	0	0

*CTCAE version 3

Table 3: Worst Hematology Laboratory Abnormalities in Patients on the Phase III MF Trial

Laboratory Parameter	Ruxolitinib (N=155)			Placebo (N=151)		
	All Grades (%)	Grade 3 (%)	Grade 4 (%)	All Grades (%)	Grade 3 (%)	Grade 4 (%)
Thrombocytopenia	69.7	9.0	3.9	30.5	1.3	0
Anemia	96.1	34.2	11.0	86.8	15.9	3.3
Neutropenia	18.7	5.2	1.9	4.0	0.7	1.3

1.6 RATIONALE FOR PATIENT SELECTION, CHANGE IN THE TREATMENT SCHEDULE AND TARGET RESPONSE RATE IN THE INITIAL PHASE II TRIAL

1.6.1 Preclinical Experiments

Eligibility for entry into the present trial is limited to patients with smoldering or chronic ATL since the ex vivo PBMCs containing ATL cells from such patients proliferated spontaneously and this proliferation was inhibited by the addition of ruxolitinib. Furthermore, the survival and proliferation of cytokine-dependent ATL cell lines were inhibited by the addition of ruxolitinib or shRNA for JAK1—a target of ruxolitinib. In contrast the ex vivo PBMCs from patients with acute and lymphomatous ATL did not proliferate spontaneously. Furthermore, the survival of cytokine-independent ATL cell lines was not inhibited by the addition of ruxolitinib or by the addition of a shRNA for JAK1. Although the Shimoyama Classification[5] System is valuable and generally accurate, there are patients who are not easily assigned to a subtype. This problem is most apparent in patients considered the chronic type, since chronic subtype patients can have lymphadenopathy, hepatomegaly and splenomegaly that are key criteria for the lymphomatous subtype patients. A key distinction that separates chronic from lymphomatous subtype is that chronic patients have an elevated absolute lymphocyte count with circulating ATL cells whereas lymphomatous patients have no evidence of leukemic involvement.

1.6.2 Results of Standard Treatment for ATL

Many of the Lymphoid Malignancies Branch chronic subtype patients will have received prior chemotherapy. Their classification will be determined on their status when they are being considered for ruxolitinib therapy rather than that at their initial presentation. While the Shimoyama classification system generally separates patients into the biologically indolent subtypes (smoldering and chronic) and the more aggressive subtypes (lymphomatous and acute), ATL is a highly variable disease and unusually poorer prognosis lymphomatous or acute subtype patients can have durable symptom free intervals following standard treatment where their disease behaves more like the indolent subtype. We believe it is appropriate to consider these patients for treatment on this protocol when there is clear evidence their ATL is quiescent and does not require urgent treatment for symptomatic progressive disease.

As noted above, there is data to suggest that the response rates to IFN and AZT in previously treated ATL patients was much lower (18% and 26%) in two reports than the response rates for treatment of naïve patients [42, 43]. Recently this resistance to AZT/IFN therapy has been demonstrated to be associated with P53 mutation and elevated IRF4 and cRel levels in the ATL cells of previously treated patients [44,45]. In the initial report by Shimoyama and coworkers, patients with chronic ATL had a median survival duration of 24.3 months. Patients with chronic ATL and adverse disease characteristics such as poorer performance status, lower neutrophil count and receive chemotherapy have poorer median survival compared to other chronic subtype patients and is reflected in the 47% 5-year survival rate noted in this long term follow up of "indolent" ATL patients [46]. Since duration of benefit is important in the treatment of chronic diseases such as chronic and smoldering ATL, survival is the clinical endpoint most often captured in these treatment reports. Response rates are frequently not sited and survival data can be difficult to compare in reports of different clinical trials based on the type of "chronic" ATL patients enrolled. One Japanese study reported a 4-year OS rate of 26.9% in patients managed with watchful waiting and then chemotherapy [47] that is very different than the survival data sited in the Journal of Clinical Oncology "Meta- analysis." The Takahasi review[46] indicates heavily pretreated patients further along in the natural history of their ATL respond less well to any form of treatment and initial target response rate of 30% in our referral patient population is an appropriate target value for response in a phase II trial[41]

1.6.3 Early Experience in this Trial

The initial 12 patients enrolled in the trial were treated with the standard 20 mg dose twice daily of off label ruxolitinib and tolerated the drug with little if any side effects, but responses were seen in only 4 of these patients. Three of these patients had more than a 50% decrease in the volume of their skin involvement and the other patient had 50% improvement in the number of circulating ATL cells, but the duration of these responses were 2 to 6 months. Serial analysis of phosphoSTAT 3 and phosphoSTAT5 on patients' samples after treatment showed only transient inhibition of the phosphorylation of STAT 5 in the immediate post-treatment period. These results are consistent with the *ex vivo* inhibition experiments performed with ruxolitinib at the 1 and 10 μM concentrations and ruxolitinib' s known pharmacokinetics (Cmax of 1.47μM ≈ 2 hours post dose and terminal elimination t ½ of ≈ 3 hours) resulting in a transient inhibition of the JAK-STAT signaling pathway that is marginally clinically active.

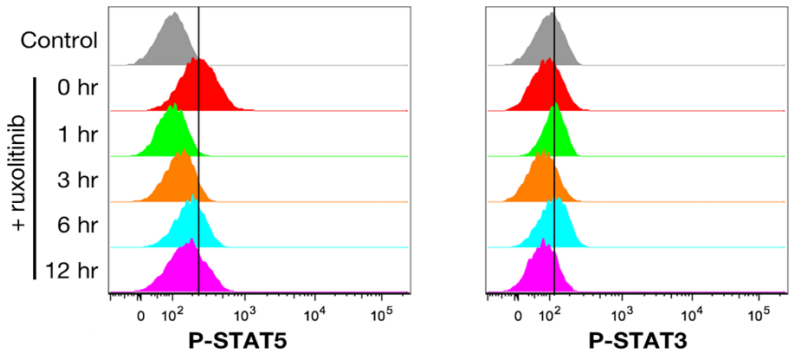
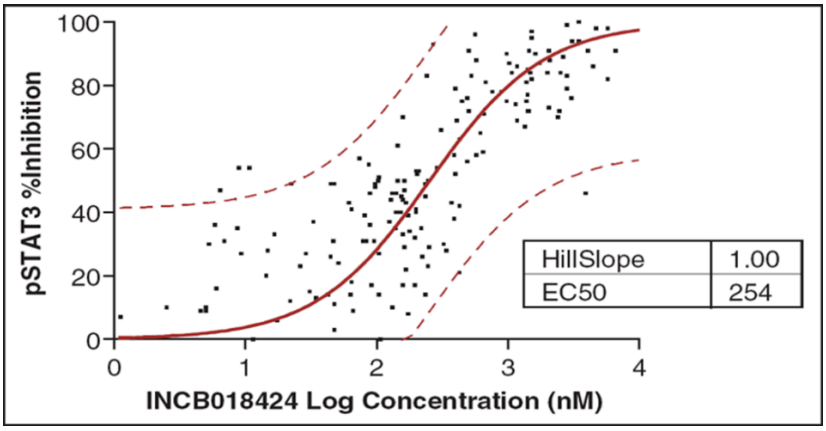


Figure 8: Effect of ruxolitinib on the endogenous phosphorylation of STAT3 and STAT 5 in ATL cells (ex vivo PBMCs isolated after oral ruxolitinib 20mg)

1.6.4 Clinical Experience with Ruxolitinib Dose Escalation in Normal Healthy Volunteers

As part of the initial clinical safety studies performed by commercial drug sponsor, patients and normal healthy volunteers (NHV) were treated with 50 mg twice daily, 100 mg once daily and in some cases individual doses of 200 mg (**Figure 9**).

A. Percent Inhibiton of STAT3 Phophorylation versus Serum concentration of Ruxolitinib



B. Serum Ruxolitinib Concentration with Augmented Dosing Regimens

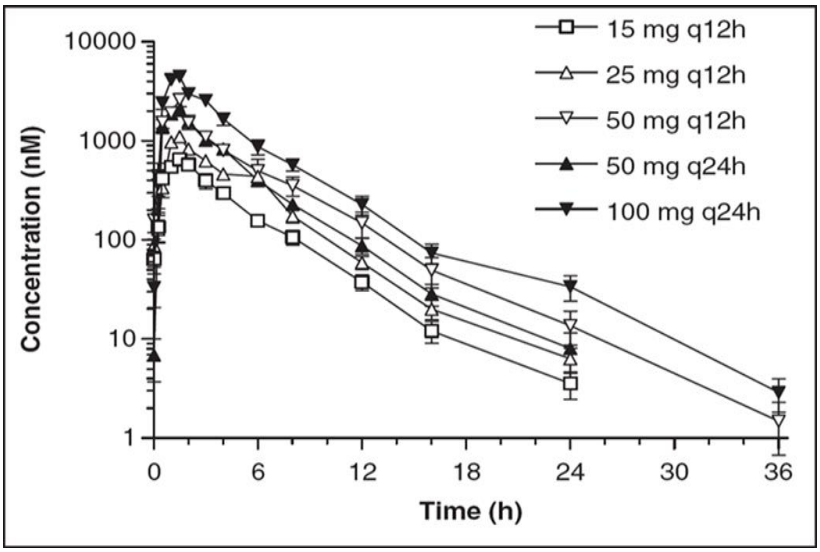


Figure 9 A and B: Percent inhibition of phosphoSTAT3 versus log concentration of (INCB018424) Ruxolitinib and serum Ruxolitinib concentrations with augmented dosing regimens[48]

Results from these NHV showed the more dose intense regimens achieved serum concentrations of ruxolitinib with greater potential to inhibit STAT3 phosphorylation without producing clinically significant AEs. In addition to monitoring for clinical toxicities, the sponsor also performed studies on cardiac conductance the was negative for QT prolongation for any of these doses. The subjects' heart rates increased maximally by 3.7 beats per minutes. Dose limiting toxicity was neutropenia at the 50 mg twice daily dose; with one of the 3 subjects having grade 4 neutropenia. A reversible increase in neutrophil counts was frequently seen at the higher doses tested and generally wore off within 24 hours of stopping treatment. The overall safety profile of ruxolitinib is good and the hematopoietic toxicities (i.e., thrombocytopenia, anemia and neutropenia) that were of greater concern in the Myelodysplastic Syndrome (MDS) patients with chronic treatment have not been observed in our ATL patients who received prolonged treatment previously as part of this protocol. This should not be that surprising with the genetic defect in MDS patients extending all the way down to their hematopoietic stem cells in contrast to the aberrant clone in ATL patients.[49]

All patients who fulfill the Shimoyama Classification for smoldering or chronic or quiescent lymphomatous, or acute ATL, at the time of evaluation for entry into this study whether they have had prior treatment or not will be deemed eligible for this study. Patients will have their burden of circulating ATL cells assessed by serial FACS analysis of their PBMCs and when appropriate have their soft tissue disease formally assessed by the (cutaneous lesions) Dermatology Service or by CT scans of their known sites of disease. New patients will begin treatment at 30 mg orally twice daily. Despite the modest clinical experience with augmented dosing in NHVs, the long-term tolerability in ATL patients will have to be established with standard phase I dose escalation plan. If the first 3 patients treated at this dose level, do not have dose limiting toxicities dosing will proceed sequentially to 40 and, then, 50 mg twice daily in a standard 3+3 fashion. A total of 9 patients will be treated at the MTD or maximum administered dose (MAD) to better evaluate the clinical activity of the augmented dosing regimen.

Patients with a transient response to previous treatment at the standard 20 mg twice daily dose and

later progressed but otherwise meet the eligibility criteria on treatment will also be eligible to restart treatment.

1.7 RATIONALE FOR MULTIPLE ASSESSMENTS OF STAT5 AND PHOSPHOSTAT5

1.7.1 Cycle 1 day 1

Additional analysis *in vitro* of inhibition of proliferation in ATL cells obtained from patients with JAK inhibitors and results of the first patients treated in this trial suggest transient inhibition of JAK signaling in the patients' malignant cells may be occurring, but is not sustained due to our inability to maintain ruxolitinib serum levels above the 1 micromolar concentration required to inhibit signaling through JAK1. To investigate this hypothesis, we will be performing serial intracellular FACS analysis of STAT5 and phosphoSTAT5 and coordinate measurement of serum ruxolitinib levels on Cycle 1 Day 1 to more accurately characterize changes in STAT5 occurring after ruxolitinib treatment. Results from these analyses may provide preliminary *in vivo* information that will allow the next iteration of this treatment strategy to be analyzed in a more advanced manner.

1.7.2 Cycle 2 and later time points

Rather than have a fixed schedule of STAT5 and phosphoSTAT5 of assessments based on hypotheses, we have chosen to make the correlative immunologic and molecular correlative laboratory assessments at these later time points optional and determined by clinically important events. The plan will be to assess several patients treated with prolonged administration of Ruxolitinib at the time of their clinical safety evaluations to detect changes in the JAK-STAT signaling pathway. If a provocative signal is detected at a particular time point, continued testing of all patients being treated on a regular basis will be instituted.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

NOTE: After approval and activation of Amendment D, patients who have failed this protocol treatment previously at the initial dose level may be eligible for re-enrollment and retreatment if they otherwise meet eligibility criteria.

2.1.1.1 Patients ≥ 18 years old with pathologically confirmed adult T- cell leukemia: smoldering or chronic, or previously treated lymphomatous or acute subtypes with clinically indolent behavior indicated by lack of significant symptoms and treatment free interval of greater than 6 months are eligible for treatment in the dose escalation and expansion cohorts (see also **APPENDIX B**).

2.1.1.2 Patients must have serum antibodies directed to HTLV-1.

2.1.1.3 Patients must have measurable or evaluable disease. Patients with > 10% of the PBMCs having the characteristic abnormal (i.e., $CD3^{dim}$, $CD4^{+}$ $CD25^{+}$ expressing) FACS profile for circulating ATL cells will be considered to have evaluable disease.

2.1.1.4 Patients must have adequate physiologic parameters:

- Absolute granulocyte count ≥ 500 K/uL, platelet count ≥ 75,000 K/uL and hemoglobin ≥ 10 g/dL.
- Bilirubin and creatinine ≤ 1.5 x institutional ULN.
- AST, ALT ≤3.0 x institutional ULN.

2.1.1.5 Karnofsky Performance Score ≥ 70% or ECOG ≤ 1 (**APPENDIX A**).

2.1.1.6 Patients must be able to understand and sign Informed Consent Form.

2.1.2 Exclusion Criteria

2.1.2.1 Patients with symptomatic leukemic meningitis, bony or GI tract involvement, serum calcium or LDH > 1.5 X the upper limit of normal will be excluded. However, patients that have both ATL and another HTLV-1 associated disease such as tropical spastic paraparesis (HAM/TSP) will be included.

2.1.2.2 Patients with symptomatic leukemic meningitis, bony or GI tract involvement, serum calcium or LDH > 1.5 X the upper limit of normal will be excluded. However, patients that have both ATL and another HTLV-1 associated disease such as tropical spastic paraparesis (HAM/TSP) will be included.

2.1.2.3 Patients who have received high doses of systemic corticosteroids for the treatment of their ATL within 4 weeks prior to the start of therapy.

2.1.2.4 Patients who have received any cytotoxic therapy, immunotherapy, antitumor vaccines or monoclonal antibodies in the 4 weeks prior to the start of the study.

2.1.2.5 Life expectancy of less than 3 months.

2.1.2.6 Documented active bacterial infections, HTLV-II infection, or hepatitis B or C as

follows:

- A positive hepatitis B serology indicative of previous immunization (i.e., HBsAb positive and HBc Ab negative), or a fully resolved acute hepatitis B infection is not an exclusion criterion.
- Patients with an indolent chronic hepatitis B infection (normal ALT, AST, albumin and no radiographic or biopsy evidence of cirrhosis) may be eligible.
- Patients with active hepatitis C are excluded. Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA.

2.1.2.7 Patients who have untreated human immunodeficiency virus (HIV) are not eligible for this study because by definition they have a defective immune response and are at much higher risk for opportunistic infections due to immune dysregulation by both HTLV-1 and HTLV-III (HIV) viruses. Patients on HIV therapy with undetectable viral loads as measured by HIV RNA quantitative real time PCR may be eligible.

2.1.2.8 Inability or refusal to practice effective contraception during therapy. Men and women of childbearing potential must use an effective method of birth control or abstinence during treatment and for 1 week after completion of the treatment.

2.1.2.9 Patient has significant and/or uncontrolled cardiac, renal, hepatic or other systemic disorders or significant psychological conditions at baseline visit that in the investigator's judgment would jeopardize subject enrollment or compliance with the study procedures.

2.1.2.10 Patients with an absolute requirement for a medication that is a strong inhibitor of P450 CYP3A4 (per **APPENDIX C**) are not eligible.

2.1.3 Recruitment Strategies

Patient accrual will be facilitated by the large number of clinical investigators who refer patients to the NCI and CCR. Contact letters or emails will be sent to the regional University referral center's Medical Oncologists informing them of this trial. In addition, this protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **10.5.2**

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols) (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Screening evaluation must be documented or performed within 28 days prior to initiating treatment:

2.2.3 Baseline history with documentation of sites of disease, physical examination, eligibility laboratory assessments. **NOTE:** For patients with skin lesions or other visually-apparent signs of disease, photographs may be taken to document and follow disease with permission.

2.2.4 Baseline imaging and circulating ATL cell count

- CT scan of the neck, chest, abdomen and pelvis
- Other imaging studies may be done to document disease as appropriate and at the direction of the PI.
- Determination of marker lesion measurements as per ATL International Consensus meeting[3].

2.2.5 FACS analysis for circulating $CD3^{dim}$ $CD4^{+}$ cells (see Section **3.4.1.6**)

2.2.6 Documentation of Karnofsky or ECOG Performance Score (**APPENDIX A**).

2.2.7 Pathology confirmed by the Laboratory of Pathology, NCI prior to registration.

- Pathological confirmation of diagnosis can be carried out on available slides from either the primary diagnostic biopsy or subsequent biopsies performed at the Clinical Center or outside institutions.
- For the subset of patients who do not have circulating abnormal cells a lymph node or skin biopsy will be obtained for diagnostic purposes and to evaluate the expression of the CD25 (Tac) on the malignant cells.
- Patients with suspected skin involvement from ATL should have skin biopsies. All skin or lymph node biopsies should be submitted fresh in saline to the Hematopathology Section of the Laboratory of Pathology.
- Biopsy specimens will be processed for routine histopathology as well as immunohistochemistry in frozen sections and/or flow cytometry for expression of CD25 (Tac) and other lymphocyte surface antigens.
- Lymph node aspirates will be handled as per the standard procedures of the cytology section.
- If the original pathological material or other diagnostic biopsies are not available, or if there is uncertainty concerning the diagnosis, a repeat biopsy may be undertaken to confirm the diagnosis of ATL.

2.2.8 Complete blood count (CBC) with differential, platelet count and reticulocyte count.

2.2.9 Acute Care panel (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine).

2.2.10 Hepatic panel (alkaline phosphatase, ALT, AST, total and direct bilirubin), LDH, and mineral panel (serum calcium, phosphate, magnesium and albumin).

2.2.11 Serum pregnancy test in women of childbearing potential.

2.2.12 Hepatitis B Surface Antigen, Hepatitis B Surface Antibody, Hepatitis B core Antibody, Hepatitis B DNA Quantitative, and Hepatitis C: HIV 1/2 and HTLV-I/II serology.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found **here.**

NOTE: Patients previously treated with the standard 20-mg twice daily dose who came off protocol for disease progression are eligible to re-enroll and be treated at the higher doses. These re-enrolled patients will be given new patient numbers to clarify their treatment outcomes for each of the 2 treatment regimens (standard vs. dose escalated).

2.3.1 Treatment Assignment and Randomization/Stratification Procedures

2.3.1.1 Cohorts

Number	Name	Description
1	T-Cell Leukemia patients (CLOSED)	HTLV-associated smoldering, chronic, or clinically indolent T-cell leukemia (ATL) (12 subjects)
2	T-Cell Leukemia patients – Dose Escalation	HTLV-associated smoldering, chronic, or clinically indolent T-cell leukemia (ATL) (up to 20 subjects)
3	T-Cell Leukemia patients – Dose Expansion	HTLV-associated smoldering, chronic, or clinically indolent T-cell leukemia (ATL) (up to 6 subjects)

2.3.1.2 Arms

Number	Name	Description
1	Experimental Treatment (CLOSED)	Ruxolitinib 20mg orally twice daily for 28 days. Patient may continue to receive treatment until PD.
2	Experimental Treatment – Dose Escalation	Ruxolitinib 30-50 mg orally twice daily for 28 days to determine the maximum tolerated dose (MTD). Patients may continue to receive treatment until PD or unacceptable toxicity.
3	Experimental Treatment – Dose Expansion	Ruxolitinib at the MTD orally twice daily for 28 days. Patients may continue to receive treatment until PD or unacceptable toxicity.

2.3.1.3 Randomization and Arm Assignment

Single arm/group: open-label and non-randomized. New patients in Cohort 2 or 3 directly assigned to treatment arm 2 or 3 based on the arm open at the time of enrollment. **NOTE:** Previously enrolled subjects are allowed to re-enroll on this study.

2.3.2 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an eligibility criterion (e.g., laboratory parameter) may be rescreened at the discretion of the investigator.

2.4 BASELINE EVALUATION

The following tests will be done after the patient is enrolled or re-enrolled in the study and within 28 days (except as indicated below) of initiating treatment with ruxolitinib. Tests do not need to be repeated if completed during screening and meet the timeframe for baseline evaluation.

2.4.1 Complete blood count (CBC) with differential, platelet count and reticulocyte count (within 2 weeks).

2.4.2 Acute Care Panel (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine) and C-Reactive Protein (within 2 weeks).

2.4.3 Hepatic panel (alkaline phosphatase, ALT, AST, total and direct bilirubin), LDH, and mineral panel (serum calcium, phosphate, magnesium and albumin) (within 2 weeks).

2.4.4 Pretreatment assessment of circulating ATL cells present in the PBMC by CellaVision differential (see Sections **3.4.1.4** and **3.4.1.6**, and **Table 4**) for T-cell receptor gamma (TCRγ) gene rearrangement to detect clonality (see Section **5.1**).

2.4.5 CMV serology and CMV antigens

2.4.6 FACS, Inflammatory marker and cytokine analysis

- Baseline flow cytometry to assess lymphocyte sets and subsets (one 10-mL purple-top tubes; see Section **3.4.1.6** and **Table 4**) to be sent to Flow Cytometry/ Immunology

Laboratory Department of Laboratory Medicine for the Clinical Center. **NOTE:** In case of scheduling conflicts, TBNK may be substituted.

2.4.7 In vitro analysis of sensitivity to JAK inhibitors and other molecular therapeutics in 6-day proliferation assay (see Section **5.1.2**)

2.4.8 Serum pregnancy test in women of childbearing potential within 3 days prior to starting the study drug.

2.4.9 **Optional Tumor Biopsy:** Patients with easily accessible tumor deposits who consent to this procedure will have a fine-needle aspiration or punch biopsy of one of their tumors for research (see Section **5.1.6**). These biopsies may be done by CT guidance.

2.4.10 Electrocardiogram

2.4.11 Bone marrow aspirate and biopsy will be obtained before treatment (biopsies performed at the Clinical Center within a year prior to treatment may be accepted at the discretion of the Principal Investigator).

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase I/II pilot open-label, trial of off label oral ruxolitinib that will enroll up to 35 patients with smoldering or chronic or biologically indolent lymphomatous or acute subtype ATL.

Each treatment cycle will be 28 days in length and new patients will begin treatment at 30 mg orally twice daily. If this dose is tolerated without exceeding the criteria for maximum tolerated dose (MTD) during the first cycle of treatment, the tolerability of treatment at 40 mg and then 50 mg twice daily will be evaluated. A total of 9 patients will be treated at the MTD or maximum administered dose (MAD) to better evaluate the clinical activity of the augmented dosing regimen. The start of subsequent treatment cycles may be delayed up to 7 days for social reasons or patients' convenience without impacting on a patient's ability to continue protocol treatment.

Patients who begin treatment with the higher dose level will stay local to the NCI Clinical Center for their initial 2 weeks of treatment. These patients will be monitored closely for the first month of treatment for the emergence of AEs. The patients will be seen in the outpatient clinic twice during the first week (day 1 and day 4 or 5) after starting treatment and on day 8 during week 2 for clinical and AE assessment, to have standard chemistry and hematology tests. If a patient appears to be tolerating their first 2 weeks of treatment well (grade 1 and 2 AEs) are considered reliable and have a referring physician willing to perform standard laboratories weekly (days 15 and 22 ± 2 days), they may be eligible to be monitored at home for the 3rd and 4th weeks of their cycle 1 treatment. Patients who have new complaints suggestive of toxicities will be seen as soon as possible for outpatient assessment of their condition. Patients who cannot meet these conditions or where there is concern that DLT will occur will remain near the NCI Clinical Center until completion of their cycle 1 treatment.

3.1.1 Dose Limiting Toxicity

Patients must complete their first cycle of treatment (28 days) without interruption or dose adjustment or DLTs to continue protocol specified treatment.

DLT will be defined as follows:

Any grade 3 or 4 toxicity, if deemed possibly, probably or definitely related to the study drug by

the PI during the first cycle of treatment, **with the following exceptions:**

- Grade 3 anemia without hemolysis
- Grade 3 or 4 granulocytopenia or leukopenia without infection
- Grade 3 thrombocytopenia without bleeding
- Grade 3 or 4 lymphopenia

Patients who tolerate treatment at these higher dose levels without DLTs will continue treatment at their initial dose level for the remainder of their protocol treatment and will not be eligible for further dose escalation. Patients who subsequently develop ≥ grade 3 toxicities after the DLT evaluation period will hold their treatment until this toxicity resolves.

The following toxicities will not require discontinuation of study drug, but treatment will be interrupted for a maximum of 10 days until the toxicity has resolved:

- Reversible grade 3 or 4 anemia without hemolysis
- Reversible grade 3 or 4 granulocytopenia or leukopenia without infection
- Reversible grade 3 or 4 thrombocytopenia without bleeding
- Reversible grade 3 or 4 non-hematologic toxicities
- Reversible grade 3 or 4 lymphopenia

These patients will be eligible to resume treatment at their starting dose one time if their AEs resolve within the 10-day treatment break; however, treatment will discontinue for recurrence of AE(s). Patients whose neutropenia does not resolve promptly may receive standard dose G-CSF to speed the recovery, but patients may not receive simultaneous G-CSF and Ruxolitinib.

Patients who have evidence of an ongoing response will continue their treatment in the absence of unacceptable toxicity or disease progression. All treatment cycles will be 28 days in length and patient's response to treatment will be assessed by FACS analysis and/or radiographic assessment of their disease performed after cycles 1, 2 and subsequent even numbered cycles. Patients will continue ruxolitinib until they experience unacceptable side effects, there is worsening of the ATL or a complete response is achieved. Response is defined in the report [4] of the 2009 International Consensus Meeting on ATL.

Patients who achieve a complete response will receive an additional 56 days (2 cycles) of treatment beyond the assessment that documented their CR and then stop treatment. These patients will continue to have disease reassessments every 3 months for evidence of relapse after completion of treatment.

3.1.2 Dose Escalation

Dose escalation will proceed in cohorts of 3-6 patients. The MTD is the dose level at which no more than 1 of up to 6 patients experience DLT during the first cycle (28 days) treatment, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level.

Dose Escalation Schedule	
Dose Level	**Dose of Ruxolitinib**
Level 1	30 mg PO BID
Level 2	40 mg PO BID
Level 3	50 mg PO BID

Dose escalation will follow the rules outlined in the table below; this study plans to treat 9 patients at the MTD/MAD:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients 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. UP to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6	This is the MTD and is generally the recommended phase 2 dose. **NOTE:** If ≤1 out of 6 patients with DLT occurs at DL3, the MTD will not have been identified and DL3 will be declared the MAD for further study.

3.1.3 Safety Assessment and Study Stopping Rules

AEs will be assessed and graded according to CTEP Common Toxicity Criteria (CTC) version 4 and the decisions regarding MTD or tolerability of the augmented dosing will be changed to follow standard phase I criteria.

Overall drug safety will be further characterized by examining adverse events, laboratory abnormalities, physical examination and ECOG performance status change from baseline. All AEs occurring within 30 days from the last study treatment will be summarized for all patients and reviewed after the 9th and 20th patients to determine if later toxicities occurring outside of the DLT evaluation period are placing the patients at greater risk for SAEs. If the protocol meets the stopping criteria, all patients will discontinue study treatment, including patients who have been treated at different, and presumably tolerable, doses.

Stopping rules for the study to address delayed clinically significant AEs are defined as ≥ 2 of the

first 9 patients experiencing grade 4 or greater toxicity attributed as possibly, probably or definitely related to ruxolitinib, or ≥ 4 of the first 20 patients experiencing similar toxicity.

Treatment will be discontinued for unacceptable toxicity defined as any grade 4 infection, febrile neutropenia treated with IV antibiotics, bleeding associated with thrombocytopenia, any evidence of sustained organ dysfunction or clinically significant biochemical laboratory abnormalities lasting more than 10 days which occurs during the treatment and is deemed by the PI to be possibly, probably or definitely related to the study drug.

3.2 DRUG ADMINISTRATION

Ruxolitinib will be given orally (PO) twice daily to newly enrolled patients at sequentially increasing dose levels of 30, 40 and 50 mg. In the absence of DLT, dose escalation will proceed until an MTD dose is defined or the 50 mg twice daily dose is shown to be tolerable.

3.3 DOSE MODIFICATIONS

There will be no modifications of the ruxolitinib dose for individual patients. Patients will be treated throughout the study with the same dose as initially administered and if this dose is not tolerable (e.g., DLT or late clinically significant toxicity) their treatment will be discontinued.

Ruxolitinib will be held for up to 10 days for the toxicities listed above in Section **3.1.3** that occur after the cycle 1 determination of the MTD until the toxicity has resolved to ≤ grade 1 or baseline and then resumed at the patient's starting dose one time.

Treatment will be discontinued for any patient has recurrence of the AEs that prompted interruption of treatment.

3.4 CLINICAL MONITORING

3.4.1 Clinical Monitoring and Assessments During Treatment

The following assessments will be performed on the patient evaluation days listed below and in the study calendar. Please refer to Section **2.4** for tests to be performed at baseline. The window for assessments is ±2 days during cycle 1 and ±5 days during cycle 2 and beyond, unless otherwise noted below or on the Study Calendar (Section **3.5**).

Patients who tolerate cycles 1 and 2 without any ≥ grade 2 toxicities or unanticipated adverse events, clinical monitoring visit may be extended to every 2 cycles for the first year of treatment. For patients continuing treatment beyond 1 year, clinical monitoring and response assessments may be extended to every 3rd or 4th cycle at the discretion of the investigator during year 2 and beyond.

Patients who have symptoms or findings suggestive of disease progression or delayed toxicity at later dates will have the appropriate laboratory and radiographic assessment to when they present to the clinic. Monitoring tests listed below will be performed once the tolerability of the treatment is established when the patient is seen in clinic.

3.4.1.1 **Vital signs:** Including weight, heart rate, blood pressure, respiration, temperature and arterial oxygen saturation (SO2) by transcutaneous pulse oximeter on whenever the patients are seen for follow-up clinical evaluations.

3.4.1.2 **Interval history, physical examination and performance status:** Days 1, 4 or 5 and 8 of cycle 1. Patients may also be seen on day 15 and 22 of cycle 1 and as needed to assess potential toxicities. Patients will be seen on day 1 of cycle 2, and at every subsequent outpatient follow-up in the clinic. NOTE: For patients with skin lesions

or other visually-apparent signs of disease, photographs may be taken to document response to treatment with permission.

3.4.1.3 **Routine chemistry and hematology tests,** including acute care panel, mineral and hepatic panels, LDH, C Reactive Protein (CRP) and CBC with differential: These labs will be obtained at any NCI outpatient visit during cycle 1; cycle 1 days 15 and 22 at home if the patient is being monitored remotely. The laboratories will be obtained on cycle 2 day 1 and at all subsequent NCI outpatient visits during the period of protocol treatment. These routine tests may also be performed at home by the patient's referring physician when the patient is not seen in the outpatient clinic or if there is suspicion of clinical progression, if feasible.

3.4.1.4 **CellaVision differential** to quantitate circulating ATL: At pre-treatment and the same schedule as routine chemistry and hematology laboratories. The CellaVision differential will be performed on a slide produced from the routine hematology sample of the day to identify atypical appearing T-cells with characteristics. Dr. Katherine Calvo or designee Hematology service/Hematopathology Bldg. 10 RM 2C418A will determine that the smear is adequate for analysis and send the processed slides to the Lymphoid Malignancies Branch for storage prior to analysis by a single hematopathologist at a later date**.**

3.4.1.5 **Radiographic restaging for response**: CT scan neck, chest, abdomen and pelvis (or other imaging studies as appropriate and at the direction of the PI) for patients with radiographically measurable or evaluable disease pre-treatment, at the end of cycles 1 and 2, every 2 cycles while the patient is on treatment (*see note), and at the end of treatment (if feasible) for all patients who have radiographically assessible disease. *NOTE: For patients continuing treatment beyond 1 year, assessments may be extended to every 3rd or 4th cycle at the discretion of the investigator. The clinical and other research assessments may also be updated accordingly without being considered a protocol deviation.

Patients who do not have radiographically evident disease at their initial evaluations are not required to have these restaging films unless there is clinical concern for disease progression. Patients with radiographically evident disease who have subjective or objective evidence (physical exam or laboratory results) that suggests disease progression may also undergo radiographic restaging to assess for new radiographic lesions.

Patients who come off treatment for a reason other than disease progression, will continue to be followed until progression (or another off-study reason per Section **3.8.2**). Assessments may be done at NIH or outside, with optional imaging at the timing discretion of the investigator.

3.4.1.6 **FACS panel restaging for response**: These samples will be collected at: pre-treatment; on day 15 and pre-cycle 2 and 3 (if feasible); at the same time as disease restaging occurs; and, at the end of treatment and off study evaluations (if appropriate). The multicolor FACS panel listed below in **Table 4** will be performed to determine percentage and absolute number of CD3^{dim} CD4^{+} ATL. Changes in CD4^{+} CD8^{+}, NK cells and T-cell central and effector memory subsets will also be assessed in these analyses (see Section **5.1.3**). Additional tubes or cell surface

markers maybe inserted or substituted into this panel later during the conduct of the trial without increasing the amount of blood sampled. **NOTE:** In case of scheduling conflicts, TBNK may be substituted.

Table 4: FACS panel surface markers and fluorokine

Tube	FITC	PE	PerCP	PE-Cy7	APC	APC-efluor 780
1	CD14					CD45 Ungated
2	IgG	IgG	IgG	IgG	IgG	CD45
3	CD45RA	CD62L	CD4	CD3	CD8	CD45
4	CD7	CD25	CD4	CD3	CD8	CD45
5	CD3/CD16+CD56		CD20			CD45
6	CD2	IL15Rα	CD4	CD3		CD45
7	CD57	MIKβ3	CD127	CD3	CD4	CD45
Initial Study Only						
8	CD3	CD25	CD194(CCR4)		CD4	CD45

3.4.1.7 **Hepatitis B serologies and quantitative DNA:** These will be repeated for patients with indolent hepatitis B infections every 3-4 months during treatment as indicated in Section **3.5** Study Calendar; and, at least yearly after treatment.

3.4.1.8 **Optional Bone marrow aspirate and biopsy** to confirm a complete response

3.4.1.9 **Optional Tumor Biopsy:** Patients with easily accessible tumor deposits who consent to this procedure will have a fine-needle aspiration (FNA) of one of their tumors during the first 28 days of treatment (between days 14 through 28) to assess for changes in their ATL infiltration and changes in gene expression associated with ruxolitinib treatment. These biopsies may be done by CT guidance. Patients who consent to additional optional FNA's may be asked to undergo an FNA during the study treatment and/or if they have progressive disease while on-study. These biopsies will help us gain additional insight into the *in vivo* effects of ruxolitinib. These samples will be used for research.

3.4.1.10 **Other Studies**

Patients may have additional laboratory or radiological studies performed as clinically indicated, in particular if there were a suspicion of disease progression that would warrant an urgent therapeutic intervention.

3.5 STUDY CALENDAR

Procedure/ Assessment	Screening	Baseline	Treatment[1]									End of Treatment and Off Study[2,17]
			Cycle 1					Cycle 2		Cycles 3 and 4, and beyond		
			D1	D4-5, D8	D15	D22	D29	D1	D29	D1	Every 2 cycles year 1, every 3rd or 4th cycle year 2+	
Windows:	*D -28 to -1*	*D -14 to -1*	*±2 days*					*±5 days*		*±10 days*		
Ruxolitinib treatment [3]			X (daily)									
Informed consent		X										
NCI pathology review/confirmation [4]	X											
Complete medical history	X											
Physical exam/interval history, Vital signs, Performance status [5]	X		X	X	X	X		X		X		X
Serum pregnancy test [7]	X	X	X					X		X		
CBC w/differential	X	X	X	X	X	X		X		X		X
Chemistries panels, LDH and CRP [6]	X	X	X	X	X	X		X		X		X
Reticulocyte count	X	X										
EKG	X											
Radiographic disease assessment [8]	X						X		X		X	(X)
Photography for skin lesions (if applicable)	X		X				X		X		X	X
CellaVision differential & FACS assessment: TBNK or lymphocyte subset analysis [9]	X		X				X		X		X	X
HIV, HTLV, Hepatitis C serology [10]	X											
Hepatitis B serology, Hepatitis B Quantitative DNA [10]	X									X (see footnote)		
Bone marrow biopsy and aspirate [11]		X										
CMV serology and CMV antigens		X										
Soluble IL-2 receptor alpha, IL-15/IL-15 receptor alpha, IL-18, TNFα [12]		X	X		X		X		X		X	X
In vitro/PBMC proliferation assays[13]		(X)	(X)		(X)		(X)			(X)	(X)	(X)
STAT5, phosphoSTAT5 and ruxolitinib levels[14]		(X)	(X)	(X)	(X)		(X)			(X)	(X)	(X)
Molecular analysis of TCRγ chain[15]		X					X				X	X
Optional tumor biopsy[16]		(X)			(X)			(X)				

(X) = Optional

NOTE: Perform additional assessments as clinically indicated. Baseline studies do not need to be repeated if completed during screening and meet the timeframe for baseline evaluation requirements.

1. Each cycle will be 28 days in length. The start of subsequent cycles may be delayed up to 7 days for social occasions or patient's convenience. The patients will be seen in the outpatient clinic twice during the first week (day 1 and day 4 or 5) after starting treatment and

on day 8. Patient who are tolerating their first 2 weeks of treatment well may be eligible to be monitored at home for the 3rd and 4th weeks of their cycle 1 treatment as stated in Section **3.1**. Patients who have new complaints suggestive of toxicities will be seen as soon as possible for outpatient assessment of their condition Patients who tolerate cycles 1 and 2 without any ≥ grade 2 toxicities or unanticipated adverse events, clinical monitoring visit maybe extended to every 2 cycles (i.e., 56 days) after cycle 4 once the tolerability of their treatment is established. For patients continuing treatment beyond 1 year, assessments may be extended to every 3rd or 4th cycle at the discretion of the investigator. The clinical and other research assessments may also be updated accordingly without being considered a protocol deviation

2. Denotes testing to be performed at the "end of study visit" (just prior to the patient being taken off study), if feasible.
3. Doses are 30, 40 and 50 mg twice daily.
4. Including biopsies of clinically suspicious skin lesions.
5. Vital signs include: weight, heart rate, blood pressure, respiration, temperature and arterial oxygen saturation (S_{O2}) by transcutaneous pulse oximeter, for follow up clinical evaluations.
6. Chemistry tests include acute care (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine), mineral (serum calcium, phosphate, magnesium and albumin) and hepatic (alkaline phosphatase, ALT, AST, total and direct bilirubin) panels, LDH and CBC will be obtained twice weekly (e.g., M, Th) during the first week and then weekly during the first month of treatment with the higher doses. C-reactive protein(CRP) will at minimum be assessed day 1 of cycle 1, day 29 of cycles 2 and 4, and every 3-4 months in follow-up for all subsequent treatment cycles. These labs may also be performed at home by the patient's referring physician when the patient is not seen in the outpatient clinic or if there is suspicion of clinical progression, if feasible.
7. Pretreatment day 1 or within 3 days prior to starting each cycle of treatment (ideally) when the patients are seen in follow-up at the Clinical Center in women of childbearing potential.
8. Radiographic restaging for response for patients with radiographically measurable or evaluable disease: CT scan neck, chest, abdomen and pelvis (or other imaging studies as appropriate) on pretreatment for all patients and then day 29 of cycles 1 and 2, and then every 2 cycles for patients who have radiographically measurable or evaluable disease.
9. CellaVision differential and FACS panel shown in **Table 4**, and Sections **3.4.1.6** and **3.4.1.4** for additional details. One 10 mL lavender top tube will be drawn for the FACS panel and sent to the Immunology Lab of the NIH Clinical Center 10/2C410. TBNK can be substituted for scheduling conflicts.
10. HIV and Hepatitis C testing only during screening. Hepatitis B testing as indicated also at screening; patients who have an indolent chronic Hepatitis B infection will have repeat serial Hepatitis B Quantitative DNA and serology every 2 cycles during treatment; this testing may be extended to every 3rd or 4th cycle at the discretion of the investigator after year 1 if the timing for other assessments is adjusted.
11. Patients who are complete responders by FACS evaluation of circulating ATL cells and/or radiographic assessment may have a second BM biopsy (optional) to confirm their response.
12. Samples to assess for IL-2Rα IL-18 and TNFα will be drawn in 5mL SST tubes at pre-treatment, on day 15 (if feasible), pre-cycle 2 and 3, at the same time as disease restaging occurs, and at the end of treatment off study visits for all patients. Samples will be held at room temperature until picked up by a Lymphoid Malignancies Branch clinical researcher who will ship the samples to a Contractor for performance of the assays. Serum samples will also be routed by the Department of Laboratory Medicine to Mayo Laboratories for CLIA-certified analysis of soluble IL-2Rα levels. See Section **5.1.3** for additional details.
13. In vitro (PBMC) proliferation assays will be an optional test for all patients to potentially be performed pre- and post-treatment on cycle 1 day 1, 15 and 29 and at the time of any subsequent follow-up visit at the PI's discretion. Up to 50 mL of whole blood will be drawn in a heparinized (green top) tubes. **NOTE:** These assays will not be performed if the absolute lymphocyte count indicates an insufficient number of cells to form these assays at these time points.

14. Assessment of the STAT 5 and phosphoSTAT 5 or other JAK-STAT pathway associated proteins in intracellular FACS analyses will be an optional test to potentially be performed on cycle 1 day 1 (pre-treatment, and 1, 3, 6 and 24 hours after treatment), days 15 and 29, and at the time of any subsequent follow-up visit at the PI's discretion. If drawn independently of the samples for in vitro proliferation assays, approximately 30 mL of blood may be drawn in heparinized (green top) tubes along with a 5 mL serum (SST tube) sample to allow for comparison of ruxolitinib level to STAT5 and phosphoSTAT5 measurements. See Section **5.1.4** for additional details.
15. Blood (4 mL) will be collected in light blue (citrate) tube at baseline, pre-cycle 2 (i.e., day 29 of cycle 1), at the same time as disease restaging occurs, and at the off-study evaluation, if feasible. The blood will be transported to room 3S247 attention to Dr. Mark Raffeld (see Section **5.1.5**).
16. Patients with easily accessible tumor deposits who consent to this procedure will have a fine-needle aspiration of one of their tumors at baseline and during the first 28 days of treatment (between days 14 through 28) to assess for changes in their ATL infiltration and changes in gene expression associated with ruxolitinib treatment. Consideration for additional tumor biopsies will be given in patients with evidence of an ongoing response, at the time of relapse and/or other clinically notable time points to gain additional insight into the *in vivo* effects of ruxolitinib. These biopsies are optional.
17. End of treatment visit will occur approximately 30 days after the last dose of study drug. If the patient cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs (specify as needed) from a local physician or laboratory. If this is not possible, patients may be assessed by telephone for symptoms. If a patient comes off treatment for a reason other than disease progression, we will continue to follow the patient until progression (or another off-study reason); in these cases, optional imaging will be at the discretion of the investigator.

3.6 CONCURRENT THERAPY

Concurrent anti-cancer therapy to include immunotherapy, immunosuppressive therapy, radiation therapy or chemotherapy, and administration of systemic corticosteroids except for physiologic replacement doses of cortisone acetate is not permitted while on the study. Initiation of medically indicated, non-oncologic medications such as anti-hypertensive agents or antibiotics for urinary tract infections that do not have significant potential for interaction with the ruxolitinib treatment are allowable with prior approval of the PI.

Concurrent use of strong CYP3A4 inhibitors is to be avoided, if possible. Per the eligibility, patients with an absolute requirement for medications that are CYP3A4 inhibitors are not eligible for enrollment. Consideration of initiation on treatment must be considered and only with the approval of the Principal Investigator. Alternative medications should be used whenever possible.

3.7 COST AND COMPENSATION

3.7.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.8 CRITERIA FOR REMOVAL FROM THE PROTOCOL TREATMENT AND OFF-STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days after the last dose of study therapy.

3.8.1 Off-Treatment Criteria

- Unacceptable toxicity as defined in Section **3.1.3** or **3.3**
- Disease progression not responsive to highest dose level that can be safely administered
- Persistent (> 14 days) treatment-related adverse events ≥ grade 2
- Investigator discretion
- Positive pregnancy test

3.8.2 Off-Study Criteria

- Completion of protocol therapy, with evidence of progression, including a 30-day safety visit
- Voluntary withdrawal of consent by the patient

- Patient non-compliance with treatment and research plans
- The institution of another anticancer treatment
- Any new medical or psychiatric condition that, in the opinion of the PI, precludes the safe continuation of the study
- Death

4 CONCOMITANT MEDICATIONS/MEASURES

Patients will be followed and managed as clinically appropriate with IV fluids, anti-emetics, anti-pyretics or other standard interventions based on the clinical experience with Ruxolitinib.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

All samples will be collected at the time points identified in the Study Calendar (Section **3.5**). The volumes for blood samples noted below may be adjusted at the time of collection based upon tube availability; in no case will the maximum amount of blood allowed to be collected from patients be exceeded.

5.1.1 Soluble IL-2 Receptor alpha chain, IL-15/IL-15 Receptor alpha (IL-15 Rα), IL-18 and TNFα assessments

Samples for IL-2Rα, IL-15/IL-15Rα, IL-18 and TNFα will be drawn in SST tubes (5 mL).

Labels listing the date and time of the blood draw as well as the patient's name will be affixed to all the tubes by the staff person who obtained the samples. Lymphoid Malignancies Branch Clinical Research personnel will arrange for these samples to be sent via courier to Clinical Support Laboratory at NCI/ Leidos Biomedical Research, Inc., for analysis. Patient identifiers will be removed from all specimens, and they will have assigned a unique number and will be labeled with this number. Serum samples will also be routed by the Department of Laboratory Medicine to Mayo Laboratories for CLIA-certified analysis of soluble IL-2Rα levels.

5.1.2 In Vitro Proliferative Assays

NOTE: These samples are optional and will be collected at the discretion of the PI. When collected, the following applies:

Samples for in vitro proliferative assays (PBMCs) will be drawn in a heparinized (green top) tubes (50 mL). The assays will not be performed if the absolute lymphocyte count indicates an insufficient number of cells to perform these assays at these time points. **If there is an insufficient number of circulating cells at these time points, the medical record will indicate this assessment was not performed due to insufficient material and will not be considered a protocol deviation.** Lymphoid Malignancies Branch Clinical Research personnel will pick up these tubes from phlebotomy.

These concentrations of the JAK inhibitors is based on the human pharmacokinetic data to represent a low but achievable *in vivo* drug level and a higher inhibitory level that cannot safely be achieved in patients to assess the drugs' potential inhibitory properties. The effect on proliferation will be assessed by addition of ^{3}H-thymidine 6 hours before the end of the 6-day cultures. Culture supernatants will be assessed by specific ELISAs for IL-2, IL-9, IL- 15 and IL-21.

These specimens may also be used to perform additional exploratory molecular analyses limited to immune response genes or genes known to be critical to the pathophysiology of HTLV-1 related ATL including but not limited to JAK1, JAK2, JAK3, IRF4, CCR4, NOTCH1 and p53.

5.1.3 FACS Analyses

FACS analyses of $CD4^+$, $CD8^+$, NK cells and T-cell central and effector memory subsets will also be assessed at the same time the patient's ATL count is assessed to explore the effects of ruxolitinib on the immune effector cells. **NOTE:** Flow cytometry samples to assess lymphocyte sets and subsets will also be sent to Flow Cytometry/Immunology Laboratory Department of Laboratory Medicine for the Clinical Center (Section **3.4.1.6**).

5.1.4 Analysis of STAT5; phosphoSTAT5 and serum ruxolitinib level

NOTE: These samples are optional and will be collected at the discretion of the PI. When collected, the following applies:

If sufficient numbers of ATL cells are obtained from the PBMC samples drawn for the proliferation assays, the cellular lysates will also be used to perform the intracellular FACS analyses for expression of STAT 5 and phosphoSTAT5 or other proteins of interest associated with the JAK-STAT pathway. When samples are collected independently of time points for PBMC samples for in vitro proliferation assays (e.g., Cycle 1 Day 1 post-treatment), blood will be drawn in a heparinized (green top) tubes. For these analyses, about 30 mL of blood is anticipated to be needed; however, may be adjusted based upon the number of cells collected and ongoing assay development with observation of the maximum amount of blood allowed to be collected within any 8-week period.

A serum sample will also be collected at each of the same time points (5 mL SST tubes) and retained to allow for comparison of ruxolitinib level to STAT5 and phosphoSTAT5 measurements.

5.1.5 Molecular Analysis for T-cell Receptor Gamma (TCRγ) Gene Rearrangement

To detect clonality and analysis of HTLV-1 viral titer, blood will be collected in light blue (citrate buffer) tubes (4 mL). The blood will be transported to room 2N116A attention to Dr. Mark Raffeld or designee. These samples will be stored at -70º C until batch analysis is performed.

5.1.6 Optional Tumor Biopsies

Patients with easily accessible tumor deposits who consent to this procedure will have a fine-needle aspiration or punch biopsy of one of their tumors at baseline and during the first 28 days of treatment (between days 14 through 28) to assess for changes in their ATL infiltration and changes in gene expression associated with ruxolitinib treatment. Patients who consent to additional optional FNA's may be asked to undergo an FNA during the study treatment and/or if they have progressive disease while on-study. These biopsies will help us gain additional insight into the *in vivo* effects of ruxolitinib. These samples will be used for research. A 23-gauge hypodermic needle will be used to obtain biopsies from the tumor mass. After insertion, multiple passes at changing angles of insertion will be made through all quadrants of the tumor. Alternatively, standard punch biopsy technique will be followed.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The

study will remain open so long as sample or data analysis continues. Samples from consenting patients will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent his/her data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2.1**.

5.2.1 Research Sample Control

- For research samples obtained for investigation, the Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled-access building and laboratory doors are kept locked at all times. Upon specimen receipt, each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID. An electronic database is used to store information related to patient samples processed by the laboratory. Vial labels do not contain any personal identifier information. Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long-term storage. These facilities are operated under subcontract to Leidos Biomedical Research, Inc.-Frederick. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH, it is the policy of the laboratory to request documentation that appropriate approvals and/or agreements, if required. are in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process. The NCI investigator responsible for the sample collection is responsible for ensuring appropriate approvals and/ or agreements are in place prior to requesting the laboratory to ship samples outside of the NIH.
- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document and the proposed research has undergone prospective IRB review and approval. If new risks are associated with the research (e.g., analysis of germline genetic mutations), the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.
- Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB-approved protocol and patient consent. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

5.2.2 Tracking

- All samples will be barcoded, with data entered and stored in the Labmatrix Data Management System utilized by the Lymphoid Malignancies Branch. This is a secure program, with access to this system limited to defined MB personnel, who are issued individual user accounts. The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number; time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the Clinical Center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).
- Barcoded samples are stored in barcoded boxes in a locked freezer at either -20°C or -80°C according to stability requirements. These freezers are located onsite in the Clinical Support Laboratory and offsite at NCI-Frederick Central Repository Services in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the Labmatrix System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB-approved protocol) and that any unused samples must be returned to the Clinical Support Laboratory.
- Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix System.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. AEs will be documented from the first study intervention, through 30 days following the last dose of study agent. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Data Collection/Recording Exceptions

6.1.1.1 Abnormal Laboratory Values

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

6.1.1.2 Hospitalizations

Hospitalizations or prolonged hospitalization for the following reasons do not require reporting:

- Technical, practical, or social reasons, in absence of an AE
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition, including scheduled therapy or standard procedure for the target disease of the study, and those required to allow efficacy measurement for the study
- Diagnostic or elective surgical procedures for preexisting conditions or a procedure that is planned (e.g., planned prior to starting of treatment on study)
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria

6.2 RESPONSE CRITERIA

Response assessment will be based on three sets of response criteria: Revised Response Criteria for lymphoma by Cheson et al[38], also by Cheson et al[39], and International Consensus Meeting Criteria for ATL[4].

6.2.1 Criteria of response for Malignant Lymphoma [57]

6.2.1.1 Complete response

The designation of a complete response (CR) requires all of the following: **(1)** Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy; **(2)** a post-treatment residual mass is permitted as long as it is PET negative. If a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (<1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy); **(3)** Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to < 1.0 cm in their short axis after treatment; **(4)** The spleen and/or liver if considered enlarged before therapy based on a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and **(5)** If the bone

marrow was involved by lymphoma before treatment the infiltrate must have cleared on repeat bone marrow biopsy.

6.2.1.2 Partial response

The designation of a partial response (PR) requires all of the following: **(1**) At least a 50% reduction in the sum of the product of the diameters of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least two perpendicular dimensions; if possible they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved; **(2)** No increase in the size of other nodes, liver, or spleen; **(3)** Splenic and hepatic nodules must regress by >50% in their SPD or for single nodules in the greatest transverse diameter; **(4)** With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present. Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. Patients who achieve a CR by the above criteria but who have persistent morphologic bone marrow involvement will be considered partial responders; **(5)** No new sites of disease; **(6)** If the pretreatment PET scan was positive, the post-treatment PET should be positive in at least one previously involved site; and **(7)** If a pretreatment PET was negative, CT criteria should be used.

6.2.1.3 Stable disease

A patient is considered to have stable disease (SD) when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for progressive disease. The PET should be positive at prior sites of disease with no new areas of involvement on the post treatment CT or PET. If the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post treatment CT scan.

6.2.1.4 Progressive disease

Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0 cm. Lymph nodes < 1.0 X < 1.0 cm will not be considered as abnormal for relapse or progressive disease. Other criteria for progressive disease include the following: **(1)** Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. **(2)** At least a 50% increase from nadir in the sum of the products of the diameters of any previously involved nodes, or in a single involved node, or the size of other lesions A lymph node with a diameter of the short axis of less than 1.0 cm must increase by >50% and to a size of 1.5 X 1.5 cm or more than 1.5 cm in the long axis. **(3)** At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis; **(4)** Lesions should be PET positive if the lesion was PET positive before therapy unless the lesion is too small to be detected by PET; **(5)** Measurable extranodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical exam, it is found to be histologically negative.

6.2.2 Criteria of Response for ATL

6.2.2.1 Complete response

Complete response (CR) is defined as disappearance of all clinical, microscopic, and radiographic evidence of disease. All nodes must have regressed to normal size (1.5 cm in their greatest transverse diameter) and previously involved nodes that were 1.1 to 1.5cm must have decreased to 1.0 cm. Because HTLV-1 carriers frequently have a small percentage of abnormal lymphocytes with polylobated nuclei, so called flower cells in peripheral blood, provided that less than 5% of such cells remained and the absolute lymphocyte count, including flower cells, was less than 4 x 10^9/L, CR is attained.

6.2.2.2 Complete response unconfirmed

A designation of unconfirmed complete response (CRu) requires a 75% reduction in tumor size with a residual mass after treatment.

6.2.2.3 Partial response

Partial response (PR) is defined as a ≥50% reduction in the sum of the products of the greatest diameters of measurable disease without the appearance of new lesions. In addition, a 50% or greater reduction in absolute abnormal lymphocyte counts in peripheral blood is required to attain PR.

6.2.2.4 Progressive disease

Progressive disease (PD) in peripheral blood is defined by a 50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of 4 x 10^9/L.

PD or relapsed disease in the other lesions is defined as a 50% increase from nadir in the sum of the products of measurable disease or the appearance of new lesions excluding skin.

6.2.2.5 Stable disease

Stable disease is defined as failure to attain CR/PR or PD.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on approximately a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level, enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

The primary goal of this study is to evaluate the safety and efficacy of ruxolitinib in patients with smoldering or chronic adult T-cell leukemia (ATL). Despite the Shimoyama Classification system[4], smoldering and chronic phase ATL patients appear to be a biologically heterogeneous group. Chronic ATL with adverse disease characteristics such as poorer performance status, lower neutrophil count receiving chemotherapy have been reported to have a poorer median survival than is generally expected for this subtype of ATL and this should be reflected in lower response rate to treatments. Picking an acceptable phase II target response rate can be difficult, due to the lack of clarity in the historical database for treated chronic or smoldering ATL. Survival is the clinical endpoint most often captured in these treatment reports and response rates are frequently not sited and, as previously discussed (Section **1.6**), survival data can be inconsistent.[47] Given the available data that indicates previously treated chronic or smoldering subtype ATL patients who are further along in the natural history of their ATL and respond less well to any form of treatment, we believe that an initial target response rate of 30% in our referral patient population is an appropriate target value for response in a phase II trial[41]. We will adhere strictly to the definition of chronic and smoldering subtypes as defined by Shimoyama for the enrolled patients.

Original Study Design: In this study for chronic or smoldering ATL will use an optimal two-stage Simon design to test for early evidence for efficacy based on complete and partial response. The design is based on the following: (a) the response proportion (partial and complete response) will be less than 5% if the treatment is totally ineffective (Po=0.05), (b) the treatment will be considered effective and worthy of future investigation if the true response proportion >30% (P1=0.30) (see Section **1.6**), (c) a type I error rate of 5% (i.e., the probability of concluding that the treatment is

effective if the true response rate is 5% is 0.05), (d) a power of 90% (i.e., the probability of concluding that the treatment is effective if the true response proportion is 30% is 0.95).

Updated Study Design (Amendment D): Although there were 4 responding patients in the first 12 patient who were treated, the activity was not sustained even in these patients; therefore, we are now changing the treatment regimen to evaluate the activity of augmented dosing (effective with Amendment D). We plan to treat a total of 9 patients at the MTD or MAD and will not proceed with the same original two-stage phase 2 dosing. Patients who discontinue treatment within the first 28 days for reasons other than toxicity will be considered inevaluable. In order to allow for this assessment of patients attempting higher treatment doses, the protocol will require an additional 15-21 patients. Accounting for the previously enrolled 12 subjects, the new design, and to assess up to 9 patients for response at the MTD/MAD level with the new increased dose levels as well as allowing for up to two (2) inevaluable patients: the new accrual ceiling will be set at 35 patients.

The major objective of this study continues to be to determine the antitumor activity of ruxolitinib with regard to response rate and time to progression. Response rate will be based on the number of patients who achieve either a complete or partial response to therapy. Time to progression will be measured from the date of registration until documentation of disease progression..

An exploratory analysis will be performed in the two subgroups of patients defined by the inhibition of their pretreatment *in vitro* ATL cells proliferation by ruxolitinib treatment. The patients will be distinguished on the basis of the results of a 6-day *ex vivo* culture analyses performed on patient peripheral blood mononuclear cells prior to drug administration. One subgroup of patients will be defined as those who fulfill the following criteria: peripheral blood mononuclear cells proliferate *ex vivo* (≥ 5,000 CPM/1μC) in 6-day culture and their thymidine uptake must be reduced by 40% by the addition of 10 μM ruxolitinib. The other group of patients would be all the remaining treated patients that do not fulfill these criteria. Since a limited number of patient samples have been assessed to date, it is not clear what percentage of smoldering or chronic stage ATL patients will fulfill the criteria for group 1.

9 COLLABORATIVE AGREEMENTS

Not applicable.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Men and women age 18 years or older of all races and ethnic groups are eligible for this trial if they meet the eligibility criteria. If differences in outcome that correlate to gender, racial, or ethnic identity were noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully. Because there is no significant preclinical information regarding the risk to a fetus or newborn infant, pregnant or breastfeeding women will be excluded from participation in this trial. Since ATL patients have an underlying defect in their immunity due to the chronic infection, T-cell dysfunction and are already at risk for opportunistic infections, patients with hepatitis B with evidence of end organ damage or dysfunction or active hepatitis C are not eligible for this trial.

10.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 years of age are not eligible to participate in this trial. The immune physiology of children and the potential toxicity in this population are felt to be sufficiently

different from those of adult patients that a separate protocol, dedicated to pediatric patients, appears warranted. Therefore, children may be the object of a separate protocol once toxicity and a dose range with biological activity are defined in adults.

10.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed. Please see section **10.5.1** for consent procedure.

10.4 Evaluation of Benefits and Risks/Discomforts

The discomforts of a 28-day or longer treatment with oral ruxolitinib are expected to be minimal. The primary risk to patients participating in this research study is from unexpected or unforeseen toxicity from ruxolitinib as an investigational agent for this indication. Patients will be carefully monitored for any adverse events and appropriate care provided.

10.4.1 Risks from Study Procedures

10.4.1.1 CT Scans

CT scans often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/ or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have more severe allergic reactions to the contrast which may include skins rashes, shortness of breath, wheezing or low blood pressure.

10.4.1.2 Radiation Exposure

On this study, patients will receive up to eight (8) CT Scans (CT Scan of neck, chest, abdomen and pelvis) maximum in annual period. Participants will undergo up to three (3) optional CT guided biopsy. The procedures for performing the CT will follow clinical policies, no special procedures will apply to these assessments for research purposes.

The total radiation dose for research purposes will be approximately 12.8 rem. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer.

10.4.1.3 Optional Tumor Biopsy

The biopsy may cause pain, bruising, bleeding and infection. The risks associated with biopsies are pain and bleeding at the biopsy site. In order to minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site. CT guidance may be used in obtaining biopsies (see **10.4.1.2** for radiation risk).

10.4.1.4 Bone Marrow Aspiration

These procedures usually cause only brief discomfort at the site from which the biopsy is taken. Rarely, infection or bleeding may occur at the needle site. A numbing agent that can cause a stinging or burning sensation may be injected at the site of your bone marrow biopsy.

The biopsy needle will go through the skin into the bone and may produce a brief, sharp pain. As the bone marrow liquid is taken from the bone, there may be a brief, sharp pain. Since the inside of the bone cannot be numbed, this procedure may cause some discomfort, however not all patients experience discomfort. The possible side effects associated with a bone marrow biopsy include pain, bleeding, bruising, and infection, as well as a reaction to the numbing agent.

10.4.1.5 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

10.4.1.6 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

10.5 Consent and Assent Process and Documentation

The informed consent document will be provided to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

10.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **10.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **10.5.**

10.5.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

11 REGULATORY AND OPERATIONAL CONSIDERATIONS

11.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

11.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have

such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

12 PHARMACEUTICAL INFORMATION

There will be no IND obtained for the use of any of the commercial agents used in this study.

This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the drugs are already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration

or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. The discussion of safety rationale is presented in section **1.6.4**

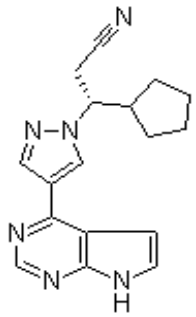
12.1 RUXOLITINIB (JAKAFI, ICNB018424)

Chemical Name: ruxolitinib phosphate

Other names: none

Classification: a Janus kinase (JAK) 1 and 2 Inhibitor

Description: 20 (elongated curved) and 15 (ovaloid curved) mg tablets embossed with NVR Ruxolitinib (Jakafi, ICNB018424) is an orally administered, potent selective inhibitor of JAK1 and JAK2 that was recently approved by the FDA for the treatment of patients with myelofibrosis (MF) [36]. Ruxolitinib, chemical name is (CAS) 1H-Pyrazole-1-propanenitrile, β-cyclopentyl-4-(7H-pyrrolo [2, 3-d] pyrimidin-4-yl)-, (βR)-, phosphate (1:1) is shown below:



The Janus-associated kinases 1 and 2 mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. Absorption, distribution, metabolism and excretion (ADME) and pharmacokinetic studies performed to support the licensing application for ruxolitinib showed rapid and near complete absorption (95%) after administration with maximum plasma concentration (C_{max}) of 1.47 μM achieved within 1 to 2 hours post dose. The mean C_{max} and total exposure (AUC) of ruxolitinib increased proportionately over the (single) dose range of 50 to 200 mg. The apparent volume of distribution of ruxolitinib at steady state is 53 to 65 L with high protein binding (97%) that is mostly to albumin. Ruxolitinib administration resulted in maximum inhibition of STATs phosphorylation 2 hours after dosing with return to near baseline by 10 hours in both healthy subjects and myelofibrosis patients. CYP3A4 is the major enzyme responsible for the metabolism of ruxolitinib. Following a single dose of [^{14}C]-labeled ruxolitinib in healthy adult subjects, elimination was predominantly through metabolism with 74% of radioactivity excreted in urine and 22% via feces [35].

12.1.1 Source

Ruxolitinib is manufactured by DSM Pharmaceuticals (Greenville NC 27834) for Incyte Corporation (Wilmington DE 19880) and has been licensed by the US FDA for the treatment of primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis. The drug will be purchased by the National Institutes of Health (NIH) Clinical Center (CC) Pharmacy. The study drug for the patients enrolled in this study will be distributed to by the NIH CC Pharmacy.

12.1.2 Toxicity

Several phase 1, 2 studies and two randomized Phase 3 studies (studies 1 and 2) were conducted in patients with MF (either primary myelofibrosis, post-polycythemia Vera myelofibrosis [PV] or post-essential thrombocytopenia-myelofibrosis [ET]) [36, 37]. Patients with a platelet count > 200 x 10^9/L were started on ruxolitinib orally 20 mg twice daily. In the 155 MF patients receiving ruxolitinib there was a 35% reduction in spleen volume in 65 (41.9%), as compared to one (0.7%) in the 154 patients in the placebo group. 148 patients receiving ruxolitinib achieved ≥ 50% improvement from baseline in the total symptoms score, in contrast to 8 of 152 patients 5.3% in the placebo-control group [(36, 37)]. The safety data base in solid tumors and hematologic malignancies consisted of 787 patients treated with ruxolitinib in 6 studies evaluating patients with MF (N=679), prostate cancer (N=22), multiple myeloma (N=13), ET and PV (N=73), of whom 617 patients received ruxolitinib. Hematologic events were the most frequent reported adverse events (AEs) primarily thrombocytopenia and anemia. The majority of these AEs are grade 1-2 and seldom led to drug discontinuation (<1% of patients). Of the 155 patients who received ruxolitinib in study one 10 (6.5%) had grade 3 thrombocytopenia and one (0.6%) had grade 4 thrombocytopenia as contrasted with one and 0 patients in the placebo group. Ten patients had grade 3 and 6 patients had grade 4 anemia compared to 5 and 0 in the placebo group. Biochemistry abnormalities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol were also reported and again the majority of these increases were grade 1 or 2 with no grade 4 observed. In this study there were 9 deaths in the ruxolitinib group and 11 in the placebo group. The safety data base which is primarily from patients with an underlying hematopoietic production defect indicates a well-tolerated drug that did cause additional decreases in blood elements.

12.1.3 Summary of Adverse Events

See Section **1.5.3** and the informed consent document for full listing.

12.1.4 Caution

Women of child-bearing age and fertile men should be advised to use adequate and effective contraception during treatment with ruxolitinib and for at least six months after therapy. The effects of ruxolitinib on embryogenesis, reproduction, and spermatogenesis are unknown.

12.1.5 Formulation and Preparation

Refer to the Jakafi package insert.

12.1.6 Stability and Storage

Refer to the Jakafi package insert.

12.1.7 Administration Procedures

Refer to the Jakafi package insert.

12.1.8 Drug Incompatibilities

None known

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14 APPENDICES

APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	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 B: DEFINITIONS OF ATL SUBSETS

	Smoldering	Chronic	Lymphoma	Acute
HTLV-1 AB	+	+	+	+
Lymphocyte (x10e9)	<4	≥4[a]	<4	*
Abnormal T cells	≥5	+[b]	≤1%	+[b]
LDH	≤1.5N	≤2N	NA	NA
Ca (mmol/L)	<2.74	<2.74	NA	NA
Histology	No	NA	+	NA
Tumor lesion				
Skin	**	*	*	*
Lung	**	*	*	*
Lymph node	No	*	Yes	*
Liver	No	*	*	*
Spleen	No	*	*	*
CNS	No	No	*	*
Bone	No	No	*	*
Ascites	No	No	*	*
Pleural effusion	No	No	*	*
GI tract	No	No	*	*

* No essential qualification except terms required for other subtypes
** No Essential qualifications if other terms are fulfilled, but histology-proven malignant lesion is required in case abnormal T-lymphocytes are less than 5% in peripheral blood
a: Accompanied by T-lymphocytosis ($3.5 \times 10^9/1$ or more)
b: In case abnormal T-lymphocytes are less than 5% in peripheral blood, histology-proven tumor lesion is required

APPENDIX C: STRONG INHIBITORS OF P450 ISOFORM CYP 3A4

acitretin	amiodarone	amprenavir
aprepitant	cimetidine	ciprofloxacin
clarithromycin	cyclosporine	danazol
delavirdine	diltiazem	diethyldithiocarbamate
efavirenz	erythromycin	ethinyl estradiol
fluconazole	fluoxetine	fluvoxamine
gestodene	grapefruit	indinavir
imatinib	isoniazid	itraconazole
ketoconazole	metronidazole	methylprednisolone
miconazole	mifepristone	nefazodone
nelfinavir	nicardipine	nifedipine
norethindrone	norfloxacin	norfluoxetine
oxiconazole	prednisone	quinine
ritonavir	roxithromycin	saquinavir
sertraline	Synercid	troleandomycin
verapamil	voriconazole	zafirlukast
zileuton		

Reference: http://medicine.iupui.edu/CLINPHARM/DDIS