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Protocol Page

Phase I-II Study of Ruxolitinib (INCB18424) for Patients with Chronic Myeloid Leukemia (CML) with Minimal Residual Disease While on Therapy with Tyrosine Kinase Inhibitors

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Protocol Body

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Phase I-II Study of Ruxolitinib (INCB18424) for Patients with Chronic Myeloid Leukemia (CML) with Minimal Residual Disease While on Therapy with Tyrosine Kinase Inhibitors

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Study Product: ***Ruxolitinib***

Protocol Number: *2012-0697*

Coordinating Center: *MD Anderson Cancer Center*
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1. OBJECTIVES

1.1. Primary Objective:

1.1.1. **Phase I**: To determine the DLT and MTD of the combination of ruxolitinib and a tyrosine kinase inhibitor (TKI) in patients with chronic myeloid leukemia (CML).

1.1.2. **Phase II**: To determine the clinical activity of the combination of ruxolitinib and a TKI in patients with CML in complete cytogenetic remission (CCyR) with minimal residual disease (MRD).

1.2. Secondary Objectives:

1.2.1. **Phase I**

1.2.1.1. To determine the clinical activity of the combination of ruxolitinib and a TKI in patients with CML

1.2.2. **Phase II**

1.2.2.1. To determine the safety of the combination of ruxolitinib and a TKI in patients with CML in CCyR with minimal residual disease.

1.2.3. **Both phases**

1.2.3.1. Determine the overall survival, event-free survival and survival free from transformation to accelerated and blast phase.

1.2.3.2. Determine the effect of therapy on bone marrow progenitors in clonogenic assays

1.2.3.3. Investigate the effect of therapy on molecular responses as assessed by genomic DNA PCR.

1.2.3.4. Determine the effect of therapy on TKI-resistant quiescent leukemic Ph+ stem cells (CFSEmax/CD34+) by flow cytometric evaluation of activated Crkl and Jak2

1.2.3.5. Assess the effect of therapy on self-renewal and/or survival of leukemic stem cells by FISH Analysis on colonies

1.2.3.6. Assess Ruxolitinib pharmacokinetics (PK) in preselected time intervals during co-administration of this agent with TKIs

2. BACKGROUND

2.1. Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a pluripotent stem cell disorder characterized by the presence of the Philadelphia (Ph) chromosome in the leukemic cells. The Ph chromosome results from a (9;22) translocation in which the c-*ABL* oncogene has

moved from chromosome 9 into the *BCR* (breakpoint cluster region) gene on chromosome 22, resulting in a chimeric *BCR-ABL* gene [1-3]. This is the causative abnormality in CML. The fused gene encodes an 8.5 kb chimeric mRNA [4,5], which is translated into a 210-kDa protein [6]. This p210 BCR-ABL protein functions as a constitutively activated tyrosine kinase and is uniquely present in the leukemic cells of CML patients [7]. The breakpoint in the *BCR* gene occurs either between *BCR* exon b2 and b3 or between *BCR* exons b3 and b4. Therefore, in the mature *BCR-ABL* mRNA, either b2 or b3 is spliced to *ABL* exon a2, which results in two alternative chimeric p210 BCR-ABL proteins, with either a B2A2 or B3A2 junction [8].

2.2. **Tyrosine Kinase Inhibitor (TKI) Therapy**

Imatinib is a low molecular weight phenylaminopyrimidine designed to selectively inhibit BCR-ABL tyrosine kinase activity [9], and is now the standard therapy for newly diagnosed patients with CML who do not undergo allogeneic stem cell transplant [10]. Results of the pivotal IRIS trial have shown that over 80% of patients achieve a CCyR, with most of these responses being durable. After 7 years of follow-up, the event-free survival rate is 81% and the survival free from transformation to accelerated or blast phase is 92%.[11] For patients who develop resistance or intolerance to imatinib, effective therapy with second generation tyrosine kinase inhibitors has been developed. Two of these agents (dasatinib and niloitinib) are currently available, and others are under development (e.g, bosutinib). Approximately 50% of patients treated with these agents after imatinib failure achieve a CCyR, and these responses are also durable in most patients.

Several reports have shown that, among patients who achieve a CCyR with imatinib or other TKI, those who achieve a MMR (ie, at 18 months from the start of therapy) have an improved EFS and survival free from transformation to accelerated or blast phase compared to those with CCyR but no MMR (7-year EFS 95% vs 86%, respectively).[12] In this same analysis, none of the patients who had achieved a MMR transformed to accelerated or blast phase. Recently, it has been suggested that achieving a complete molecular response (CMR) further improves the long-term outcome of patents after treatment with imatinib. In one series, patients who had an MMR had a shorter relapse-free survival (median 44 months) compared to those that achieved CMR (median not reached, with a hazard ratio for relapse 11%.[13] Similar results were reported from our institution, where patients with sustained complete molecular response have an estimate EFS at 7-years of 95%, compared to 85% for those with sustained MMR but no CMR, and 50% for those with CCyR but no MMR.[14] Thus, improving the molecular response of patients who have already achieved a CCyR with TKI has become an important goal of therapy. It has been suggested that patients who achieve CMR may discontinue therapy with imatinib, an important goal for patients. However, discontinuation of imatinib among patient who achieve CMR results in relapse in over 50% of patients.[15] Thus additional measures are needed to make treatment discontinuation a safer proposition.

2.3. **The role of JAK2 in CML**

The JAK family of kinases comprises four proteins (JAK1, JAK2, JAK3, and TYK2) that can associate with cytokine receptor subunits, phosphorylate them, and in doing so create docking sites on the receptors for binding of SH2-containing proteins. In general, JAKs consist of several domains (JH1-JH7), including a tyrosine kinase domain, and the functional significance of these domains has been characterized by mutational analysis. JAKs are able to associate with the cytokine receptors as well as with each other. Dimerization/oligomerization of cytokine receptor subunits as a result of ligand binding leads to juxtaposition of JAKs. This results in transphosphorylation and activation of their kinase activity and the phosphorylation of downstream signaling proteins such as STATs, *Src*-kinases, and adaptors such as Shc, Grb2, and Cbl.

JAK proteins activate a number of intracellular signaling proteins, among which STATs are the best defined. Binding of a cytokine to its receptor rapidly induces tyrosine phosphorylation of the cytoplasmic domains of the receptor by activated JAK kinases, thus providing a docking site for STAT proteins, which are then phosphorylated. This phosphorylation of STATs leads to their homo- or heterodimerization and translocation to the nucleus, followed by DNA binding and gene activation. The specificity for STAT phosphorylation is determined by the receptor docking sites and not the JAK kinases. Also, different STAT proteins have different DNA-binding affinities, resulting in activation of specific genes. STATs also interact with other transcription factors such as the p300/cyclic AMP-responsive element binding protein family of coactivators to activate genes. The transcriptional activity of STATs may also be regulated by the phosphorylation of their serine and threonine residues, although the implications of such regulation are not known.

STATs mediate diverse and sometimes opposite cellular events affecting growth, differentiation, and apoptosis. For example, STATs can mediate both growth arrest and cellular proliferation. Specifically, STAT1 mediates the growth-inhibitory effects of IFN-γ, through the induction of the CDKI $p21^{waf1}$, whereas STAT5 mediates proliferative effects of IL-3 and GM-CSF. Similarly, phosphorylation of STAT3 can result both in IL-6- and IL-10-induced growth arrest, and in GM-CSF- and IL-3-induced proliferation. STATs also modulate cellular differentiation and apoptosis. STAT3 and STAT5 mediate the antiapoptotic effects of IL-6 and IL-2, respectively. STAT1 activates the caspase cascade through up-regulation of Fas and FasL expression in response to IFN-γ. The exact mechanisms underlying these diverse effects are being elucidated.

The JAK-STAT signaling pathway is constitutively activated in a spectrum of human malignancies including CML, AML and ALL. Jak2 was first reported to be involved in Bcr-Abl transformation by Xie et al in 2001.[16] The C-terminal domain of Abl within Bcr-Abl is involved in complex formation with Jak2 and a kinase-inactive Jak2 mutant blocked the colony forming ability of K562 cells.[16] In Bcr-Abl positive cells, Jak2 activation mediates an increase of c-Myc RNA expression and interferes with proteasome-dependent degradation of c-Myc protein.[17] Importantly, Jak2 inhibition induced apoptosis and reduced colony formation in imatinib-sensitive and imatinib-

resistant Bcr-Abl mutant cell lines, and induced apoptosis in CML cells from patients in blast phase but not in normal hematopoietic cells.[18] Jak2 inhibition is able to overcome drug resistance in blast phase.[19] Recent studies have suggested that Jak2 activation is critical for the survival of the CML leukemic stem cell. Inhibition of Jak2 activity significantly reduces LTC-ICs, impairs β-catenin activity, and induces apoptosis of Ph^+ quiescent hematopoietic stem cells. Ph^+ HSC survival/self-renewal requires expression but not activity of BCR-ABL1, which is important for PP2A inhibition and Jak2 recruitment/activation. Other studies using a retroviral mosyse model have not been able to confirm this potential synergistic effect.[20] However, this concept has not yet explored in vivo or in a human setting.

We hypothesize that adding ruxolitinib to a TKI may improve the molecular response of patients with CML with CCyR but no MMR. If a significant number of patients convert to a CMR, we will explore whether TKI can be safely discontinued with minimal risk of relapse. Throughout the study we will measure the effect of therapy with ruxolitinib (administered in combination with continuing TKI therapy) on numerous correlative pathobiological parameters by performing experimental testing of peripheral blood and bone marrow samples (as described in Appendix A of this protocol.

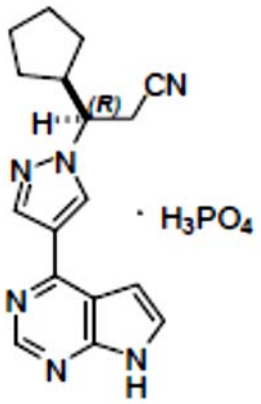
2.4. **Ruxolitinib**

2.4.1. ***Overview of Ruxolitinib***

Ruxolitinib (also known as INCB018424 phosphate; IND# 109051, NSC# 752295) represents a novel, potent, reversible and selective inhibitor of JAK1 and JAK2- STAT signaling that is currently under development for treatment of myeloproliferative neoplasms (MPNs) and advanced hematologic malignancies.

2.4.1.1.**Structure and molecular weight**

The chemical name of ruxolitinib is (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate.
The chemical structure of ruxolitinib is shown below:



Molecular formula: C17H18N6●H3PO4

Molecular weight: 404.36

2.4.2. **Preclinical Studies**

Ruxolitinib inhibited the splenomegaly and morbidity/mortality in mice resulting from intravenous inoculation of cells expressing the same mutated JAK2 (V617F) implicated in the pathogenesis of the majority of Philadelphia chromosome negative MPNs. Ruxolitinib inhibited erythroid colony formation from mononuclear cells derived from PV subjects (IC_{50} of 223 nM compared to 407 nM for normal donors). Growth factor independent colony formation, a unique characteristic of PV and other MPNs, was inhibited more potently with an IC_{50} of 67 nM for ruxolitinib in cells bearing the JAK2 V617F mutation compared to cells bearing the wild-type JAK2.

Effects of ruxolitinib noted in 6 month rat and 12 month dog repeat dose toxicology studies were primarily those associated with the mechanism of action of ruxolitinib (inhibitor of JAK-STAT signaling). Genetic toxicology assessments (evaluations of ruxolitinib in the bacterial mutagenicity assay, in vitro chromosome aberration assay, and in vivo micronucleus assay) in rats were negative. In safety pharmacology evaluations, an adverse decrease in minute volume in a respiratory study in female rats only was noted at the highest dose. In a cardiovascular evaluation of ruxolitinib in dogs, electrocardiogram (ECG) parameters and ventricular repolarization were unaffected at all doses; whereas the compound lowered blood pressure and increased heart rate compared to vehicle control at the highest dose evaluated. In embryo-fetal assessments in rat and rabbit, maternal toxicity and minimal embryo-fetal toxicity were noted at the highest doses evaluated. Ruxolitinib was not teratogenic in either rat or rabbit. No effects were noted on reproductive performance or fertility in male or female rats. Increases in post-implantation loss were noted at the higher doses.

More detailed information on pharmacology of ruxolitinib, single and multiple dose pharmacokinetic (PK) studies conducted in multiple species and nonclinical safety evaluations can be found in the Investigator's Brochure (IB).

2.4.3. **Clinical Pharmacology of ruxolitinib**

Following oral, single-dose administration of ruxolitinib capsules in the fasted state, ruxolitinib was absorbed rapidly, typically attaining peak plasma concentrations within 1 to 3 hours after administration for all doses. After attaining C_{max}, the ruxolitinib plasma concentrations declined with a mean terminal-phase disposition $t_{1/2}$ of approximately 3-5 hours. The mean ruxolitinib C_{max} and AUC increased with approximately linear proportionality to dose for the entire dose range evaluated of 5 to 200 mg. There was no significant food effect on absorption or exposure. A double-blind, randomized, placebo-controlled, single dose escalation study (INCB 18424-131 study) has been conducted to investigate the food effect. T_{max}, C_{max}, AUC in particular were determined.

The main conclusion was that overall magnitude of the food effect on the ruxolitinib exposure is not expected to be clinically significant.

Ruxolitinib is metabolized in the liver by the cytochrome (CYP) P450 metabolizing enzyme system, predominantly by the 3A4 isozyme. Systemic exposure of ruxolitinib was appreciably increased (AUC 2-fold higher) when given in combination with ketoconazole, a potent CYP3A4 inhibitor, with a similar effect observed on the PD activity (cytokine-induced STAT3 phosphorylation). CYP3A4 inducers significantly decreased the exposure to ruxolitinib, with essentially no difference observed on the PD activity (cytokine-induced STAT3 phosphorylation). This suggests that CYP3A4 induction with rifampin results in metabolism of ruxolitinib to active metabolites which also inhibit JAKs.

Ruxolitinib was given as a single 25 mg dose to subjects with varying degrees of renal function (INCB 18424-142 study) and to subjects with varying degrees of hepatic dysfunction or with normal hepatic function (INCB 18424-137 study). Mild, moderate or severe impairment of renal function had no statistically significant effect on PK or PD parameters; subjects requiring dialysis showed decreased ruxolitinib clearance. The mean total AUCs of ruxolitinib were 88%, 29% and 66% higher, respectively, in subjects with mild, moderate and severe hepatic impairment compared to subjects with normal hepatic function. Terminal half-life of ruxolitinib was increased in subjects with hepatic impairment by approximately 2-fold compared to healthy controls. The subjects with severe hepatic impairment showed modestly protracted PD activity compared to the other hepatically impaired subjects who displayed PD activity similar to the healthy controls.

Additional details as to the clinical pharmacology of ruxolitinib may be found in the IB.

2.4.4. **Ruxolitinib Clinical Safety in Healthy Volunteers**

Ruxolitinib has been administered in single or multiple doses to over 200 healthy subjects. In single dose studies, ruxolitinib has been safe and well tolerated with adverse events (AEs) generally mild in intensity, reversible and of similar incidence following ruxolitinib treatment compared with placebo or with other control treatments.

In a 10-day multiple dose study, a total of 71 healthy volunteers in 6 cohorts received doses of 50 mg qd, 100 mg qd, 15 mg bid, 25 mg bid or 50 mg bid ruxolitinib or

placebo (Study INCB 18424-132). Ruxolitinib was well tolerated in the study, with most adverse events were reported equally by both ruxolitinib-treated and placebo-treated subjects. Neutropenia was noted in 3 subjects receiving the highest dose of ruxolitinib, 50 mg bid. Neutropenia at the Grade 4 level led to study drug discontinuation on Day 5 in one subject and was reported as a Serious Adverse Event. There was a decline in mean absolute neutrophil count (ANC) and to a lesser extent, mean white blood cell count (WBC) values with ruxolitinib doses of 15 mg bid or higher. In general ANC or WBC returned to Baseline levels within 1 to 2 days following the last dose of study drug. Doses of 25 mg bid and 100 mg qd were determined to be the maximum tolerated doses (MTDs) in this study based on the dose limiting toxicity of neutropenia.

A definitive QT study was carried out in 50 healthy volunteers, evaluating the effects of single doses of 25 mg or 200 mg ruxolitinib compared with placebo and 400 mg moxifloxacin (positive control). The overall conclusion is that there appears to be no adverse impact on ECG signaling (little change in heart rate, QRS duration, QTcF interval, and no clinically significant slight increase in PR interval) with the administration of ruxolitinib.

For additional details related to studies conducted in Healthy volunteers, consult the IB.

2.4.5. **Ruxolitinib Clinical Safety and Efficacy in Myelofibrosis**

Safety data were extracted from the clinical database (cut-off for COMFORT-II or NVSCINC424A2352: 04-Jan- 2011; cut-off for COMFORT-I or INCB 18424-351: 02-Nov-2010). AEs by preferred term, and AEs leading to discontinuation/dose reduction/dose interruption were updated 120 days after the initial cutoff of the clinical database. Cumulative suspected unexpected serious adverse reactions (SUSARs) reported from any studies, including the Phase III studies were extracted from the Novartis safety database (cut-off for SAEs: 02-Aug-2011).

The Phase III population includes MF patients recruited into the two Phase III studies (COMFORT-I and –II) presents available safety data collected during the randomized treatment phase of those studies. Patients were treated with either ruxolitinib (at starting doses of 15 mg or 20 mg b.i.d. and their dose was optimized up or down depending on tolerability and efficacy: COMFORT-I; n=155, COMFORT-II; n=146) or placebo (COMFORT-I; n=151) or BAT (COMFORT-II; n=73).

All patients treated in these Phase III studies are included in this population, but only patients randomized to ruxolitinib are pooled (n=301 patients, ruxolitinib-treated population). The extension phases are not included in the Phase III patient population. Data obtained for patients randomized to placebo or BAT who crossed over to ruxolitinib are not included.

2.4.5.1. **Common adverse events**

A summary of most frequently (≥5%) reported AEs in the Phase III population regardless of study drug relationship by preferred term is presented in the table below.

The comparison of the control groups to the ruxolitinib patients showed that headache was more frequent in ruxolitinib-treated patients (13.6% vs. 6.0% on placebo and 5.5% on BAT). Most AEs of headache were Grade 1 or 2. Similarly, dizziness (12.0% vs. 6.6% on placebo and 6.8% on BAT) was more frequent in ruxolitinib-treated patients, again mostly Grade 1 or 2. When adjusted for patient-year exposure, the differences are still present for headache and dizziness.

Weight increase was also more frequent in ruxolitinib-treated patients than in the control groups (9.6% vs. 1.3% on placebo and 1.4% on BAT). Although some of these patients had co-reported AEs of edema, many had a past medical history of weight loss and the weight gain usually gradually accumulated over the course of one year of treatment. The majority of weight gain AEs were Grade 1 and 2. It is worth noting that weight gain may be a beneficial effect in patients with MF, given the catabolic nature of the disease and the frequency of weight loss reported as a constitutional symptom.

Other preferred terms with increased frequency in the ruxolitinib arms included bruising (2.6% vs. 1.3% on placebo in COMFORT-I only), contusion (8.6% vs. 5.3% on placebo and 1.4% on BAT), urinary tract infection (7.3% vs. 4.6% on placebo and 2.7% on BAT), herpes zoster (4.0% vs. 0.7% on placebo and 0% on BAT) and flatulence (3.3% vs. 1.3% on placebo and 0% on BAT).

Abdominal pain was more frequent in the control groups than in the ruxolitinib group (43% on placebo and 13.7% on BAT vs. 12% on ruxolitinib), as were weight decrease (8.6% on placebo and 8.2% on BAT vs. 1% on ruxolitinib), early satiety (8.6% on placebo and 0% on BAT vs. 0.3% on ruxolitinib) and splenic infarction (6.0% on placebo and 0% on BAT vs. 1.0% on ruxolitinib).

TABLE: Most frequently reported adverse events regardless of study drug relationship by preferred term in Phase III patients (safety set)

Preferred term	Study INCB 18424-351 Ruxolitinib N=155 n (%)	Study INCB 18424-351 Placebo N=151 n (%)	Study CINC424A2352 Ruxolitinib N=146 n (%)	Study CINC424A2352 BAT N=73 n (%)	Total Ruxolitinib N=301 n (%)
Any	152 (98.1)	149 (98.7)	145 (99.3)	67 (91.8)	297 (98.7)
Thrombocytopenia	58 (37.4)	14 (9.3)	65 (44.5)	9 (12.3)	123 (40.9)
Anemia	49 (31.6)	22 (14.6)	61 (41.8)	10 (13.7)	110 (36.5)
Diarrhea	37 (23.9)	35 (23.2)	38 (26.0)	11 (15.1)	75 (24.9)
Edema peripheral	31 (20.0)	36 (23.8)	34 (23.3)	19 (26.0)	65 (21.6)
Fatigue	43 (27.7)	54 (35.8)	19 (13.0)	8 (11.0)	62 (20.6)
Dyspnea	28 (18.1)	28 (18.5)	24 (16.4)	13 (17.8)	52 (17.3)
Nausea	23 (14.8)	29 (19.2)	21 (14.4)	5 (6.8)	44 (14.6)
Headache	24 (15.5)	9 (6.0)	17 (11.6)	4 (5.5)	41 (13.6)
Pyrexia	19 (12.3)	12 (7.9)	22 (15.1)	7 (9.6)	41 (13.6)
Cough	18 (11.6)	13 (8.6)	22 (15.1)	12 (16.4)	40 (13.3)
Pain in extremity	22 (14.2)	16 (10.6)	17 (11.6)	3 (4.1)	39 (13.0)
Arthralgia	18 (11.6)	14 (9.3)	19 (13.0)	7 (9.6)	37 (12.3)
Abdominal pain	19 (12.3)	65 (43.0)	17 (11.6)	10 (13.7)	36 (12.0)
Dizziness	25 (16.1)	10 (6.6)	11 (7.5)	5 (6.8)	36 (12.0)
Vomiting	20 (12.9)	17 (11.3)	16 (11.0)	1 (1.4)	36 (12.0)
Asthenia	8 (5.2)	12 (7.9)	26 (17.8)	7 (9.6)	34 (11.3)
Nasopharyngitis	7 (4.5)	9 (6.0)	27 (18.5)	10 (13.7)	34 (11.3)
Constipation	21 (13.5)	19 (12.6)	11 (7.5)	4 (5.5)	32 (10.6)
Weight increased	13 (8.4)	2 (1.3)	16 (11.0)	1 (1.4)	29 (9.6)
Hemoglobin decreased	23 (14.8)	6 (4.0)	4 (2.7)	3 (4.1)	27 (9.0)
Insomnia	18 (11.6)	15 (9.9)	9 (6.2)	5 (6.8)	27 (9.0)
Back pain	11 (7.1)	13 (8.6)	15 (10.3)	9 (12.3)	26 (8.6)
Contusion	23 (14.8)	8 (5.3)	3 (2.1)	1 (1.4)	26 (8.6)
Platelet count decreased	15 (9.7)	4 (2.6)	11 (7.5)	2 (2.7)	26 (8.6)
Muscle spasms	11 (7.1)	11 (7.3)	14 (9.6)	5 (6.8)	25 (8.3)
Night sweats	12 (7.7)	18 (11.9)	13 (8.9)	6 (8.2)	25 (8.3)
Abdominal pain upper	10 (6.5)	13 (8.6)	12 (8.2)	4 (5.5)	22 (7.3)
Bronchitis	4 (2.6)	2 (1.3)	18 (12.3)	5 (6.8)	22 (7.3)
Urinary tract infection	12 (7.7)	7 (4.6)	10 (6.8)	2 (2.7)	22 (7.3)
Epistaxis	9 (5.8)	8 (5.3)	12 (8.2)	5 (6.8)	21 (7.0)
Abdominal distension	13 (8.4)	17 (11.3)	5 (3.4)	1 (1.4)	18 (6.0)
Cardiac murmur	12 (7.7)	5 (3.3)	6 (4.1)	3 (4.1)	18 (6.0)
Hematoma	4 (2.6)	0	14 (9.6)	3 (4.1)	18 (6.0)
Pneumonia	13 (8.4)	11 (7.3)	4 (2.7)	5 (6.8)	17 (5.6)
Rash	9 (5.8)	8 (5.3)	8 (5.5)	1 (1.4)	17 (5.6)
Dyspnea exceptional	6 (3.9)	5 (3.3)	10 (6.8)	2 (2.7)	16 (5.3)
Paraesthesia	6 (3.9)	4 (2.6)	10 (6.8)	4 (5.5)	16 (5.3)
Dyspepsia	9 (5.8)	8 (5.3)	6 (4.1)	4 (5.5)	15 (5.0)

AEs with frequency of ≥5%
Preferred terms are sorted in descending order of frequency, as reported in the <Total> column.

A patient with multiple occurrences of an AE under one treatment is counted only once in the AE category for that treatment.
A patient with multiple AEs is counted only once in the total row.
AEs occurring more than 28 days after the discontinuation of study treatment are not summarized.
Includes patients from safety clinical database-1
In the table above, Study Incyte INCB 18424-351 is also known as COMFORT-I, while NVS study CINC424A2352 is also known as COMFORT-II.

The most frequently occurring Grade 3 and 4 AEs regardless of study drug relationship were hematologic including anemia (14%) and thrombocytopenia (8%). Non-hematologic Grade 3-4 AEs were infrequent and rarely reported more frequently than in the control arms. Two patients (0.7%) had febrile neutropenia. In general, the pattern of AEs was similar between the two ruxolitinib arms in both studies, although there were some differences in frequency for specific AEs.

2.4.5.2. **AEs leading to treatment discontinuation, dose interruption or dose reduction**

In the ruxolitinib-treated Phase III population, the overall frequency of AEs leading to study drug discontinuation was 11%. This frequency was similar across both studies. None of the AEs leading to discontinuation was reported in more than two patients in any group.

In the ruxolitinib-treated Phase III population, the overall frequency of AEs requiring dose reduction or interruption was 59.8%. This frequency was higher than in the control groups (placebo: 27.2%, BAT: 15.1%). The most frequently reported AEs requiring dose reduction or interruption in ruxolitinib-treated patients were thrombocytopenia (36.9%), platelet count decreased (7.6%) and anemia (5.6%). The high frequency for thrombocytopenia is due to protocol-mandated dose reductions and interruptions. Although there were no protocol-specified guidelines for dose reductions secondary to anemia, some investigators chose to reduce a patient's dose in the setting of anemia to minimize this particular cytopenia. The frequency of these AEs was higher than in the control groups. All other AEs requiring dose reduction or interruption occurred with a frequency of 1.3% or less in the ruxolitinib-treated patients. This information is summarized in the Tables below:

Summary of adverse events leading to discontinuation, dose interruption or dose reduction

Category	Study INCB 18424-351		Study CINC424A2352		Total
	Ruxolitinib N=155 n (%)	Placebo N=151 n (%)	Ruxolitinib N=146 n (%)	BAT N=73 n (%)	Ruxolitinib N=301 n (%)
AEs leading to discontinuation	17 (11.0)	17 (11.3)	16 (11.0)	6 (8.2)	33 (11.0)

Category	Study INCB 18424-351 Ruxolitinib N=155 n (%)	Placebo N=151 n (%)	Study CINC424A2352 Ruxolitinib N=146 n (%)	BAT N=73 n (%)	Total Ruxolitinib N=301 n (%)
Suspected to be drug-related	5 (3.2)	4 (2.6)	4 (2.7)	0	9 (3.0)
Other significant AEs	137 (88.4)	128 (84.8)	134 (91.8)	55 (75.3)	271 (90.0)
AEs requiring dose interruption and / or reduction	85 (54.8)	41 (27.2)	95 (65.1)	11 (15.1)	180 (59.8)
Thrombocytopenia requiring dose interruption and / or reduction	50 (32.3)	9 (6.0)	61 (41.8)	1 (1.4)	111 (36.9)
Platelet decrease requiring dose interruption and / or reduction	13 (8.4)	3 (2.0)	10 (6.8)	2 (2.7)	23 (7.6)
Anemia requiring dose interruption and / or reduction	10 (6.5)	3 (2.0)	7 (4.8)	1 (1.4)	17 (5.6)

In the tables above, Study Incyte INCB 18424-351 is also known as COMFORT-I, while NVS study CINC424A2352 is also known as COMFORT-II.

2.4.5.3. **Events suspected to be drug-related (as per investigator's assessment)**

The adverse events reported below were considered at least possibly related by individual investigator assessment. In the ruxolitinib-treated patients, the overall frequency of AEs (preferred terms, maximum Grade) with suspected relationship to study drug was 77.1%. In COMFORT-I, related AEs were higher in the ruxolitinib-treatment group than in the placebo group (74.2% vs. 55.6%). Similarly in COMFORT-II, their frequency was higher in the ruxolitinib group than in the BAT group (80.1% vs. 19.2%). The majority of related AEs were Grade 1 or 2.

Thrombocytopenia (36.2%) and anemia (27.2%) were the most commonly reported related AEs and the frequency was higher in the ruxolitinib arms than in comparator arms for both. This information is summarized in the tables below:

Frequent adverse events with suspected study drug relationship by preferred term (at least 5% in any group) and maximum Grade in Phase III patients (Safety set)

Preferred term Maximum Grade	Study INCB 18424-351 Ruxolitinib N=155 n (%)	Placebo N=151 n (%)	Study CINC424A2352 Ruxolitinib N=146 n (%)	BAT N=73 n (%)	Total Ruxolitinib N=301 n (%)
Any preferred term	115 (74.2)	84 (55.6)	117 (80.1)	14 (19.2)	232 (77.1)
Grade 3	31 (20.0)	25 (16.6)	31 (21.2)	1 (1.4)	62 (20.6)
Grade 4	12 (7.7)	0	2 (1.4)	0	14 (4.7)
Thrombocytopenia	47 (30.3)	8 (5.3)	62 (42.5)	1 (1.4)	109 (36.2)
Grade 3	10 (6.5)	1 (0.7)	9 (6.2)	0	19 (6.3)
Grade 4	1 (0.6)	0	1 (0.7)	0	2 (0.7)
Anemia	38 (24.5)	9 (6.0)	44 (30.1)	3 (4.1)	82 (27.2)
Grade 3	10 (6.5)	5 (3.3)	13 (8.9)	0	23 (7.6)
Grade 4	6 (3.9)	0	0	0	6 (2.0)
Diarrhea	17 (11.0)	9 (6.0)	12 (8.2)	1 (1.4)	29 (9.6)
Grade 3	1 (0.6)	0	0	0	1 (0.3)
Fatigue	19 (12.3)	20 (13.2)	5 (3.4)	1 (1.4)	24 (8.0)

	Study INCB 18424-351		Study CINC424A2352		Total
	Ruxolitinib	Placebo	Ruxolitinib	BAT	Ruxolitinib
Preferred term Maximum Grade	N=155 n (%)	N=151 n (%)	N=146 n (%)	N=73 n (%)	N=301 n (%)
Grade 3	3 (1.9)	1 (0.7)	0	0	3 (1.0)
Platelet count decreased	14 (9.0)	2 (1.3)	10 (6.8)	1 (1.4)	24 (8.0)
Grade 3	2 (1.3)	0	1 (0.7)	0	3 (1.0)
Edema peripheral	9 (5.8)	10 (6.6)	9 (6.2)	0	18 (6.0)
Hemoglobin decreased	13 (8.4)	2 (1.3)	4 (2.7)	1 (1.4)	17 (5.6)
Grade 3	8 (5.2)	2 (1.3)	0	0	8 (2.7)
Grade 4	2 (1.3)	0	1 (0.7)	0	3 (1.0)
Nausea	10 (6.5)	10 (6.6)	6 (4.1)	0	16 (5.3)
Grade 3	0	1 (0.7)	0	0	0
Weight increased	5 (3.2)	0	10 (6.8)	0	15 (5.0)
Grade 3	0	0	2 (1.4)	0	2 (0.7)
Headache	8 (5.2)	2 (1.3)	6 (4.1)	0	14 (4.7)
Grade 3	0	0	1 (0.7)	0	1 (0.3)
Dizziness	8 (5.2)	3 (2.0)	2 (1.4)	0	10 (3.3)
Asthenia	1 (0.6)	3 (2.0)	8 (5.5)	1 (1.4)	9 (3.0)
Grade 3	0	1 (0.7)	0	0	0
Abdominal pain	3 (1.9)	13 (8.6)	5 (3.4)	1 (1.4)	8 (2.7)
Grade 3	1 (0.6)	3 (2.0)	1 (0.7)	0	2 (0.7)

Single cases of Grade 5 AEs were reported, these are included in category "Grade 4"

The adverse events reported in the table were considered at least possibly related by individual investigator assessment.

In the tables above, Study Incyte INCB 18424-351 is also known as COMFORT-I, while NVS study CINC424A2352 is also known as COMFORT-II.

2.4.5.4. **Deaths and other serious adverse events**

In the Phase III population, there were 34 deaths in total, 27 of which were on-treatment deaths: 20 deaths in COMFORT-I (9 in the ruxolitinib group, 11 in the placebo group) and 7 deaths in COMFORT-II (4 in the ruxolitinib group, 3 in the BAT group). The reasons for death (infections, intestinal perforation, disease progression and events probably due to disease progression, bleedings events) were similar in the ruxolitinib and the placebo groups.

In the ruxolitinib-treated Phase III population, the overall frequency of SAEs was 28.9%. This frequency was similar across both studies. The most frequently reported SAEs in ruxolitinibtreated patients were anemia (4.0%) and pneumonia (3.7%). Pneumonia was the only SAE that was reported in more than 5% in any treatment group COMFORT-I ruxolitinib group with 6.5% and COMFORT-II BAT group with 5.5%). When evaluating all lower respiratory tract infection AEs grouped by MedDRA higher level group term (MedDRA: Medical Dictionary for Regulatory Activities), there was no appreciable difference across the arms of the studies COMFORT-I: ruxolitinib 10.3% vs. placebo 7.3%; COMFORT-II: ruxolitinib 13.1% vs. BAT 18%). Most other SAEs were reported in three patients or fewer in any group, with the following exceptions: in the placebo group, abdominal pain was

reported as an SAE in six patients (4.0%), and splenic infarction in four patients (2.6%); in the ruxolitinib-treated patients, fatigue, gastrointestinal hemorrhage and pyrexia were reported in four patients (1.3%) each.

Frequent serious adverse events regardless of study drug relationship by preferred term (at least 1% in any group) in Phase III patients (safety set)

	Study INCB 18424-351		Study CINC424A2352		Total
	Ruxolitinib	Placebo	Ruxolitinib	BAT	Ruxolitinib
Preferred term	N=155	N=151	N=146	N=73	N=301
Any preferred term	43 (27.7)	53 (35.1)	44 (30.1)	21 (28.8)	87 (28.9)
Anemia	5 (3.2)	3 (2.0)	7 (4.8)	3 (4.1)	12 (4.0)
Pneumonia	10 (6.5)	5 (3.3)	1 (0.7)	4 (5.5)	11 (3.7)
Fatigue	4 (2.6)	0	0	0	4 (1.3)
Gastrointestinal hemorrhage	2 (1.3)	2 (1.3)	2 (1.4)	0	4 (1.3)
Pyrexia	1 (0.6)	1 (0.7)	3 (2.1)	1 (1.4)	4 (1.3)
Abdominal pain	0	6 (4.0)	3 (2.1)	1 (1.4)	3 (1.0)
Diarrhea	1 (0.6)	0	2 (1.4)	0	3 (1.0)
Dyspnea	1 (0.6)	1 (0.7)	2 (1.4)	3 (4.1)	3 (1.0)
Hemoglobin decreased	3 (1.9)	0	0	0	3 (1.0)
Thrombocytopenia	3 (1.9)	1 (0.7)	0	1 (1.4)	3 (1.0)
Varices esophageal	0	1 (0.7)	3 (2.1)	0	3 (1.0)
Acute myeloid leukemia	2 (1.3)	0	0	0	2 (0.7)
Asthenia	2 (1.3)	1 (0.7)	0	1 (1.4)	2 (0.7)
Atrial fibrillation	1 (0.6)	1 (0.7)	1 (0.7)	1 (1.4)	2 (0.7)
Bronchitis	0	0	2 (1.4)	1 (1.4)	2 (0.7)
Cardiac failure	0	1 (0.7)	2 (1.4)	0	2 (0.7)
Cerebral hemorrhage	0	0	2 (1.4)	0	2 (0.7)
Fall	2 (1.3)	2 (1.3)	0	0	2 (0.7)
Gastroenteritis	0	1 (0.7)	2 (1.4)	0	2 (0.7)
General physical health deterioration	0	0	2 (1.4)	1 (1.4)	2 (0.7)
Lung infection	0	0	2 (1.4)	0	2 (0.7)
Renal failure	1 (0.6)	2 (1.3)	1 (0.7)	1 (1.4)	2 (0.7)
Renal failure acute	0	2 (1.3)	2 (1.4)	1 (1.4)	2 (0.7)
Respiratory tract infection	0	0	2 (1.4)	0	2 (0.7)
Splenic infarction	1 (0.6)	4 (2.6)	1 (0.7)	0	2 (0.7)
Squamous cell carcinoma of skin	0	0	2 (1.4)	1 (1.4)	2 (0.7)
Urinary tract infection bacterial	0	0	2 (1.4)	0	2 (0.7)
Cardiac failure congestive	1 (0.6)	3 (2.0)	0	0	1 (0.3)

	Study INCB 18424-351		Study CINC424A2352		Total
	Ruxolitinib	Placebo	Ruxolitinib	BAT	Ruxolitinib
Preferred term	N=155	N=151	N=146	N=73	N=301
Colitis	1 (0.6)	3 (2.0)	0	0	1 (0.3)
Disease progression	0	3 (2.0)	1 (0.7)	0	1 (0.3)
Pleural effusion	0	1 (0.7)	1 (0.7)	1 (1.4)	1 (0.3)
Portal vein thrombosis	0	0	1 (0.7)	1 (1.4)	1 (0.3)
Respiratory failure	1 (0.6)	0	0	2 (2.7)	1 (0.3)
Sepsis	1 (0.6)	2 (1.3)	0	0	1 (0.3)
Urinary tract infection	0	2 (1.3)	1 (0.7)	0	1 (0.3)
Actinic keratosis	0	0	0	2 (2.7)	0
Aortic aneurysm	0	0	0	1 (1.4)	0
Aortic thrombosis	0	0	0	1 (1.4)	0
Ascites	0	2 (1.3)	0	2 (2.7)	0
Atrial flutter	0	0	0	1 (1.4)	0
Bronchopneumonia	0	0	0	1 (1.4)	0
Campylobacter infection	0	0	0	1 (1.4)	0
Coagulopathy	0	0	0	1 (1.4)	0
Constipation	0	0	0	1 (1.4)	0
Gout	0	2 (1.3)	0	0	0
Hepatomegaly	0	0	0	1 (1.4)	0
Hypotension	0	2 (1.3)	0	0	0
Ileus paralytic	0	0	0	1 (1.4)	0
Inguinal hernia	0	0	0	1 (1.4)	0
Lung neoplasm malignant	0	0	0	1 (1.4)	0
Myelofibrosis	0	1 (0.7)	0	1 (1.4)	0
Peritoneal hemorrhage	0	0	0	2 (2.7)	0
Pulmonary hypertension	0	2 (1.3)	0	0	0
Pulmonary edema	0	3 (2.0)	0	1 (1.4)	0
Renal failure chronic	0	0	0	1 (1.4)	0
Renal impairment	0	0	0	1 (1.4)	0
Renal infarct	0	0	0	1 (1.4)	0
Respiratory distress	0	1 (0.7)	0	1 (1.4)	0
Splenomegaly	0	0	0	1 (1.4)	0
Subileus	0	0	0	1 (1.4)	0
Supraventricular tachycardia	0	0	0	1 (1.4)	0
Vertigo	0	0	0	1 (1.4)	0

In the tables above, Study Incyte INCB 18424-351 is also known as COMFORT-I, while NVS study CINC424A2352 is also known as COMFORT-II.

2.4.5.5. Clinical Laboratory Evaluations

In ruxolitinib-treated patients, the most frequent newly occurring or worsening hematology abnormality of any Grade was decreased Hgb (81.7%) and decreased platelet count (67.4%). The majority of decreased platelet counts were Grade 1 or 2. Approximately half of the newly occurring or worsening decreased Hgb was Grade 1 or 2. These hematology results are consistent with the observed AE profile. The most frequently observed Grade 3-4 hematology laboratory abnormalities were anemia (40.5%), thrombocytopenia (10.6%) and neutropenia (6.3%).

A majority of patients entered the pivotal studies with Grade 1 and 2 abnormal Hgb at baseline. Those patients receiving ruxolitinib mostly worsened to the grade immediately worse than their baseline grade. Few worsened to Grade 4 at any time during the study (11% on COMFORT-I and 8.2% on COMFORT-II).

The Kaplan-Meier estimates of time to onset of first episode of Grade 2 or higher Grade anemia show that the majority of Grade 2 or higher Grade new onset or worsening anemia occurs within the first 3 months of the study with an estimate of 0.66 (0.60, 0.71) and a median time to onset of 1.45 months (95% CI: 1.41 – 1.87).

The majority of patients entered the pivotal studies with normal or slightly abnormal (Grade 1) platelet counts. Those patients receiving ruxolitinib either remained normal throughout the study (30.3% and 32.2% of patients in the COMFORT-I and –II studies respectively, remained at Grade 0) or had new onset or worsening to Grade 1 or 2; few patients had new onset or worsening platelet counts to Grade 3 (9.0% and 6.2% in the COMFORT-I and –II studies, respectively) or Grade 4 (3.9% and 2.1%, respectively).

The majority of Grade 3 or 4 thrombocytopenia occurred within the first 3 months of the study. Furthermore, the Kaplan-Meier estimates of time to resolution of first episode of Grade 3 or 4 thrombocytopenia show that the median time to resolution was 2 weeks (95% CI: 1.29– 2.14).

Transfusions of PRBCs are a relatively common occurrence for MF patients, but increased rates of anemia resulted in an increase in transfusion requirements for some ruxolitinib-treated patients. The COMFORT-I and –II studies are consistent in demonstrating an increased frequency of patients requiring transfusion of one or more units of PRBCs while on treatment compared with the comparator arm (59.4% vs. 37.1% for patients randomized to ruxolitinib vs. placebo, respectively, and 51.4% vs. 38.4% for patients randomized to ruxolitinib vs. BAT, respectively.) However, there is a relatively small difference in the mean number of units transfused per month when comparing patients randomized to ruxolitinib vs. placebo who received any transfusion (0.92 vs. 0.75, respectively) and similarly when comparing patients randomized to ruxolitinib vs. BAT who received any transfusion (0.86 vs. 0.91, respectively). Anemia led to discontinuation in only one patient (0.3%) across these Phase III studies.

In the ruxolitinib-treated patients, the most frequent newly occurring or worsening biochemistry abnormality was increased ALT and gamma Glutamyltransferase (γGT). No newly occurring or worsening Grade 4 biochemistry abnormality was reported and very few Grade 3 abnormalities were observed; in ruxolitinib-treated patients, two patients (0.7%) reported Grade 3 increased bilirubin, four patients (1.3%) Grade 3 increased ALT and two patients (0.7%) Grade 2 increased AST.

Common terminology criteria for adverse events (CTCAE) Grade 1-2 hypercholesterolemia was also frequent in the ruxolitinib-treated patients (16.6%). No CTCAE Grade 3 or 4 hypercholesterolemia were reported.

There were few abnormal vital signs in the pivotal studies. Vital signs were comparable between treatment groups. The only relevant change in any of the parameters includes an increase >25% compared with baseline in systolic blood pressure in the ruxolitinib arms (15.5% vs. 7.3%, ruxolitinib vs. placebo, respectively in COMFORT-I, and 17.5% vs. 10.1%, ruxolitinib vs. BAT, respectively in COMFORT-II).

In the ruxolitinib-treated patients, three patients (1%) presented with a QTcF>500 ms compared to one patient (0.7%) on placebo and none on BAT. One ruxolitinib-treated patient (0.3%) presented with a QTcF increase from baseline >60 ms compared to six patients (4.0%) on placebo and none on BAT. No relevant changes in heart rate were observed. No relevant changes in any other parameters were observed except for an increase in the PR interval >25% and to a value >200 ms (3.3% for ruxolitinib vs. 0.7% for placebo in COMFORT-I and 5.7% for ruxolitinib vs. 1.6% for BAT in COMFORT-II). The largest increase in QTcF at any timepoint in the combined ruxolitinib group was 4.4 ms at Week 24.

2.4.6. **Investigational Agent Supply**

2.4.6.1 Ruxolitinib will be provided by Incyte as 5 mg, 10 mg, 15 mg or 20 mg tablets.

2.4.6.2 Unused or expired drug must be returned to MDACC. Unused or expired drug will be disposed per institutional policy.

2.5. **Supply of other CML-specific treatments**

Imatinib, dasatinib and nilotinib are commercially available. Product information may be found in the product information for each product available through the following links:

Imatinib: http://www.pharma.us.novartis.com/product/pi/pdf/gleevec_tabs.pdf

Nilotinib: http://www.pharma.us.novartis.com/product/pi/pdf/tasigna.pdf

Dasatinib: http://packageinserts.bms.com/pi/pi_sprycel.pdf

3. Criteria for Patient Eligibility

3.1. **Inclusion Criteria**

3.1.1. Patients 18 years or older with Philadelphia chromosome (Ph)-positive or BCR/ABL-positive CML (as determined by cytogenetics, FISH, or PCR).

3.1.2. Patients must be on continuous TKI therapy for management of their CML. Any commercially available and FDA- approved TKI can be used, i.e., imatinib mesylate (IM), nilotinib (NIL) or dasatinib (DAS). Patients may be receiving TKI at entry in the frontline or salvage setting, including patients currently on imatinib after α-interferon failure or on dasatinib or

nilotinib after failure to prior therapy including imatinib.

3.1.3. Patients must have received the current TKI for at least 18 months and not have increased their dose in the last 6 months.

3.1.4. For the phase I portion of the study, patients may be included without a CCyR provided they remain in chronic or accelerated phase CML and have at least a CHR. For the phase II portion of the study patients must be in complete cytogenetic remission (CCyR), regardless of the stage of disease they had at the time they started therapy with TKI.

3.1.5. Patients must have detectable BCR-ABL transcript levels meeting at least one of the following criteria:

3.1.5.1. Patient has never achieved a major molecular response (MMR, as defined by a BCR-ABL/ABL ≤0.1% in the international scale (currently equivalent to 0.28 in the MDACC molecular diagnostic laboratory), and transcript levels have shown in at least two consecutive measures separated by at least 1 month to have increased by any value, or

3.1.5.2. Achieved a major molecular response which has been lost, with an interim increase in transcript levels by at least one-log, confirmed in two consecutive analyses separated by at least 1 month, or

3.1.5.3. The patient has received therapy for at least 2 years and lacks a sustained major molecular response, or

3.1.5.4. The patient has received therapy for at least 5 years and lacks a sustained complete molecular response (CMR, defined as transcript levels still detectable in the MDACC molecular diagnostic laboratory).

3.1.6. Patients must not have had a known interruption of TKI therapy of greater than 21 consecutive days or for a total of 6 weeks in the 6 months prior to enrollment.

3.1.7. Patients must be able to understand and sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the institutional policies.

3.1.8. ECOG performance status ≤2.

3.1.9. Adequate organ function defined as: bilirubin <2x upper limit of normal

(ULN) (unless associated with Gilbert's syndrome), and ALT or AST ≤2.5x ULN.

3.1.10. ANC ≥1 $x10^9$/L and platelets ≥100 $x10^9$/L.

3.1.11. Serum creatinine < 1.5 mg/dL or creatinine clearance greater or equal than 60 cc/min as defined by the Cockcroft-Gault Equation: Males(mL/min):(140-age)*IBW(kg) / 72*(serum creatinine (mg/dl)); Females (mL/min):0.85*(140-age)*IBW(kg) / 72*(serum creatinine (mg/dl))

3.1.12. Women of childbearing potential should be advised to avoid becoming pregnant while on therapy with Ruxolitinib and for 30 days after the last dose and practice effective methods of contraception. Men should be advised not to father a child while receiving treatment with Ruxolitinib and for 30 days after the last dose. Effective methods of contraception for this study include barrier methods (e.g., condoms, diaphragm); spermicidal jelly or foam; oral, depo provera, or injectable hormonal contraceptives; intrauterine devices; tubal ligation; and abstinence.

3.2. **Exclusion Criteria**

3.2.1. For the phase I portion of the study, patients in blast phase. For the phase II portion of the study, patients in accelerated or blast phase.

3.2.2. Patients receiving any other investigational agents

3.2.3. Patients who are pregnant or breast-feeding

3.2.4. Patients with clinically significant heart disease (NYHA Class III or IV)

3.2.5. Patients with QTc >480msec.

3.2.6. Patients taking a potent CYP3A4 inhibitor that cannot be changed to an alternate drug.

3.2.7. Known or suspected hypersensitivity to ruxolitinib.

3.2.8. Patients with advanced malignant hepatic tumors.

3.2.9. Patients with known active hepatitis B or C, or HIV infection.

3.2.10. Patients with other medical conditions or concomitant medications that in the opinion of the principal investigator may interfere with the therapeutic treatment

4. Treatment Plan

4.1. **TKI**: Patients will continue receiving commercially available TKIs (IM, NIL or DAS) at the dose they had been receiving during the last 6 months.

4.1.1. **Phase I:** For the phase I portion of the trial, only patients receiving imatinib will be included first at the dose the patient had been receiving for the past 6 months.

4.1.2. Once the MTD is defined, 6 patients each may be treated with dasatinib and nilotinib in combination with the same dose of ruxolitinib established as MTD for the combination with imatinib.

4.1.3. The following dose levels will be used for dose adjustments:

Table 1: **Dose adjustment levels for TKI**

Dose level	Imatinib (mg/d)	Dasatinib (mg/d)	Nilotinib (mg)
-5	N/A	20	150 QD
-4	200	40	200 QD
-3	300	50	150 BID
-2	400	70	200 BID
-1	600	80	300 BID
0	800	100	400 BID

4.2.**Ruxolitinib:**

Phase I: patients will be treated in cohorts of 3. The starting dose will be dose level 0 (5 mg orally, twice daily; Table 1).

4.2.1. At least 3 patients will be entered at each level. All patients treated at any one dose level must have been observed for at least four weeks before escalating to the next dose level. Patients who come off study prior to the first four weeks and have not experienced a DLT will be replaced.

Table 2: **Dose adjustment levels for ruxolitinib**

Dose level	Ruxolitinib (mg, orally, twice daily)
-1	5 once daily
0	5
1	10
2	15
3 (Target dose)	20

4.2.2. Dose escalation will be done as follows:

Dose-limiting toxicity in	**Result**
0/3	Escalate to next level

1/3	Enter 3 more patients
1/6	Escalate to next level
≥2/3 or ≥ 2/6	MTD exceeded. This dose level will be declared the maximally administered dose. Expand previous level to include a total of 6 patients if not already accrued to this number.

4.2.2.1 For the phase 1 aspect of the protocol: Prior to advancing dose levels, a cohort summary must be completed and submitted to the Clinical Research Monitor in the IND Office.

4.2.3. **Definition of Dose-Limiting Toxicity (DLT)**

4.2.3.1. Dose limiting toxicity will be defined in the first 28 days of therapy.

4.2.3.2. The toxicity profile of these agents is well known. Non-hematologic DLT will be defined as grade 3 or 4 elevation of ALT or AST possibly related to TKI.

4.2.3.3. Hematologic DLT is defined as grade 4 neutropenia, anemia, and/or thrombocytopenia lasting for 4 weeks or more.

4.2.4. If dose-level 0 exceeds MTD, subsequent patients will be treated at dose level -1. Additional cohorts at lower doses (down to dose level -2) may be included if higher doses exceed MTD. If dose level -2 exceeds MTD, the study will be terminated.

4.3. **Phase II:** The target dose for the phase II portion of the study is 20 mg orally twice daily, unless MTD is defined at a lower dose level in the phase I portion of the study. Other dose levels shown in Table 1 will be used for dose adjustments for toxicity during therapy.

4.4. Once MTD is defined for imatinib in the phase I portion of the study the phase 2 portion of the study will start with imatinib only. The phase I portion of the study with dasatinib and nilotinib will open once MTD is defined for the imatinib-based combination.

4.4.1. Opening of phase I for dasatinib and nilotinib may occur simultaneously with opening of phase 2 portion of the study for the imatinib-based combination.

4.4.2. Once and only when an MTD is defined for dasatinib and nilotinib-based combinations, patients may be treated with these combinations in the phase 2 portion of the study.

4.4.3. When all three combinations are entered into the phase 2 portion of the study, patients may be treated with any of these combinations depending on the TKI they were receiving prior to entering this study. The aggregate response will be calculated for all patients, independent of the TKI being received.

4.5. Treatment with ruxolitinib (in both phase I and phase II) will continue uninterrupted unless there are indications for dose delays/modifications as shown in Section 4.5.

4.6. **Dosing Delays/Dose Modifications**

4.6.1. Patients experiencing unacceptable toxicity directly attributable to the study drugs should temporarily stop treatment according to the guidelines in the dose adjustment schema.

4.6.2. Toxicity grading will be according to the NCI CTCAE, v4. The following guidelines for dose adjustment for drug-related toxicities are recommended.

4.6.3. **TKI:** dose modifications and treatment interruptions will be performed according to institutional guidelines and standard clinical practice. Briefly, dose adjustments will be as follows:

4.6.3.1. Non-Hematologic Toxicity

4.6.3.1.1. **Persistent Grade 2:** Patients with persistent grade 2 toxicity that is considered clinically significant, unresponsive to appropriate therapy, may have treatment held until the toxicity has resolved to grade ≤1. Therapy may then be resumed at the same dose the patient was receiving at the time treatment was interrupted. If the grade 2 toxicity recurs, therapy may be held until the toxicity has resolved to grade 1. Treatment may then be resumed with a one dose level reduction.

4.6.3.1.2. **Grade 3-4**: If a patient experiences Grade 3-4 toxicity that is considered clinically significant and possibly related to TKI, therapy must be withheld until the toxicity has resolved to Grade ≤1. Treatment may then be resumed with a one dose level reduction. If toxicity recurs, additional dose reductions may be implemented following the same general guidelines.

4.6.3.2. Hematologic Toxicity

4.6.3.2.1. If neutrophils are <0.5 x 10^9/L and/or platelets are <50 x x10^9/L, hold therapy until granulocytes are above 0.5 x10^9/L and platelets are above 50 x10^9/L, then resume therapy at 1 dose level reduction from the dose the patient was receiving at the time therapy was interrupted.

4.6.3.2.2. If a similar degree of toxicity returns, a further dose reduction by one dose level can be performed, using the above procedures.

4.6.3.2.3. There will be no making up for missed doses.

4.6.4.

4.6.5. **Ruxolitinib**: dose modifications for toxicity will be done according to the following guidelines:

4.6.5.1. Non-Hematologic Toxicity

4.6.5.1.1. **Persistent Grade 2:** Patients with persistent grade 2 toxicity that is considered clinically significant, unresponsive to appropriate therapy, may have treatment held until the toxicity has resolved to grade ≤1. Therapy may then be resumed at the same dose the patient was receiving at the time treatment was interrupted. If the grade 2 toxicity recurs, therapy may be held until the toxicity has resolved to grade 1. Treatment may then be resumed with a one dose level reduction.

4.6.5.1.2. **Grade 3-4**: If a patient experiences Grade 3-4 toxicity that is considered possibly related to ruxolitinib, therapy must be withheld until the toxicity has resolved to Grade ≤1. Treatment may then be resumed with a one dose level reduction. If toxicity recurs, additional dose reductions may be implemented following the same general guidelines.

4.6.5.2. Hematologic Toxicity

4.6.5.2.1. If neutrophils are <0.5 x 10^9/L, and/or platelets are <50 x x10^9/L, and/or hemoglobin <8 g/dL, hold therapy until granulocytes are ≥0.5 x10^9/L and platelets are ≥50 x10^9/L and hemoglobin ≥8 g/dL, then resume therapy.

4.6.5.2.2. For neutropenia:

4.6.5.2.2.1.If recovery to 0.5 to less than 1 x 109/L, re-start therapy with two dose level reduction for at least 2 weeks; if stable, may escalate the dose by one dose level.

If recovery to >1 x10^9/L, re-start therapy with one dose level reduction for at least 2 weeks; if stable, may escalate the dose by one dose level.

4.6.5.2.3. If a similar degree of toxicity returns, a further dose reduction by one dose level can be performed, using the above procedures.

4.6.5.2.4. There will be no making up for missed doses.

4.6.5.3.For thrombocytopenia:

4.6.5.3.1. If recovery to 50 to <75 x10^9/L, resume therapy at 5 mg twice daily for at least 2 weeks; if stable may increase the dose to 10 mg twice daily.

4.6.5.3.2. If recovery to 75 to 100 x10^9/L, resume therapy at 10 mg twice daily for at least 2 weeks; if stable may increase the dose to 15 mg twice daily.

4.6.5.3.3. If recovery to >100 to 125 x10^9/L, resume therapy at 15 mg twice daily for at least 2 weeks; if stable may increase the dose to 20 mg twice daily.

4.6.5.4.For anemia:

4.6.5.4.1.1.If recovery to 8 to less than 10 g/dL, re-start therapy with two dose level reduction for at least 2 weeks; if stable, may escalate the dose by one dose level.

4.6.5.4.2. If recovery to >10 g/dL, re-start therapy with one dose level reduction for at least 2 weeks; if stable, may escalate the dose by one dose level.

4.6.5.5.If more than one cytopenia meets criteria for interruption and dose adjustment, use the greatest dose reduction indicated.

4.6.5.6.Modifications of dose schedules other than the above will be allowed within the following guidelines:

4.6.5.6.1. Alternative dose reductions can be made to keep clinically significant, ruxolitinib-related toxicity grade ≤2. However, the lowest acceptable dose level is -2 and the highest is what is established as MTD (and no greater than 20 mg

twice daily).

4.6.5.6.2. Dose adjustments by more than 1 dose level at a time can be considered when judged in the best interest of the patient (e.g., neutropenia with sepsis, bleeding requiring platelet transfusions) when toxicity has resolved. The reason for this reduction will be discussed with the PI and the sponsor and documented in the medical record.

4.6.5.6.3. A patient who has had a dose reduction because of any of the reasons mentioned above may have their dose re-escalated provided the patient has remained free of toxicity requiring dose adjustments as defined above for at least 1 month. Escalation will be made by 1 dose-level increments only, and not more frequent than every month.

4.6.6. **Dose Escalation**: Patients that do not achieve an improvement in molecular response after 3 months (e.g., from major to complete or from non-major to major) and have experienced no grade ≥3 toxicity may have the dose of ruxolitinib escalated up to the dose determined to be MTD.

4.6.6.1. Dose escalation of Ruxolitinib will be done in increments of 5 mg per day at a time and no more frequent than once every 4 weeks. Dose escalations will be from 5 mg BID, to 5 mg in am and 10 mg in pm (or vice versa), to 10 mg BID, to 10 mg in am and 15 mg in pm (or vice versa), to 15 mg BID, to 15 mg in am and 20 mg in pm (or vice versa), to 20 mg BID.

4.6.6.2. Patients who have had a prior dose reduction of ruxolitinib and who have no toxicity for at least 1 month of therapy may increase the dose of ruxolitinib following the guidelines mentioned above. Dose escalation of the TKI is not allowed.

4.6.7. Occasional missed doses of TKI will not be considered a deviation. Missed dosed of ≥ 2 weeks without adequate justification will be considered a protocol deviation.

4.6.8. **Duration of Therapy:** Patients may receive therapy for six months. After completion of the 6th month, patients may continue therapy if in the opinion of the investigator there is clinical benefit provided there has been no grade 3 or higher non-hematologic toxicity attributable to ruxolitinib at the doses being administered. In these instances therapy may continue for up to 2 years form the start of study therapy.

4.6.8.1. Patients who achieve a complete molecular remission sustained for 2 years with at least 2 PCR assessments per year can hold therapy with ruxolitinib and/or TKI and be monitored with PCR. If there is reappearance of transcripts detectable by PCR, therapy with one or both agents can be resumed at the doses being administered at the time

treatment was interrupted.

4.6.9. **Concomitant Medications**

4.6.9.1. Patients may not receive any other treatment for CML while on study. This includes the use of hydroxyurea and anagrelide.

4.6.9.2. The use of other medications for management of comorbidities or adverse events is allowed, as clinically indicated.

4.6.9.3. Concomitant medications will be captured as part of the medical record but will not be entered in the case report form.

4.6.9.4. Potent CYP3A4 inhibitors should be avoided whenever possible. Concomitant administration of these agents should be discussed with the principal investigator.

5. Evaluation During Study

5.1. **Pretreatment Evaluation** (within 7 days from treatment start, unless otherwise specified)

5.1.1. A complete history and physical examination including performance status.

5.1.2. CBC, platelet count and differential (differential not required if WBC <0.5 $x10^9$/L), total bilirubin, SGPT (or SGOT), and creatinine within 1 week.

5.1.3. Bone marrow aspirate for morphology (if not done within 6 months).

5.1.4. Cytogenetic analysis or FISH in bone marrow or peripheral blood (if not done within 3 months).

5.1.5. Pregnancy test (blood or urine) for female patients of childbearing potential within 7 days before initiation of study drug dosing.

5.1.6. Peripheral blood and/or bone marrow for PCR.

5.1.7. Peripheral blood and/or bone marrow for correlative studies (optional – Appendix A, & B). Not all optional samples may be collected.

5.1.8. EKG

5.2. **Evaluation During Study**

5.2.1. Physical exam and evaluation of toxicity (clinic visit or telephone interview) after 1 week (±3 days), 4 weeks (±1 week) from the start of therapy, then every

3 months (±1 month) for the first 6 months, then every 6 to 12 months.

If the evaluation for toxicity is done by phone, a report of physical exam from home physician is acceptable provided there is a physical exam at MDACC at least every 6 months (+/- 1 month) during the first year and every 12 months (+/- 1 month) thereafter.

5.2.2. Through interview, we will review the patient's adherence with therapy (TKI and ruxolitinib).

5.2.3. CBC, platelet, differential every 1-2 weeks (+/-1 week) for 8 weeks from the start of therapy, then every 3 months (±3 weeks). Differential not required if WBC $\leq 0.5 \times 10^9$/L.

5.2.4. Bone marrow aspirate with cytogenetics or FISH every 3-6 months in year 1, then as clinically indicated.

5.2.5. Total bilirubin, SGPT or SGOT, and creatinine every 1-2 weeks (+/- 1 week) for 8 weeks from the start of therapy, then every 3 months (±3 weeks).

5.2.6. PCR (peripheral blood and/or bone marrow) every 4 weeks (±1 week) for 3 months, then every 3-6 months until one year, then every 6-12 months.

5.2.6.1. Patients who permanently discontinue therapy with TKI will have PCR every 4 weeks (±1 week) for the first 6 months after discontinuation of TKI, then every 3 months (±3 weeks) for 12 months, then every 6 months (±2 months).

5.2.7. Peripheral blood and bone marrow for correlative studies every 3-6 months in year 1, then every 6-12 months. Not all optional samples may be collected. (Optional – Appendix A & B).

5.2.8. Pharmacokinetics (PK) (optional): blood for PK for ruxolitinib at baseline, 0.5, 2 and 4 h from dosing on days 1, and before your next dose at the 3, 6, 9 and 12 month (±1 month) visits. Occasional missed PK samples will not constitute a protocol deviation/violation.

5.2.9. CBC, blood chemistries, EKG's may be done at outside facilities. Under exceptional circumstances other tests might be done at outside facilities after discussion with PI. All outside lab results will be faxed or mailed to MDACC and reviewed and signed by the investigator.

5.2.10. Patients that come off therapy will continue follow-up for toxicity (clinic visit or telephone interview) for 30 days (+/- 5 days) after end of therapy.

* Every effort will be made to collect optional procedures at all time points for all patients; however, missing collection in one or more of these time points in occasional patients will not be considered a protocol deviation/violation.

Outside Physician Participation During Treatment

1. MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician.
   *This will be documented in the patient record

2. A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (Appendix EE)
3. Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it.
4. Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.
5. A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.
6. Documentation to be provided by the local physician will include drug administration records, progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.
7. The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
8. Patients will return to MDACC every 3 months for 6 months then every 6-12 months thereafter for evaluation. If necessary, patients may have the first 3 month evaluation conducted through their local physician if approved by the MDACC physician investigator.

6. Criteria for Response

6.1. A favorable response for the primary endpoint is defined as a greater than a one-log reduction of BCR-ABL transcript levels from the baseline level or disappearance of BCR-ABL transcripts (i.e., includes complete molecular response).

6.2. For the purposes of this study, progression will be defined as a confirmed loss of complete cytogenetic response (CCyR) (i.e., >0% Ph+ metaphases among 20 metaphases counted by karyotype, or >10% positive by FISH) for patients who enter the study with this response. “Confirmed” is defined here as assessed in two consecutive cytogenetic analyses separated by at least a month.

6.3. Survival endpoints (overall, event-free and free from blastic transformation) will be measured from the time of start of ruxolitinib therapy.

7. Criteria for Removal from Study

7.1. The patient receives CML-directed therapy other than TKI and ruxolitinib.

7.2. The patient develops disease progression defined as confirmed loss of CCyR, confirmed loss of CHR or transformation to accelerated or blast phase of CML.

7.3. The dose of TKI is increased beyond the dose being used at the time of study entry or new therapies for their leukemia are added.

7.4. The patient has a continuous, non-planned interruption of TKI therapy for greater than 8 weeks (e.g., for non-compliance).

7.5. The patient withdraws consent.

7.6. The treating physician considers it in the best interest of the patient.

7.7. Pregnancy or suspected pregnancy

8. Investigational Agent (Ruxolitinib) Packaging and Labeling

8.1. Ruxolitinib 5 mg tablets are packaged as 60 count in high-density polyethylene (HDPE) bottles. The bottles will include labeling "New Drug - Limited by Federal (USA) Law to Investigational Use".

8.2. Preparation

8.2.1. Tablets will be provided to the site and no specific preparation of study medication is required prior to administration.

8.3. Handling and Storage: Investigational product must be dispensed or administered according to procedures described herein. Only subjects enrolled in the study may receive investigational product, in accordance with all applicable regulatory requirements. Only authorized site staff may supply or administer investigational product. All investigational products must be stored in a secure area with access limited to the Principal Investigator and authorized site staff and under physical conditions that are consistent with investigational product-specific requirements. The bottles of tablets should be stored at room temperature, 15°C to 30°C (59°F to 86°F). Stability studies will be conducted on all clinical batches to support the clinical trial. Any unused or expired investigational product will be returned to MDACC to be disposed per institutional policy.

8.4. Importantly, for the purposes of this study, the TKI agents (IM, NIL, DAS) are not considered investigational agents and they will be supplied by commercial sources (per routine clinical care).

9. Adverse Event Reporting

9.1. Adverse events will be documented in the medical record and entered into PDMS/CORe. PDMS/CORe will be used as the electronic case report form for this protocol.

9.2. Adverse event is any untoward medical occurrence that may present during treatment with a pharmaceutical product but which does not necessarily have a causal relationship with this treatment.

Adverse drug reaction (ADR) is a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function.

Assessing causal connections between agents and disease is fundamental to the understanding of adverse drug reactions. In general, a drug may be considered a contributory cause of an adverse event if, had the drug not been administered, 1) the event would not have happened at all, 2) the event would have occurred later than it actually did, or 3) the event would have been less severe.

The Investigator (or physician designee) is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

9.3. Adverse Events (AEs) will be evaluated according to the latest CTC version and documented in medical record. Only unexpected AEs will be recorded in the Case Report Form (CRF). The Leukemia Specific Adverse Events Recording and Reporting Guidelines will be utilized. Expected events during leukemia therapy are:

9.3.1. **Myelosuppression-Related Events (due to disease or leukemia therapy)**

9.3.1.1. febrile or infection episodes not requiring management in the intensive care unit

9.3.1.2. epistaxis or bleeding except for catastrophic CNS or pulmonary hemorrhage

9.3.1.3. anemia, neutropenia, lymphopenia, thrombocytopenia, leukopenia

9.3.2. **Disease-Related Events**

9.3.2.1. symptoms associated with anemia

9.3.2.2. fatigue

9.3.2.2.1. weakness

9.3.2.2.2. shortness of breath

9.3.2.3. electrolyte abnormalities (sodium, potassium, bicarbonate, CO2, magnesium)

9.3.2.4. chemistry abnormalities (LDH, phosphorus, calcium, BUN, protein, albumin, uric acid, alkaline phosphatase, glucose)

9.3.2.5. coagulation abnormalities

9.3.2.6. disease specific therapy (induction, maintenance, salvage, or stem cell therapy)

9.3.2.7. alopecia

9.3.2.8. bone, joint, or muscle pain

9.3.2.9. liver function test abnormalities associated with infection or disease progression

9.3.2.10. disease progression

9.3.2.11. abnormal hematologic values

9.3.3. **General Therapy Related Events**

9.3.3.1. catheter related events

9.3.3.2. renal failure related to tumor lysis syndrome or antibiotic/ antifungal therapy

9.3.3.3. rash related to antibiotic use

9.3.3.4. Hospitalization for the management of any of the above expected events

9.4. Abnormal hematologic values will not be recorded on the case report form. For abnormal chemical values, the apogee or nadir (whichever is appropriate) will be reported per course on the case report form.

9.5. **Serious Adverse Event Reporting (SAE)**

Serious Adverse Event Reporting (SAE) for M. D. Anderson-Sponsored IND Protocols

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death

- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical

recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Investigator Communication with Supporting Companies:

Serious adverse event will be reported to Incyte via fax to Telerx: 1-866-726-9234.

9.6. **Annual Reports**

If the FDA has granted an IND number, it is a requirement of 21 CFR 312.33, that an annual report is provided to the FDA within 60-days of the IND anniversary date. 21 CRF 312.33 provides the data elements that are to be submitted in the report. The Annual Report (AR) should be filed in the study's Regulatory Binder, and a copy provided to Incyte as a supporter of this study as follows:

Kathy Lenard Roberts, Executive Director Pharmacovigilance
Incyte Corporation
Experimental Station - E400
RT 141 & Henry Clay Road
Wilmington, DE 19880
Bus 302.498.6727
E-mail: kroberts@incyte.com

10. Statistical Considerations

10.1. This is a single-arm, open-label, phase I-II trial to evaluate the toxicity and efficacy of ruxolitinib in CML patients in complete cytogenetic remission but with minimal residual disease while continuing to receive TKI therapy. A total of 48 patients will be accrued at a rate of 1 to 2 patients per month.

Patients lost to follow-up or those who stop therapy for personal or financial reasons

need to be replaced and do not count as failure. Patients who discontinue because of unacceptable toxicity are included in the stopping rule for toxicity and will also be counted as failures in efficacy calculations.

10.2. **Phase I**

This is a 3+3 phase I trial to determine the MTD for Ruxolitinib while patients are treated with tyrosine kinase inhibitors (TKIs). Below are the 3+3 algorithm rules.

Dose-Escalation Algorithm:

- Enroll 3 patients at dose level 0.
- If no DLT is observed in at least 3 evaluable patients during the first cycle, the dose will be considered a well-tolerated dose and dose escalation will continue to the next dose level.
- If any DLT is observed in 1 of 3 evaluable patients during the first cycle, additional patients will be recruited to expand the cohort up to 6 evaluable patients at the same dose level.
- If any DLT is observed in only 1 of 6 evaluable patients during the first cycle, the dose will be considered tolerated and dose escalation will continue to the next dose level.
- If any DLT is observed in ≥ 2 of 3 or 2 of 6 evaluable patients during the first cycle, this dose is considered a non-tolerated dose and dose escalation will discontinue and the MTD has been exceeded.
- Once the MTD has been exceeded, treat another 3 patients at the previous dose level if there were only 3 patients treated at that dose level.
- The MTD is the highest dose level at which 6 patients were treated and at most 1 patient experienced a DLT.
  Since there are only 3 doses to be considered and we will have a maximum of 6 patients at each dose for each patient group, the maximum number of patients enrolled in this phase I trial will be 18 patients. If dose level zero exceeds the MTD additional patients may be required for exploration of lower doses.

10.3. **Phase II**

The part of the study will be a phase II trial with a Bayesian design with early stopping rules for toxicity. The phase II portion of the study will use the highest dose level that induced DLT in ≤1/6 patients in phase I. A total of 30 patients will be treated in this phase II study. The accrual rate is about 2 to 3 patients per month.

The primary endpoint (response) of this trial is to determine if the residual disease as measured by PCR can decrease by at least 1 log or become undetectable within 12 months from the start of study therapy. We will determine the proportion of patients with response after 12 months of treatment. In this setting, it would be difficult to do an interim monitoring for futility. Patients who lose CCyR within 12 months will be counted as treatment failures.

We plan to recruit 30 CML patients to achieve a 88% power to detect that the difference between a proportion of 0.20 response rate is significantly different from 0.05 (alpha=0.05). Summary statistics will be used to describe the clinical and demographic characteristics of the study population.

10.3.1. **Toxicity Monitoring during Phase II**

With the concern of treatment related unacceptable toxicity defined as grade 3 or 4 toxicity that prevents patients from continuing therapy with ruxolitinib despite optimal management of adverse events. The following rules apply for each arm. Denote the probability of toxicity by P_E. We assume $P_E \sim$ beta (0.2, 1.8). Our stopping rule is given by the following probability statement: $Pr(P_E > 0.20 \mid data) > 0.80$. That is, we will stop the trial if, at any time during the study, we determine that there is more than 95% chance that the unacceptable toxicity is more than 20%. The study will be stopped early if (The number of unacceptable toxicities)/ (The number of patients evaluated) ≥ 4/9, 5/12, 5/15, 6/18, 7/21, 7/24, 8/27, 9/30. We will apply these stopping boundaries starting from the ninth patient and in cohorts of 3. The operating characteristics are summarized in table 2.

Table 2: Operating characteristics for monitoring of toxicities

True Toxicity Rate	Early Stopping Probability	Sample Size		
		25th percentile	Median	75th percentile
0.10	0.020	30	30	30
0.20	**0.274**	**24**	**30**	**30**
0.30	0.698	9	15	30
0.40	0.940	9	9	15
0.50	0.994	9	9	12

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Appendix A. Correlative studies (including assessment of stem cell renewal factors)

CORRELATIVE/SPECIAL STUDIES:

1. Investigate the effect of therapy on molecular responses as assessed by genomic DNA PCR.

2. Determine the effect of therapy on TKI-resistant quiescent leukemic Ph+ stem cells (CFSEmax/CD34+) by flow cytometric evaluation of activated Crkl and Jak2

3. Assess effect of therapy on self-renewal and/or survival of leukemic stem cells by FISH Analysis on colonies

Rationale:

Given that 1) quiescent Ph+ stem cell (HSC) show innate resistant to TKIs (IM, NIL, DAS) monotherapy and, TKIs force proliferating primitive progenitors into quiescence[21]; and 2) we have evidence that BCR-ABL1 expression is essential for recruiting and activating JAK2 which, in turn, controls survival and self-renewal of Ph+ CFSEmax/CD34+ HSCs[22,23], it is plausible that treating TKI-treated CML patients in CMR with a JAK2 inhibitor (such as ruxolitinib) may result in eradication of CML through induction of apoptosis of the quiescent leukemic HSC.

Specifically we will:

1) Assess changes in the number of leukemic stem cells by BCR-ABL1 genomic PCR in BM CD34+ cells before and after ruxolitinib treatment. We do expect a marked decrease in the levels of genomic PCR BCR-ABL1 positivity if JAK2 activity controls survival of leukemic HSCs.
2) Determine whether the ruxolitinib + TKI combination exerts its anti-leukemic activity towards Ph+ HSCs by direct suppression of JAK2 activity in the quiescent TKI-resistant CML HSCs by CFSE-labelling of CD34+ BM cells before and after treatment and monitoring of JAK2 activity by intracellular flow cytometry on the gated CFSEmax cells. As controls, pCRKL levels will be also monitored on the same cell fractions. It is expected a decreased number of quiescent HSCs expressing both high levels of pJAK2 and pCrkl (as read-out of BCR-ABL1 activity).
3) To formally determine whether suppression of JAK2 by ruxolitinib inhibits self-renewal and survival of the primitive leukemic HSC we will assess a) the serial replating colony forming efficiency (CFC/replating assay) of CD34+/CD38- cell from untreated and Ruxolitinib-treated patients, and b) the frequency of long term culture-initiating cells (LTC-IC assays), respectively. D-FISH and/or RT-PCR for BCR-ABL1 will be performed on plucked colonies to specifically determine the percentage of Ph+ colonies. If JAK2 plays a pivotal role in the regulation of self-renewal and survival of leukemic HSC we do expect the emerging colonies to be Ph-negative or to have a marked decrease of the Ph-positive colonies.

Methods:

Tracking of cell division using CFSE staining

CD34+ cells from CML primary samples will be stained with 0.25µM of carboxyfluorescein diacetate succimidyl diester (CFSE, CellTrace CFSE proliferation Kit, Invitrogen). CFSE-stained cells were then FACS-sorted to isolate a narrow peak of CFSE fluorescence, in order to be able to resolve discreet populations corresponding to cell divisions. Non-dividing cells, whose cell cycle is arrested by the addition of Colcemid (100ng/ml, Sigma) in the medium, were used to determine the $CFSE^{max}$ population after 3 to 6 days in culture. The cells were harvested after 6 days in culture, stained with anti-CD34 PE (Beckman Coulter), the viability stain 7-AAD (BD Bioscience) to determine the number of viable quiescent cells (CFSEmax/CD34+/7-AAD- cells). The calculation of the recovery of quiescent cells, expressed as a percentage of the absolute number of cells used to establish the culture at time zero, will performed as described in [24].

Intracellular flow cytometry.

The CFSE+/CD34+ fraction from 3-6 day cultured CFSE-stained CML samples and the will be stained with anti-CD34 PE (BD), anti-CD38 PEcy7 (eBioscience) then fixed and permeabilized with the BD Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences). Cells will then stained with either a specific primary antibody or an isotype matched control and a secondary goat F(ab') anti-rabbit conjugated to Alexa Fluor 647 (Invitrogen). Stained samples will be analyzed on a LSRII flow cytometer (BD). The primary antibodies used will be: rabbit anti-pCRKL (pY207) and rabbit anti-phospho-JAK2 (pY1007/1008) (Cell Signaling Technology).

Long-term culture-initiating assays (LTC-IC assay)

$2x10^6$ bone marrow cells from CML patients will be cultured on 35mm dishes with a 1:1 mix of irradiated (80Gy) M2-10B4 (producing IL-3 and G-CSF) and SI/SI (producing IL-3 and KL) murine fibroblasts in MyeloCult H5100 medium (StemCell Technologies) supplemented with 10µM hydrocortisone. The long-term cultures are then incubated for 5 weeks with weekly half-medium changes. Adherent and floating cells will be harvested and $50x10^3$ cells plated in colony-forming assays with MethoCult H4435 (containing rhKL, rhG-CSF, rhGM-CSF, rhIL-3, rhIL-6, rhEpo) semisolid medium (StemCell Technologies). The colonies, derived from long-term culture-initiating cells (LTC-ICs), will be scored after an additional 14 days in culture[25,26].

Colony-forming cell and self-renewal assay (CFC/replating assays).

Methylcellulose colony formation assays will be carried out by plating 10^3 CD34+/CD38- cell sorted from CML patient samples in 0.9% MethoCult H4435 (containing rhKL, rhG-CSF, rhGM-CSF, rhIL-3, rhIL-6, rhEpo) (Stem Cell Technologies). Colonies will be scored after 14 days in culture. To assess self-renewal individual colonies will be plucked in 50µl of IMDM/2%FBS, mixed with 150µl of Methocult and replated in 96-well plates and analyzed 14 days later. Single wells containing at least one secondary colony will be scored as positive for self-renewal as previously described[27].

Shipping Instructions:

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Appendix B. Pharmacokinetic (PK) Studies for Ruxolitinib Plasma Level Determination

- **PK Blood Processing, Storage, and Shipment**

1. Collect PK blood samples to analyze ruxolitinib plasma concentrations or potentially, metabolites of ruxolitinib using 4 mL **EDTA(lavender top) Vacutainer tubes** at the time points indicated in the protocol.
2. After obtaining the PK blood sample, gently mix evacuated-collection tube thoroughly by slowly inverting the collection tube several times.
3. Place the collection tube in an **ice/water bath**.
4. Within 45 min of sample collection, process collection tubes in a refrigerated centrifuge set at approximately 2000 x g for 15 minutes at approximately 5 ºC.
5. Transfer plasma into two aliquots (Aliquots A and B) of approximately equal volume, using standard laboratory technique, into 2 appropriately-labeled storage tubes.
6. Label each tube with the patient's study registration number, the study I.D., cycle, dose and the date and time the sample was drawn.
7. Ship the samples to the laboratory address listed below with a 2 day supply of **dry ice**. Samples must be stored in a freezer set to maintain a temperature of **-20°C to -80°C** until ready for shipment.

Note: Samples may be batched but they should only be shipped on Monday through Wednesday.

- PK Sample Shipping Instructions:

Ship the plasma samples with a 2-day supply of dry ice. On the day of shipment, **notify Incyte of the pending shipment by email**, and include the protocol number (**I-RUX-11-09**), sample type (*e.g.,* plasma), number of samples, and tracking information.

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