PHASE I/II STUDY OF RUXOLITINIB PLUS DECITABINE IN PATIENTS WITH POST MYELOPROLIFERATIVE NEOPLASM - ACUTE MYELOID LEUKEMIA

Short title: Phase I/II study of RUXOLITINIB plus decitabine in patients with acute myeloid leukemia

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1.0 OBJECTIVES

1.1. For phase I portion of study*:*

To determine the tolerability of the combination of decitabine and RUXOLITINIB (DI) in patients with leukemia.

1.2. For phase II portion of study:

Primary objective - To determine the efficacy of RUXOLITINIB in increasing and prolonging response induced by decitabine alone in patients with post myeloproliferative neoplasm AML (post MPN-AML) alternatively referred to as (myeloproliferative neoplasm- Blast phase; MPN-BP).(Compared to historical response rate with decitabine alone)

Secondary objective - To compare whether there is a difference in response rate patients with post-MPN AML with JAK2 mutations and patients without JAK2 mutations.

1.3. Endpoints:

1. Assessment of DLT and MTD

2. Proportion of patients achieving overall response (CR + CRi). We will also assess response by the MPN criteria (including ALR-C and ALR-P, see section 11.00)

3. Proportion of patients with post-MPN AML with JAK2 mutations and the proportion of patients without JAK2 mutations and any difference in response rate.

2.0 BACKGROUND AND RATIONALE

RUXOLITINIB phosphate is an inhibitor of the Janus kinase family of protein tyrosine kinases (JAKs) that has been recently approved for the treatment of patient with myelofibrosis and is undergoing further evaluation for the treatment of other myeloproliferative disorders under IND No. 77,456. Unless otherwise noted, RUXOLITINIB phosphate is referred to throughout this protocol as RUXOLITINIB. RUXOLITINIB Phosphate Tablets 5 and 25 mg (free base equivalent), as disclosed in IND No. 77,456, will be used in the proposed studies described in this IND. Additional details of the CMC characterization of RUXOLITINIB may be found in the Clinical Investigators Brochure (CIB).

We propose to investigate the combination of decitabine and RUXOLITINIB in patients with high-risk MDS/MPN and post-MPN AML. In the phase I portion of the trial, the aim is to determine the tolerability of the combination. In the phase II portion of the trial, the number and duration of responses in patients with post-MPN AML and high-risk MDS/MPN ($\geq$ 20% blasts) will be determined.

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.[1] 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. [1]

Abnormalities of Jak function have been associated with a number of disorders. For example, chromosomal translocations resulting in TEL-JAK2 constructs lead to the constitutive activation of STAT5, IL-3-independent cellular proliferation, and leukemogenesis.[2-4] The translocation t(9;12)(p24;p13) results in the fusion of the kinase catalytic region of JAK2 with the transcription factor TEL generating the constitutively active TEL-JAK2.[5] Similarly, infection with oncogenic viruses such as human T-cell lymphotrophic virus, type I, and Abelson murine leukemia viruses results in enhanced kinase activity of Jaks, possibly accounting for their leukemogenic potential.

The STAT transcription factors are coded by six known mammalian genes and include 10 different STAT proteins including different isomers of STATs 1, 3, 4, and 5. Like other transcription factors STATs have a well-defined structure including a DNA-binding domain, a conserved NH_2-terminal domain, a COOH-terminal transactivation domain, and SH2 and SH3 domains. Their activation through tyrosine phosphorylation results in their dimerization and translocation into the nucleus where they activate specific genes.[1]

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. Reconstitution of STAT1 in STAT1-null U3A cells (which do not respond to TNF-α) restores basal caspase expression and renders them sensitive to TNF-induced apoptosis. Conversely, 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.[1]

Abnormalities of the JAK-STAT pathways have been described in a variety of leukemias and their inhibition can be a goal for leukemia therapy. Myelofibrosis (MF) is a clonal stem cell disorder with the potential to transform to acute leukemia (AML), referred to as myelofibrosis in blast phase (MF-BP).[6] The outcome of patients with MF-BP is grave with a median survival of only 2.7 months. MF-BP is largely refractory to conventional chemotherapy and intensive induction therapy fails to have a significant impact with a median survival of 3.9 months.[7]

Decitabine (5-aza-2′deoxycytidine) is a deoxycytidine analog originally synthesized in the 1960s. The compound is phosphorylated by deoxycytidine kinase prior to incorporation into DNA, and it is an S-phase–specific agent. Decitabine also inhibits DNA methyltransferases (DNMTs) by forming irreversible covalent bonds with DNMTs at cytosine sites targeted for methylation. At low doses, hypomethylation of DNA with subsequent gene reactivation and induction of cellular differentiation occur. At higher doses, cellular cytotoxicity predominates.

DNA hypermethylation of promoter-specific CpG islands is a well-known mechanism of epigenetic silencing, occurring frequently in cell cycle regulatory genes such as $p15^{INK4B}$ in a variety of hematologic malignancies, including MDS and AML. In MF, $p15^{INK4B}$ and $p16^{INK4A}$ hypermethylation has been reported to occur as the disease advances into accelerated and blast phases. Hypermethylation of the calcitonin gene (used as a marker of methylation at the 11p15 chromosome locus where several tumor-suppressor genes appear to cluster) has been reported in MF even in the chronic phase of the disease compared with other Philadelphia chromosome–negative chronic myeloproliferative diseases. In addition, the retinoic acid receptor β (*RARβ*) gene has been found to be a target of epigenetic silencing in MF and has been proposed as a candidate tumor-suppressor gene in this disease. In clinical trials, decitabine has clinical activity in MDS at lower doses. Furthermore, a decrease in hypermethylation of the $p15^{INK4B}$ gene was observed in some patients with MDS following treatment with decitabine and correlated with clinical response.Significant evidence of clinical activity was also reported in a recent phase I study of decitabine at low doses in hematologic malignancies, but this study found no evidence of a

correlation of clinical responses with *p15*^{INK4B} methylation, suggesting that the biologic effects of the drug may not be mediated solely through *p15*^{INK4B} demethylation.

Our group has previously conducted a phase-II study to evaluate the activity of DNA methyltransferase inhibitor, 5-azacitidine, in patients with MF.[8] Thirty-four patients (76% previously treated) received 5-azacitidine at 75 mg/m(2) subcutaneously daily for 7 days, every 4 weeks. Twelve (35%) patients had abnormal cytogenetics and 19 (70%) of 27 evaluable patients had JAK2(V617F) mutation. Responses occurred in 8 (24%) patients after a median of 5 months (range, 3-10). Partial response occurred in 1 (3%) patient (duration 22+ months) and clinical improvement in 7 (21%) patients (median duration 4 months; range, 2-8.5). Myelosuppression was the major adverse effect, with grade 3-4 neutropenia in 10 (29%) patients. Global DNA methylation assessed by the long interspersed nucleotide element (LINE) bisulfite/pyrosequencing assay decreased from 53% pretherapy to 44% on day 14 (P=0.0014) and returned to 50% at the end of the first 28-day cycle (P=0.016). 5-azacitidine is relatively well tolerated and results in induction of global hypomethylation in patients with MF, but results in limited clinical activity.[9]

In another recently published study, 54 patients with Philadelphia-negative myeloproliferative neoplasm (MPN, including 21 essential thrombocythemia [ET], 21 polycythemia vera [PV], 7 primary myelofibrosis, and 5 unclassified MPN) who had progressed to AML (n = 26) or MDS (n = 28) were treated with azacitidine in a patient-named program.[10] Overall response rate was 52% (24% complete response [CR], 11% partial response [PR], 8% marrow CR or CR with incomplete recovery of cytopenias, 9% hematologic improvement) and median response duration was 9 months. Prognostic factors were for overall response the underlying MPN (71% vs 33% responses in ET and PV, respectively; P = .016); prognostic factors for CR achievement were the underlying MPN (14% CR for PV vs 43% for ET; P = .040) and World Health Organization classification at transformation (36% vs 12% CR in MDS and AML, respectively, P = .038). Recurrence of chronic phase features of the initial MPN was observed in 39% of the responders. Median overall survival was 11 months. The authors concluded that azacitidine gives encouraging results in Ph-negative MPN having progressed to AML or MDS, but response duration is short, and consolidation treatments have to be evaluated.[10]

We and others have also evaluated decitabine in the treatment of MF and have demonstrated significant activity.[11] In a study from Mount Sinai School of Medicine, Four out of seven patients (57%) with MF-BP treated with decitabine reported subjective improvement in symptoms of fatigue/global weakness and left upper quadrant abdominal fullness/pain.[7] All seven patients experienced an objective reduction in spleen size on physical examination with a mean 35% reduction of palpable splenomegaly (range 11–61%). Four out of seven patients (57%) had a decrease in RBC transfusion requirements and a single patient achieved transfusion independence after three cycles of therapy. The three patients who remain alive currently are in a morphologic complete response with incomplete platelet recovery (CRi) by IWG response criteria for AML

We have recently conducted a phase II study of RUXOLITINIB in patients with relapsed/refractory leukemias for which no standard therapies were anticipated to result in a durable remission. Patients with acceptable performance status (0-2) with adequate organ function and no active infection received RUXOLITINIB 25mg orally twice a day for 4 weeks (1 cycle). Response was assessed after every 2 cycles of treatment. Responding patients or patients with stable disease were allowed to continue until progression. Dose escalation to 50 mg twice daily was permitted in patients demonstrating a benefit. 38 patients with median age 69 (range 45-88) with relapsed and refractory leukemias were treated. The median number of prior therapies was 2 (range 1-6). 12 patients had JAK2V617F mutation. Patients who completed 2 cycles of INCB with clinical improvement were defined as having stable disease (SD). Patients received a median of 2 cycles of therapy (range 1-22). Three patients with AML developing after prior MF (MF-BP) showed remarkable response with 2 achieving complete remission (CR) and one remission with insufficient platelet count (CRi). 12 patients had SD. Responding patients have had a significant reduction in their spleen size. Overall INCB was tolerated very well.

Rationale:

There is no standard medical treatment for MF-BP or other post-MPN-AML. We believe that the combination of ruxolitinib and decitabine is a candidate approach to the treatment of MF-BP that is worthy of exploration based on the current understanding of the biology of disease. The molecular pathogenesis of MPN and progression to blast phase is almost certainly due to a complex combination of gene mutations (JAK2V617F, MPL) and epigenetic alterations (IDH1/2, IKZF1, EZH2, TET2) that culminate in the emergence of leukemic clones. Recent evidence indicates that the JAK2V617F protein can localize in the nucleus and influence global DNA methylation patterns which may lead to genomic instability and disease progression (40, 41). The inhibition of JAK-STAT mediated cell proliferation and survival in conjunction with the reversal of DNA hypermethylation of tumor suppressor genes would be predicted to have at least an additive if not synergistic effect in inducing apoptosis of cells belonging to the malignant myeloid clone. Correlative studies conducted within a trial of combination JAK2 inhibitor and DMNT1 inhibitor in patients with MPN-BP would explore the effect on methylation status of various gene promoters as well as the influence on gene expression of chromatin related proteins and ultimately leukemic cell survival. The sequential administration of a JAK2 inhibitor followed by a DNMT inhibitor would also potentially serve to overcome the JAK2-independent effects of epigenetic lesions that lead to MPN-BP.

3.0 STUDY DESIGN

3.1 Design

In this non-randomized Phase I/II investigator-initiated, single center trial, cohorts of patients will be treated with increasing doses of ruxilitonib in combination with a decitibine at a dose of 20mg/m2 daily intravenously over 5 days. We will use an intitial dose of ruxolitinib of 10 mg orally twice daily. Patients will receive ruxolitinib for the first 28 days in addition to decitabine on days 1-5 of each cycle. Cycles will be repeated every 4-6 weeks depending on tolerance and the recovery of the counts. Patients who become eligible for allogenic stem cell transplant may leave the study at any time after the first cycle.

After the first cycle of therapy, modifications to the dose of Ruxolitinib felt to be in the best interest of the patient are allowed after discussion with the Principal Investigator of the study and with the documentation of the discussion in the patient's medical records.

For the purposes of assessing safety, DLTs will be defined as those adverse events occurring in the first cycle (i.e. 28 days) of therapy that are not clearly related to disease, intercurrent illness or concurrent medication. These events will be defined based on the NCI CTCAE version 4 as follows:

- Any Grade 3 or higher non-hematologic toxicity (with the exceptions of Grade 3 or higher nausea, vomiting, or diarrhea, which will be considered DLTs only after optimal prophylactic measures have been prescribed; Grade 3 elevations of AST/ALT must be sustained for more than 4 days or reach Grade 4 to be considered a DLT. Grade 3 elevations in bilirubin must occur in the setting of a direct bilirubin >1 to be considered a DLT. Fatigue must be Grade 4 to be considered a DLT)

- Any Grade 4 hematologic toxicity lasting at least 42 days from the first day of the last cycle of therapy with a bone marrow cellularity of ≤5% and no evidence of leukemia. No dose modification will occur until any potential DLTs is fully assessed

MTD is defined as the highest dose studied for which the incidence of DLT is less than or equal to 17% (1 out of 6).

3.2 Intervention

Ruxolitinib will be administered as 5 mg (or if necessary, 25 mg) tablets taken orally at doses indicatedin table 1 on page 14, approximately every 12 hours for 28 days every cycle. Decitabine is administered intravenously at a dose of 20mg/m2 daily for 5 days per cycle. Subsequent cycles may be administered at 4-6 week intervals as clinically tolerated with a provision to start early if in the best interest of the patient after discussion with the prinicipal investigator.

4.0 THERAPEUTIC AGENTS

4.1 Ruxolitinib

Ruxolitinib represents a novel, potent, and selective inhibitor of JAK1 (IC50 = 3.3 ± 1.2 nM) and JAK2 (IC50 = 2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (IC50 = 19 ± 3.2 nM) and JAK3 (IC50 = 428 ± 243 nM), respectively. INCB018424 is inactive (ie, < 30% inhibition) against 28 additional kinases when tested at 200 nM.

Ruxolitinib has high solubility and permeability, (ie, it is designated as a Class I molecule in the Biopharmaceutical Classification System (BCS)) and exhibits moderate- to-high clearance, volume of distribution and oral bioavailability in preclinical species. The apparent elimination half-life is short (< 5 hr) in all species. The primary clearance pathway is oxidative metabolism. The metabolism by human liver microsomes is catalyzed predominantly by CYP3A4. In a study using 14C-Ruxolitinib in healthy volunteers, unchanged Ruxolitinib was the predominant circulating drug-related entity with 2 major circulating metabolites observed, both of which are mono-oxidation products. The metabolites of Ruxolitinib retain varying degrees of JAK-related pharmacological activity. Excretion was fairly rapid by both urinary and fecal routes with parent drug accounting for < 1% of the administered dose. Tissue distribution studies in rats indicate rapid and complete elimination of radioactivity in most tissues. Ruxolitinib exhibits high plasma protein binding in humans with an unbound fraction of 3.3%.

4.1.1 Chemistry

The chemical name of ruxolitinib, previously known as INCB018424 (also referred to as INC424) phosphate is (*R*)-3-(4- (7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate. INCB018424 phosphate has a molecular formula of C17H21N6O4P and a molecular weight of 404.36.

4.1.2 Physical Properties

Ruxolitinib is a white to off-white powder.

4.1.3 Stability

Based on available stability data, the drug product should be stored in high density polyethylene (HDPE) bottles with induction sealing and child-resistant closure between 15°C and 30°C.

4.1.4 Formulation

Ruxolitinib is provided as 5 mg, 10 mg, 15 mg, 20 mg and 25 mg strength tablets. The tablet formulations contain the active ingredient and may include the following commonly used excipients: microcrystalline cellulose, lactose, stearic acid, magnesium stearate, colloidal silicone dioxide, sodium starch glycolate, Povidone and hydroxyl propyl cellulose. All excipients are of US and EuPh compendial grade.

4.1.5 FDA approval status and IND issues

Ruxolitinib was approved by the FDA with the trade name, Jakafi, for the treatment of patients with intermediate or high-risk myelofibrosis.

4.1.6 Decitabine

Decitabine is a cytidine antimetabolite analogue that is believed to exert its antineoplastic effects after phosphorylation and direct incorporation into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. Decitabine inhibits DNA methylation *in vitro,* which is achieved at concentrations that do not cause major suppression of DNA synthesis. Decitabine-induced hypomethylation in neoplastic cells may restore normal function to genes that are critical for the control of cellular differentiation and proliferation. In rapidly dividing cells, the cytotoxicity of decitabine may also be attributed to the formation of covalent adducts between DNA methyltransferase and decitabine incorporated into DNA. Non-proliferating cells are relatively insensitive to decitabine.Decitabine is approved by the FDA for the treatment of patients with myelodysplastic syndromes.

4.1.7 Chemistry and Physical Properties

Decitabine is also known as 5-aza-2'-deoxycytydine, an analogue of the natural nucleoside 2'-deoxycytidine. Decitabine is a fine, white to almost white powder with themolecular formula of C8H12N4O4 and a molecular weight of 228.21. Its chemical name is 4-amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one. Decitabine is slightly soluble in ethanol/water (50/50), methanol/water (50/50) and methanol; sparingly soluble in water and soluble in dimethylsulfoxide (DMSO).

4.1.8 Formulation and Stability

Decitabine for injection is supplied as Dacogen™, a sterile lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Dacogen™ (decitabine) for Injection is a sterile lyophilized powder supplied in a clear colorless glass vial. Each 20 mL, single dose, glass vial contains 50 mg decitabine, 68 mg monobasic potassium phosphate (potassium dihydrogen phosphate) and 11.6 mg sodium hydroxide **(NDC 58063- 600-50).** Vials are to be stored at 25°C (77°F) with excursions permitted to 15-30°C (59-86°F). Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C - 8°C) infusion fluids and stored at 2°C - 8°C (36°F - 46°F) for up to a maximum of 7 hours until administration.

4.1.9 FDA approval status

Decitabine was approved as Dacogen™ by the FDA for treatment of patients with myelodysplastic syndromes (MDS) including previously treated and untreated, de novo and secondary MDS of all French-American-British subtypes (refractory anemia, refractory anemia with ringed sideroblasts,

refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) and intermediate-1, intermediate-2, and high-risk International Prognostic Scoring System groups. The indication of refractory anemia with excess blasts in transformation has been included in the definition of acute myeloid leukemia according to the most recent WHO classification. As a result, decitabine is a common treatment of AML, and has been incorporated into standard treatment pathways for AML by the National Comprehensive Cancer Network, especially in patients who are unfit for standard therapies, such as those over age 60 years.

5.0 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

1. Diagnosis of AML (WHO classification definition of $\geq$ 20% blasts).

2. In the phase I portion of the study all patients with relapsed or refractory AML are eligible. For the Phase II portion of the study, patients must have AML progressing from prior MPN (MPN-BP) or have MDS/MPN with more than 20% blasts.. Temporary prior measures to control blood counts, such as apheresis or hydrea are allowed. Patients with newly diagnosed or previously treated disease are eligible as long as prior therapy does not include hypomethylating agents.Prior therapy for RUXOLITINIB for MPN is allowed.

3. Serum biochemical values with the following limits unless considered due to leukemia:

- creatinine $\leq$ 1.5 mg/dl
- total bilirubin $\leq$ 1.5 mg/dL, unless increase is due to hemolysis or congenital disorder
- transaminases (SG PT) $\leq$ 2.5x ULN

4. Ability to take oral medication.

5. Ability to understand and provide signed informed consent.

6. Performance status ≤ 3, unless directly related to disease process as determined by the Principal Investigator

7. Age ≥ 18 years

5.2 Exclusion Criteria

1. Any coexisting medical condition that in the judgment of the treating physician

is likely to interfere with study procedures or results including uncontrolled severe infections, as well as uncontrolled cardiac disease, or other organ dysfuction. Patients with history of tuberculosis, HIV or hepatitis B and C are excluded.

2. Nursing women, women of childbearing potential with positive blood pregnancy test within 30 days of study start, or women of childbearing potential who are not willing to maintain adequate contraception (such as birth control pills, IUD, diaphragm, abstinence, or condoms by their partner) over the entire course of the study..

3. Incomplete recovery from any prior surgical procedures or had surgery within 4 weeks prior to study entry, excluding the placement of vascular access.

4. Active clinically serious and uncontrolled infection

6.0 RECRUITMENT PLAN

Patients will be recruited among the patients referred to the Leukemia Department at the University of Texas – MD Anderson Cancer Center. There will be no direct advertising for this study. The study will be available to the public and the details of the inclusion criteria, exclusion criteria and study design will be posted at www.clinicaltrials.gov. A maximum of 36 patients (up to 12 patients in phase I and up to 24 patients in the phase II) will be enrolled in the study. Up to 6 patients can be replaced in the phase I portion of the study.

7.0 PRETREATMENT EVALUATION

The following tests must be done within 30 days of entry:

- A complete history and physical exam including ECOG performance status assessment and manual measurement (in cm) of the liver and spleen from the costal margin, if palpable.
- CBC with differential, comprehensive metabolic panel, LDH, direct bilirubin, phosphorus, albumin, uric acid, coagulation profile (PT, aPTT, fibrinogen), and ßHCG (for pre-menopausal females),
- HIV and Hepatitis B and C testing
- Chest X-ray (PA and lateral)
- Abdominal imaging (only if clinically indicated, e.g. organomegaly)
- Bone marrow aspirate
    - Cytogenetics, and if necessary, FISH analysis (if insufficient aspirate, these tests can be performed in peripheral blood samples)
    - $JAK2^{V617F}$ and $MPL^{W515L/K}$ mutation analysis and allele burden (if insufficient aspirate, these tests can be performed in peripheral blood samples)
- Bone marrow biopsy (including reticulin stain to assess the degree of fibrosis)

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- Peripheral blood sample for scientific correlative studies: one red top tube (10ml) and two purple top tubes (10ml each)
- ECG, MUGA/Echo
- CT/MRI abd/pelvis without contrast, if clinically indicated
- Pregnancy test performed only on women of child-bearing potential.

8.0 TREATMENT/INTERVENTION PLAN

8.1 Treatment Schedule

Phase I

Assuming MTD is not achieved, the first 9 patients on study will receive RUXOLITINIB for 28 days during induction therapy at escalating doses per the table below, plus

Decitabine 20 mg/m^2 IV daily for 5 days

Dose of RUXOLITINIB will be as follows:

Table 1 – RUXOLITINIB dose

Dose level	RUXOLITINIB (mg PO) (x 28 days)	No of evaluable patients
0	10 BID	3
1	15 BID	3
2	25 BID	3
3	50 BID	3

We will utilize the above design format to determine the tolerable dose of RUXOLITINIB to be used in combination with decitabine, with 25 mg PO BID as the target dose. The enrollment of participants will be staggered; all the participants in each cohort will finish the first cycle of therapy followed by 7 days of observation (i.e. day 35 of therapy) before proceeding to the next cohort. Subsquence cycles will begin on the first day of decitabine therapy and, in the absence of toxicity, RUXOLITINIB administration will not be interrupted.Patients who come off the study during the first cycle for reasons other than development of dose limiting toxicity (e.g. disease progression) will be replaced in order to ensure adequate chance for the assessment of DLT.

If grade 3-4 non-hematological RUXOLITINIB-related toxicities are observed in ≥ 2/6 patients, this dose level would exceed the MTD.

If grade 3-4 RUXOLITINIB-related toxicities are observed in 0-1/6 patients, the study will continue at the RUXOLITINIB dose of 15, 25, and 50 mg P.O. BID x 28 days plus chemotherapy, as outlined above. We will examine the dose of 50 mg BID to assess its tolerability and if well tolerated, this will be the dose to move to phase II, However, the target dose for phase II is 25 mg po BID x 28 days.

Cycles will be repeated approximately every 4 to 6 weeks, depending on the recovery of the blood counts and will continue until clinically significant progression, withdrawal of consent or unacceptable toxicity for a maximum of 24 cycles (see section 13.0).

Toxicities to be monitored include any grade 3 or 4 non-hematological toxicity felt to be related to RUXOLITINIB and prolonged cytopenias (lasting for more than 42 days after the initiation of the treatment) felt to be related to the treatment and not the underlying disease. Patients with prolonged cytopenias should have a bone marrow exam around day 42 to establish whether this is due to the treatment or whether there is persistent disease. In the case of the former, treatment should be discontinued.

Prior to advancing/changing dose levels, a cohort summary must be completed and submitted to the IND Medical Monitor for review.

Each cohort will have up to 6 patients enrolled.

Phase II

Twenty four patients (not including any patients from the phase I portion of the study) will receive cycles of therapy according to the following starting schedule:

RUXOLITINIB at dose defined by Phase I portion of the study, on days 1-28 of each cycle

Decitabine 20 mg/m^2 IV daily for 5 days

Cycles will be repeated every 4 to 6 weeks depending on tolerance and the recovery of counts.

RUXOLITINIB will be provided by the sponsor (Incyte) free of charge to the participating patients for a maximum period of up to 24 months. Any used or expired study drug will be disposed of accordance to the MD Anderson policy.

Dose modifications during induction therapy in phase I and II:

Patients who experience RUXOLITINIB-specific (defined as determined by the principal investigator) grade 3-4 extramedullary toxicities during induction cycle #1, and who are not in CR after cycle #1, may receive a second induction course at the next lower dose with respect to RUXOLITINIB (as defined in table1 on page 14). Other dose modification schedules felt to be in the best interest of the patient may be permitted, after discussion with the principal investigator with the documentation of the discussion in the patient's medical records. There will be no dose modifications of decitabine for either induction cycle #1, or second induction course, secondary to myelosuppression.

Adjustment of dose of RUXOLITINIB to 15 mg BID or 10 mg BID are acceptable in patients who have toxicity but are having benefit from the treatment. If patients develop significant myelosuppression that is thought to be specifically related to RUXOLITINIB, it can be held for a week and then resumed at a lower dose (5-10 mg per day less than the prior dose).

Toxicities to be monitored include any grade 3 or 4 non-hematological toxicity felt to be related to RUXOLITINIB and prolonged cytopenias (lasting for more than 42 days after the initiation of the treatment) felt to be related to the treatment and not the underlying disease. Patients with prolonged cytopenias should have a bone marrow exam around day 42 to establish whether this is due to the treatment or whether there is persistent disease. In the case of the former, treatment should be discontinued.

Number of patients:

A maximum of 36 patients (up to 12 patients in phase I and up to 24 patients in the phase II) will be enrolled in the study.

Phase I study

Nine to 12 patients will be treated. Open study to phase II if first cycle RUXOLITINIB attributable toxicity in ≤2 of 6. If first cycle RUXOLITINIB-related DLTs are observed in more than 2/6 patients, this dose level would exceed the MTD, and 3 patients will be treated at the next lower dose level according to Table 1.

Phase 2 study

We will accrue a maximum of 24 patients at a rate of 2 patient per month. The primary outcome for this trial is overall response rate.

8.2 Dose escalation scheme for phase I

Cohorts of 3-6 patients each will be treated with increasing doses of ruxolitinib in a progression of 10mg, 15mg, 25 mg and 50 mg twice daily.

At least 3 patients will be treated at each dose level. All patients treated at the preceding dose level will be observed a minimum of 5 weeks before dose escalation occurs. Dose escalation will proceed as follows:

- If none of the initial 3 evaluable patients in a cohort experiences first cycle dose limiting toxicity (DLT), then a new cohort of 3 patients will be treated at the next higher dose level.
- If one of 3 evaluable patients in a cohort experiences DLT, then up to an additional 3 patients will be treated at the same dose level. Escalation will continue if only one of the 6 patients experiences a DLT.
- In the event that 2 of 3 evaluable patients, or 2 of 6 patients in the first cohort experiences a DLT, then the next cohort of patients will be treated at a lower dose level (dose level -1) and no further dose escalation with occur in this step. Three patients will be enrolled at this dose level, if 0 or 1 patients experiences a DLT, a further 3 patients will be enrolled at this dose level. If overall 0 or 1 patient experiences a DLT at this dose level, this dose will be established as the maximum tolerated dose (MTD).
- If two or more evaluable patients in the cohort experience DLT, then the maximum tolerated dose (MTD) will have been exceeded, and no further dose escalation will occur. The previous dose will be considered as the MTD.
- If only three patients were treated at the dose level under consideration as the MTD, then up to an additional 3 patients will be accrued. If no more than one patient at that dose level experiences DLT, then that dose level will be confirmed as the MTD. If two or more patients in that cohort experience DLT, then the previous dose level will be studied in the same fashion.

8.3 Definition of DLT and MTD:

For the purposes of assessing safety, DLTs will be defined as those adverse events occurring in the first cycle (i.e. 28 days) of therapy that are not clearly related to disease, intercurrent illness or concurrent medication. These events will be defined based on the NCI CTCAE version 4 as follows:

- Any Grade 3 or higher non-hematologic toxicity (with the exceptions of Grade 3 or higher nausea, vomiting, or diarrhea, which will be considered DLTs only after optimal prophylactic measures have been prescribed; Grade 3 elevations of AST/ALT must be sustained for more than 4 days or reach Grade 4 to be considered a DLT. Grade 3 elevations in bilirubin must occur in the setting of a direct bilirubin >1 to be considered a DLT. Fatigue must be Grade 4 to be considered a DLT)

- Any Grade 4 hematologic toxicity lasting at least 42 days from the first day of the last cycle of therapy with a bone marrow cellularity of ≤5% and no evidence of leukemia. No dose modification will occur until any potential DLTs is fully assessed

MTD is defined as the highest dose studied for which the incidence of DLT is less than 17% (1 out of 6).

Table 2 - Criteria for dose modifications of RUXOLITINIB due to study drug-related toxicity **– These must be clearly related to ruxolitinib and not the underlying disease; to be determined by PI and treating physician**

Worst Toxicity **CTCAE Grade* unless otherwise specified (Value)**		**Dose Modification Guidelines** **At any time during a cycle of therapy (including intended day of dosing)**
HEMATOLOGICAL TOXICITIES		
Thrombocytopenia	< 20 x 10^9/L	Interrupt study treatment dosing until resolved to ≥ 20 x 10^9/L, then restart study treatment at reduced level as per Table 5
	> 20 x 10^9/L + grade 3 bleed	Interrupt study treatment dosing until bleeding is resolved and platelet count is at level before bleed was noted, then resume study treatment at reduced level as per Table 5
Neutropenia (ANC)	Grade 4 (ANC < 0.5 x 10^9/L)	Interrupt study treatment dosing until resolved to ≤ grade 2, or baseline, then: • If resolved within 7 days restart study treatment at an unchanged dose level • If resolved in more than 7 days then restart study treatment at reduced level as per Table 5
	Febrile neutropenia (ANC < 1.0 x 10^9/L, fever ≥ 38.5°C)	Interrupt study treatment dosing until fever resolved and ANC ≤ grade 2, then restart study treatment at reduced level as per Table 5

Worst Toxicity **CTCAE Grade* unless otherwise specified (Value)**		**Dose Modification Guidelines** **At any time during a cycle of therapy (including intended day of dosing)**
Anemia# only requires dose reductions after cycle 6	Doubling of transfusion frequency from baseline or hemoglobin	Hold Ruxolitinib only and continue Decitabine, if hemoglobin (or transfusion frequency) returns to baseline within 28 days restart Ruxolitnib at same dose, if this recurs again then reduce dose as pre Table 5
NON-HEMATOLOGICAL TOXICITIES		
GASTROINTESTINAL		
Diarrhea	Grade 2 (4-6 stools/day over baseline, etc) despite the use of optimal antidiarrheal medications	Hold Ruxolitinib dosing until resolved to ≤ grade 1, or baseline, then restart at unchanged dose level
	Grade 3 (≥ 7 stools/day over baseline, etc) despite the use of optimal antidiarrheal medications	Hold Ruxoltinib dosing until resolved to ≤ grade 1, or baseline, then restart study treatment at reduced level as per Table 5
	Grade 4 (life-threatening consequences, hemodynamic collapse, etc) despite the use of optimal antidiarrheal medications	Permanently discontinue study treatment dosing
Vomiting/Nausea* **	Grade 1 & 2 not requiring treatment **or** controlled using standard anti-emetics	Maintain dose level
	Grade 3 or 4 vomiting or Grade 3 nausea that cannot be controlled despite the use of standard anti-emetics	Interrupt study treatment dosing until resolved to ≤ grade 1, or baseline, then restart study treatment at reduced level as per Table 5
HEPATIC		
Total Bilirubin	Grade 3 or 4	Interrupt study treatment dosing until resolved to ≤ grade 2, or baseline, then restart study treatment at reduced level as per Table 5

Worst Toxicity **CTCAE Grade* unless otherwise specified (Value)**		**Dose Modification Guidelines** **At any time during a cycle of therapy (including intended day of dosing)**
Note: If Grade 3 or Grade 4 hyperbilirubinemia is due to the indirect component only, and hemolysis as the etiology has been ruled out as per institutional guidelines (e.g., review of peripheral blood smear and haptoglobin determination), then reduction of one dose level and continuation of treatment is at the discretion of the Investigator.		
AST/SGOT, ALT/SGPT	> 5-10 x ULN	Interrupt study treatment dosing until resolved to ≤ grade 1 (or ≤ grade 2 if liver infiltration with tumor is present), or baseline, then: • If resolved within 7 days, then: • restart study treatment at unchanged dose level • If resolved in more than 7 days, then restart study treatment at reduced level as per Table 5
	> 10 x ULN	Interrupt study treatment dosing until resolved to ≤ grade 1, or baseline, then: • restart study treatment at reduced level as per Table 5
All dose modifications should be based on the worst preceding toxicity.		
* Common Terminology Criteria for Adverse Events (CTCAE Version 4.0)		
** It is critical that electrolyte abnormalities be followed closely and corrected prior to dosing		
*** See also concomitant medication section		

If Ruxoltinib is required to be held based on the dose holding and modification rules above, then it is permissible to use a prednisone taper starting at a dose of 20mg or more a day at the discretion of the investigator to reduce the rebound symptoms that have been well documented in previous studies with Ruxolitinib.

9.0 EVALUATION DURING TREATMENT/INTERVENTION

Response assessment

Response assessment (RA) can be performed at the end of any cycle after cycle 1. Specific events that will prompt response assessment include of resolution of splenomegaly on exam and clearance of blasts from the peripheral blood, which will prompt bone marrow aspirate and biopsy. Patients continuing to maintain at least SD by the response criteria shown below should continue to receive ruxolitinib and decitabine until progression of disease, unacceptable toxicity or patient withdrawal. Patients may receive up to 24 cycles in this study. Patients may proceed to allogeneic stem cell transplant at any time after Cycle 1. Dose modification of ruxolitinib will be allowed after Cycle 1. RUXOLITINIB will be provided by the sponsor for a maximum of up to 2 years, after which it will be required to procure it from commercial sources.

Adverse events will be captured as long as patients are on study and will be used to fully characterize the tolerability and toxcicity of this combination therapy. These events will be defined based on the NCI CTCAE version 4. Patients who withdraw from the study for any reason will be followed and seen 28 days (+/- 5 days) for an end of study visit to capture all adverse events.

Adverse events will only be captured after the first dose of RUXOLITINIB has been administered.

Table 3 - Schedule of study procedures

Evaluation	Baseline screening	Cycle 1	Cycle 2 and beyond	Response assessment	End of Study
		At least once weekly (+/- 3 days)	At least once every 2 weeks (+/- 3 days)		(+/- 5 days)
History	x				
physical exam	X	X	X		X
Vital signs	X	X	X		X
Concomitant medications [5]	X	X	X		X
Adverse event assessment		X	X		X
HIV, HBV, HCV serology	X				
MUGA or Echo	X				
ECG	X				X
CT/MRI abd/pelvis without contrast,	X			X[3]	

if clinically indicated					
Pregnancy test	**X**				
CBC with differential	**X**	**X**	**X**		**X**
Chemistry [4]	**X**	**X**	**X**		**X**
Bone marrow biopsy	**X**			**X[3]**	**X**
Bone marrow aspirate	**X**			**X[3]**	**X**
Jak2/MPL allele burden[1]	**X**				
Correlative studies[2]	**X**				**X**
Chest X-ray	**X**				

1. Preferentially performed on the marrow, but will be performed on the peripheral blood if a specimen cannot be obtained. Allele burden will not be repeated if neither Jak2 nor MPL is mutated. The sample will be obtained in 5 ml EDTA tube.
2. After one cycle of therapy, after 3 cycles, and then every 3 cycles while on the study (in addition to base-line and end of the study samples), peripheral blood will be collected for measurements of inflammatory cytokine levels, expression of different proteins related to intracellular signaling, and methylation of genes. This will include collection of: one red top (10ml) and two purple top tubes (10ml each). All samples will be submitted to the laboratory of Dr. Srdan Verstovsek at MD Anderson.
3. There are 2 milestone events that will prompt response evaluation. Resolution of palpable splenomegaly will prompt imaging of the abdomen and pelvis. Clearance of blasts from the peripheral blood will prompt bone marrow aspirate and biopsy.
4. Direct Bilirubin, Phosphorus, Albumin, PT/PTT, Alk Phos, Total Bilirubin, Total Protein, Glucose, Calcium, Magnesium, Na, K, Cl, HCO3, BUN, creatinine, AST, ALT, LDH and uric acid
5. Comcomitant medications will not be captured in CRF. Available in Clinic Station.

Testing in outside labs are allowed and the PI or treating physician must review lab results, determine clinical significance for abnormal labs and sign/date the report.

End of Study Visit (Within 6 weeks of the last dose of decitabine)

- An interval history and physical exam including ECOG PS assessment and manual measurement (in cm) of the liver and spleen from the costal margin, if palpable.Record concomitant medications
- Record Adverse Events
- Vitals (temperature, HR, BP and RR)
- ECG
- Bone marrow aspirate and biopsy (A peripheral blood sample may be substituted in patients with more than 10% blasts in the peripheral blood)
- Laboratory Assessments
    - Hematology: CBC with differential, platelets

- Chemistry: Na, K, Cl, Mg, CO_2, BUN, Cr, CPK, glucose (random), Ca, Phosphorus, AST, ALT, Alk Phos, total bilirubin, LDH, total protein, albumin, uric acid
- Peripheral blood sample for scientific correlative studies: one red top tube (10ml) and two purple top tubes (10ml each).

Outside Physician Participation During Treatment

- 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.
- A letter ot the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (Appendix D)
- Protocol required evaluation 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.
- 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.
- A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.
- 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 hosptializations.
- The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
- Patients will return to MDACC every two to three months for evaluation.

10.0 TOXICITIES/SIDE EFFECTS

Adverse Event: An adverse event is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of the clinical trial, whether associated with drug or placebo and whether or not considered drug related. All of the following are to be considered adverse events:

- An exacerbation of a pre-existing condition
- An intercurrent illness
- Any drug interaction
- Any event related to a concomitant medication
- Development of an abnormal laboratory value or a significant change from baseline in a laboratory value within the range of normal, considered by the investigator to be clinically important
- An unexpected significant worsening of the cancer under treatment. Anticipated day-to-day fluctuations in the activity of the cancer or the anticipated progression of the cancer (other than death) should not be considered an adverse event.

Serious Adverse Event: A serious adverse event is one that is fatal or life-threatening (see below), is temporarily or permanently disabling, requires inpatient hospitalization (initial or

prolonged), or is associated with a congenital anomaly, a new cancer or a drug overdose (either accidental or intentional). In addition, any event suggesting a significant hazard, contraindication, side effect or precaution should also be considered serious. The MDACC criteria for adverse events in Leukemia will be used for reporting (Appendix E).

Life-threatening: means an immediate risk of death from the reaction as it occurred. Life-threatening does not include a reaction that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.
The following potential adverse reactions may occur: bone marrow suppression with risks of neutropenia, anemia, and thrombocytopenia. It is anticipated that there will be a risk of infection both from myelosuppression and immunosuppression. There is a risk for developing a secondary neoplasm such as acute leukemia or myelodysplastic syndrome (MDS). Specific side effects of the drugs given during treatment are listed below:

The main anticipated side effect of treatment with Decitabine is myelosuppression. This is both expected and acceptable in this clinical setting and will be managed with standard of care practice including appropriate anti-microbial prophyalxis for bacterial, fungal and viral infections. Red blood cell transfusions are acceptable for a hemoglobin <8g/dL or higher if the investigator believes the transfusion will ameliorate symptoms of anemia. Platelet transfusions are acceptable in cases of acute bleeding or routinely for a platelet count below 20 x 10^9/L.

Treatment with Ruxolitinib would also expect to induce myelosuppresion and the same standard practices listed above would apply. The clinical adverse event profile of Ruxolitinib as has been described in the published phase I/II would indicate minimal gastrointestinal and other non-hematological toxicity.

11.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Response assessment will follow the standard response assessment for AML defined by Cheson et al [12]:

Complete Response (CR)

- The subject must be free of all symptoms related to leukemia and have an absolute neutrophil count ≥ 1 x 10^9/L, no need for red blood cell transfusion, platelet count ≥ 100 x 10^9/L, and normal marrow differential (≤ 5 % blasts) in a normo- or hypercellular marrow

CRi

- As per CR but incomplete count recovery.

Partial Response (PR)

- CR with 6 – 25 % abnormal cells in the marrow or 50 % decrease in bone marrow blasts.

In addition, we will also use the response criteria recently published by Mascarenhas, et al [13]:

Table 4 - Response criteria for myeloproliferative neoplasm in blast phase (MPN-BP)[13]

Complete Molecular Response (CMR)

Description	Complete remission of both leukemia and MPN
Hematologic profile	ANC>1000 Hemoglobin>10g/dL Platelets >100 $X10^9$/L Absence of leukoerythroblastosis[1]
Spleen	Non-palpable
Bone Marrow	Celullarity appropriate for age Loss of abnormal morphology Blasts ≤5%[2] ≤ grade 1 marrow fibrosis
Cytogenetics	Normal karyotype[3]
Molecular markers	Loss of any previously documented markers associated with either the leukemic or MPN clone

Complete Cytogenetic Response (CCR)

Description	Complete remission of both leukemia and MPN
Hematologic profile	ANC>1000 Hemoglobin>10g/dL Platelets >100 $X10^9$/L Absence of leukoerythroblastosis[1]
Spleen	Non-palpable
Bone Marrow	Celullarity appropriate for age Loss of abnormal morphology Blasts ≤5%[2] ≤ grade 1 marrow fibrosis
Cytogenetics	Normal karyotype[3]

Molecular markers	Residual expression of MPN mutation (e.g. Jak2V617F)

Acute Leukemia Response- Complete (ALR-C)

Description	Complete remission of leukemia with residual MPN features
Hematologic profile	Absence of blasts [1]
Spleen	<25% increase in spleen size by palpation or imaging if baseline spleen <10cm or <50% if baseline spleen ≥ 10cm
Bone Marrow	Blasts ≤5%[2]
Cytogenetics	Loss of cytogenetic abnormality associated with leukemic clone, may have persistent abnormality associated with MPN
Molecular markers	Loss of previously identified markers in leukemic clone, may have persistent molecular markers associated with MPN

Acute Leukemia Response-Partial (ALR-P)

Description	Decrease in leukemic burden but without resolution of peripheral blood or bone marrow blasts and residual MPN features
Hematologic profile	>50% reduction in blasts
Spleen	<25% increase in spleen size by palpation or imaging if baseline spleen <10cm or <50% if baseline spleen ≥ 10cm
Bone Marrow	>50% reduction in blasts
Cytogenetics	No new abnormalities

Molecular markers	No new abnormalites

Stable Disease (SD)

Description	Failure to achieve at least ALR-P, but no evidence of progression for at least 8 weeks.

Progressive Disease (PD)

Description	Progression of leukemia and/or background MPN
Hematologic profile	For patients with 10-20% blasts: ≥50% increase to >20% blasts For patients with >20% blasts: ≥50% increase to > 30% blasts
Spleen	>25% increase in spleen size by palpation or imaging if baseline spleen <10cm and >50% if baseline spleen ≥ 10cm
Bone Marrow	For patients with 5-10% blasts: ≥50% increase to >10% blasts For patients with 10-20% blasts: ≥50% increase to >20% blasts For patients with >20% blasts: ≥50% increase to > 30% blasts

12.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may voluntarily withdraw from the study or be removed at the discretion of the investigator at any time. If such withdrawal occurs, or if the patient fails to return for visits, the investigator must determine the primary reason for a patient's premature withdrawal from the study and record this information on the appropriate eCRF. Patients may be withdrawn from the study prematurely for one of the following reasons:

- Adverse event(s)
- Abnormal laboratory value(s)
- Abnormal test procedure result(s)
- Protocol deviation
- Subject withdrew consent
- Lost to follow-up
- Administrative problems

- Death
- Initiation of new cancer therapy
- Disease progression
- The investigator determines that further therapy is not in the patient's best interest (e.g., due to non-compliance, toxicity etc.).

Patients who experience disease progression, start of new anti-cancer therapy, or withdraw consent, must be permanently discontinued from the study.

For patients who are lost to follow-up, the investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient, e.g., dates of telephone calls, registered letters, etc.

13.0 STATISTICAL DESIGN

Basis for sample size:

Phase I

First, a phase I study is performed to assess the safety of different dosing regimens for RUXOLITINIB combined with decitabine. Four combination dose levels are defined (table 1). A 3+3 design will be used to for dose escalation. Detailed dose escalation rules are described in the following section. A maximum of 18 evaluable patients will enroll in the phase I study.

Dose Escalation Procedures:

The dose of treatment agent will be escalated in successive cohorts of patients. The starting dose is at dose 0 as shown in table 1. Enroll 3 patients at the dose level and proceed to the next higher dose level with a cohort of 3 patients until at least 1 patient experiences a dose-limiting toxicity (DLT). If only 1 of 3 patients experiences a DLT at a given dose level, enter 3 additional patients at the current dose level. If only 1 of 6 patients experiences a DLT at a given dose level, proceed to the next higher dose level with a cohort of 3 patients. If at least 2 of 3 or 2 of up to 6 patients experience a DLT at a given dose level, then the MTD has been exceeded (stopping dose). Once the MTD has been exceeded, treat another 3 patients at the MTD if there were only 3 patients treated at that dose level. The MTD is defined as the highest dose level in which 6 patients were treated with at most 1 experiencing a DLT during the 1st cycle. One cycle of therapy is defined 5 days of decitabine and 28 days of RUXOLITINIB but treatment with RUXOLITINIB can be continued until the next cycle (start of decitabine) in the absence of toxicity, beyond the day 28 and without interruption.

Table 5 - Dose levels of decitabine and RUXOLITINIB

Dose level	Decitabine (mg/m^2/d x 5 days)	RUXOLITINIB (mg PO) (x 28 days)
0 (starting dose)	20	10 BID
1	20	15 BID
2	20	25 BID
3	20	50 BID

Phase II

Phase II part is a single arm study using the dosage level recommended from phase I. The patients treated at the MTD in phase I will not be included in phase II part, and the sample size for phase II is a maximum of 24 patients. The trial will be continuously monitored for efficacy and toxicity (non-hematological ≥grade 3 and hematological ≥grade 4). The method of Thall, Simon, and Estey will be used to perform interim efficacy and safety monitoring.

Efficacy and Toxicity Monitoring

The primary endpoint is the overall response (CR+CRi) after three cycles of treatment. Overall response (OR) and toxicity will be monitored simultaneously using the Bayesian approach of Thall, Simon, Estey (1995, 1996) and the extension by Thall and Sung (1998. The historical data suggested the overall response rate is 10% and the target overall response rate with the experimental treatment is 30%. This regimen of the combination treatment will be considered worthy of further investigation if it elicits an increase in OR to 20% with acceptable toxicity. A >20% toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions above, were constructed that meet the following two conditions,

$$\Pr(0.2 < \theta_{E,\,Toxicity} \mid \text{data}) > 0.80 \text{ and}$$

$$\Pr(\theta_{E,\,OR} > 0.3 \mid \text{data}) < 0.025.$$

where $\theta_{E,\,Toxicity}$ and $\theta_{E,\,OR}$ are the true toxicity and overall response for the combination treatment, respectively. The first rule provides for stopping the study if excessive toxicity is highly probable (i.e., probability >80.0%) for the combination treatment. The second condition will stop the study early if the data suggest that it is unlikely (i.e., probability < 2.5%) that OR rate of the combination treatment is 30%. Monitoring for toxicity and futility will not begin until 4 patients have been evaluated, and cohort size for future evaluations is 2.

The monitoring rule for the toxicity rate, based on these assumptions and monitoring conditions above is found in Table 7. For example, accrual will cease if 3 or more patients experience toxicities among the first 6 patients.

Table 7. Stop accrual if the number of toxicities is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	2-4	6-8	10-12	14-16	18-20	22	24
# patients with toxicities	2-4	3-8	4-12	5-16	6-20	7-22	Always stop with this many patients

Monitoring the OR rate, based on the above assumptions and monitoring conditions is found in Table 8. For example, accrual will cease if 0 or fewer patients experience a response in the first 8 patients treated.

Table 8. Stop accrual if the number with OR is less than or equal to indicated (i.e., # patients with OR) among the number of patients evaluated

# patients evaluated	2-6	8-12	14-18	20-22	24
# patients with OR	Never stop with this many patients	0	0-1	0-2	Always stop with this many patients

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 9). In order to utilize the software for the design, a 30% response rate and beta (0.6, 1.4) priors were assumed for the standard treatment response distribution and experimental treatment response prior distribution, respectively. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity constant rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true response rate of the combination treatment was not better than 30% and the true toxicity rate is 20% was 44%. Probabilities of stopping early for high true toxicity rates (i.e., 40%) were 10% when the true OR rate was 10% and 39% when true OR rate was 20%.

Table 9. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True OR	True Toxicity Rate	True Probability Vector (OR/Tox, OR/NoTox, NoOR/Tox, NoOR/NoTox)	Probability of Stopping	Average number of patients treated
10%	10%	(0.01, 0.09, 0.09, 0.81)	0.7689	14
10%	20%	(0.02, 0.08, 0.18, 0.72)	0.8437	12
10%	30%	(0.03, 0.07, 0.27, 0.63)	0.9325	9
10%	40%	(0.04, 0.06, 0.36, 0.54)	0.9827	7
20%	10%	(0.02, 0.18, 0.08, 0.72)	0.3679	19
20%	20%	(0.04, 0.16, 0.16, 0.64)	0.5724	15
20%	30%	(0.06, 0.14, 0.24, 0.56)	0.8153	11
20%	40%	(0.08, 0.12, 0.32, 0.48)	0.9526	7
30%	10%	(0.03, 0.27, 0.07, 0.63)	0.1676	21
30%	**20%**	**(0.06, 0.24, 0.14, 0.56)**	**0.4369**	**17**
30%	30%	(0.09, 0.21, 0.21, 0.49)	0.7568	12
30%	40%	(0.12, 0.18, 0.28, 0.42)	0.9379	8
40%	10%	(0.04, 0.36, 0.06, 0.54)	0.1036	22
40%	20%	(0.08, 0.32, 0.12, 0.48)	0.3936	18
40%	30%	(0.12, 0.28, 0.18, 0.42)	0.7381	12
40%	40%	(0.16, 0.24, 0.24, 0.36)	0.9328	8

Analysis method

Data analysis will be performed using SAS or S-plus, as appropriate. All patients who received at least 1 dose of the combined agents will be included in the intent-to-treat analysis for efficacy. Demographic and disease characteristics of the patients at registration will be summarized using descriptive statistics such as mean, standard deviation (SD), median and range. Overall response rates will be presented with 95% confidence intervals.

The association between response and patient and disease characteristics will be examined by two-sample t-test (or Wilcoxon rank-sum test) or Chi-square test.

The data from all patients who received the combined therapy during the study will be included for safety analysis. Safety data will include laboratory, physical exam, and adverse event reports on study patients. These descriptive summaries will be provided for all patients for each safety parameter by cycle, grade, and relationship to treatment.

14.0 PROTECTION OF HUMAN SUBJECTS

14.1 Serious Adverse Event (SAE) Reporting

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 events (SAE) will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug. The investigator will inform Incyte of any SAE or pregnancy within 24 hours of being aware of the event via email and/or fax. Respective companies will to be informed based on the attribution of a given event:

1. Due to RUXOLITINIB alone – These SAEs will only to be reported to INCYTE.
2. Due to both the RUXOLITINIB and decitabine – These SAEs will be reported to INCYTE
3. All pregnancies will be reported to INCYTE If the investigator can't associate causality with one or the other alone then causality needs to be associated with the combination of the products. These SAEs will go to INCYTE.

Adverse events will be documented in the Leukemia-specific AE Recording and Reporting Guidelines attached in Appendix E. medical record and entered into the case report form. PDMS/ CORE will be used as the electronic case report form for this protocol. All adverse events will be captured in CRF during Phase I portion of the study. In Phase II, only Grade 3 and higher related will be captured in the CRF. 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. SAEs must be documented using the MDACC IND eSAE electronic form.This form must be completed and supplied to Incyte within 24-hours/1-business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up MDACC IND eSAE reporting form.

15.0 CORRELATIVE STUDIES

After one cycle of therapy, after 3 cycles, and then every 3 cycles while on the study (in addition to baseline and end of study samples), peripheral blood will be collected for measurements of inflammatory cytokine levels, (including but not limited to IL6, TNF-alpha, CRP and IL10), expression of different proteins related to intracellular signaling, (including but not limted to STAT3, STAT5, and JAK2), and methylation of genes (including but not limited to p15, ER, and ECAD). This will include collection of: one red top (10ml) and two purple top tubes (10ml each). All samples will be submitted to the laboratory of Dr. Srdan Verstovsek at MD Anderson.

All research samples may not be drawn at all times, missed samples will not be considered a deviation.

16.0 CONCOMITANT MEDICATIONS

The use of standard agents deemed necessary for patient supportive care are permitted (antibiotics, allopurinol, GCSF). Only other chemotherapy agents or investigational agents are prohibited. Temporary prior measures to control blood counts, such as apheresis or hydrea are allowed.

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