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Protocol Page

EVALUATION OF RUXOLITINIB AND PRACINOSTAT COMBINATION AS A THERAPY FOR PATIENTS WITH MYELOFIBROSIS
2014-0445

Core Protocol Information

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(●) The Clinical Research Committee - (CRC)

Protocol Body

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EVALUATION OF RUXOLITINIB AND PRACINOSTAT COMBINATION AS A THERAPY FOR PATIENTS WITH MYELOFIBROSIS

1.0 HYPOTHESIS AND OBJECTIVES

Hypothesis

Ruxolitinib (also known as INCB018424), a JAK1/2 inhibitor, and pracinostat (a histone deacetylase inhibitor; HDACi) are effective and tolerable treatments for patients with myelofibrosis (MF). Combination of these agents in patients with MF can improve the overall clinical response to therapy without causing excessive toxicity.

Objectives

Primary:

- to determine the efficacy/clinical activity of the combination of ruxolitinib with pracinostat as therapy in patients with MF

Secondary:

- to determine the toxicity of the combination of ruxolitinib with pracinostat as therapy in patients with MF

Exploratory:

- to explore time to response and duration of response
- to explore changes in bone marrow fibrosis
- to explore changes in JAK2V617F (or other molecular marker) allele burden or changes in cytogenetic abnormalities

2.0 BACKGROUND AND RATIONALE

2.1 Myelofibrosis

Myelofibrosis (MF) is a rare clonal proliferative disorder of a pluripotent stem cell. This clone subsequently induces fibrogenic cytokines and/or growth factors in the marrow, which stimulate the deposition of extracellular matrix proteins by polyclonal fibroblasts. Megakaryocytic hyperplasia-dysplasia is frequently observed. Invasion of the blood stream and colonization of extramedullary sites ensues, resulting in organomegaly and splenomegaly. Extensive marrow fibrosis and osteosclerosis may be observed in advanced MF.

The entity of MF can be either idiopathic (primary) or representative of end-stage myeloproliferative diseases such as polycythemia vera (PV) or essential thrombocythemia (ET). MF occurs in about 15-25% of patients with PV and in 5-10% of patients with ET. In the early cellular phase of MF with minimal marrow fibrosis, the differential diagnosis includes Philadelphia-positive chronic myeloid leukemia (CML), PV, and ET that must be distinguished based on cytogenetics and clinicopathologic features. Other entities that can induce myelophthisis include myelodysplastic syndrome (MDS), metastatic malignancies, lymphoma, Hodgkin's disease, and plasma cell dyscrasias. MF must be differentiated from acute megakaryocytic leukemia (AML, M7

of the French-American-British classification) and MDS with fibrosis. In acute megakaryocytic leukemia patients usually present with severe constitutional symptoms and pancytopenia but without organomegaly or peripheral blood myelophthisis.

The clinical picture of MF involves constitutional symptoms (e.g., cachexia, night sweats, fatigue, fever), splenomegaly, anisopoikilocytosis with teardrop erythrocytes, progressive anemia, immature myeloid and erythroid precursors in the peripheral blood, elevated lactate dehydrogenase (LDH) levels, and fibrosis of the marrow (as evaluated by reticulin and trichome [collagen] stains). The leukoerythroblastic picture is postulated to be related to both the intramedullary sinusoidal marrow and splenic hematopoiesis.

The disease generally occurs in adults, with the median age of 65 years. In 40% of the patients, constitutional symptoms are present, including fever, weight loss, nocturnal sweating, pruritus, and bone pain. Splenomegaly is present in 85% of the patients at diagnosis and is massive in 10%. Hematologic disease features include anemia in 50% to 70% at diagnosis and 25% will have severe anemia with hemoglobin level < 8.0 g/dL. Approximately half of the patients present with an elevated white cell count (WBC), 28% with thrombocytosis (platelet count > 500 x 10^9/L), and 37% with thrombocytopenia (platelet count < 150 x 10^9/L). Circulating blast cells are present in one-third of the patients.

Complications of MF are varied. Thrombotic obliteration of intrahepatic veins and splenomegaly may lead to portal hypertension; severe cases may be associated with ascites and/or variceal bleeding. Left upper quadrant pain may herald splenic infarction; episodes are usually self-limited and may persist for several days. Supportive care measures such as analgesics and hydration are usually sufficient; refractory cases may require splenectomy or irradiation. Extramedullary hematopoiesis (EMH) may occur in locations other than the liver or spleen; involvement of such sites may be managed by low-dose irradiation. Liver involvement is associated with increased levels of plasma alkaline phosphatase. Clinical manifestations of EMH include cardiac tamponade, papular skin nodules, pleural effusions, and spinal cord compression.

Autoimmune phenomena have been observed, including Coomb's positive autoimmune hemolytic anemia, nephrotic syndrome, antinuclear antibodies, rheumatoid factor, and lupus-type anticoagulant. Postulated etiologies include clonality of the lymphoid population or activation by abnormal monocyte-macrophage interaction with the immune system.

Adverse prognostic factors for survival include older age and anemia (hemoglobin < 10 g/dL). The etiology for the latter finding is usually multifactorial and related both to marrow failure and hypersplenism. Poor prognosis has also been correlated with leukocytosis, leukopenia, circulating blasts, increased numbers of granulocyte precursors, thrombocytopenia, abnormal karyotype, and hypercatabolic symptoms. The course of the disease is highly variable. Median survival from time of diagnosis ranges from 5 to 6 years. Progressive marrow failure, transformation into acute myeloid leukemia, and portal hypertension lead to demise.

Several therapies are available for MF, however no one therapy has demonstrated an ability to produce sustained remissions. In addition, currently available therapies are often limited by their myelosuppressive effects. Ruxolitinib, a JAK1/2 inhibitor, produces symptom and spleen-size responses, but may result in anemia and/or thrombocytopenia in a significant proportion of patients. Hydroxyurea is another commonly used agent in the proliferative phases of the disease. Interferon-alpha had yielded hematologic responses and reductions in splenomegaly (definitions varying among studies) especially those with proliferative phase; however, this agent tends to be poorly tolerated. Agents used for the management of anemia include androgens and/or erythropoietin. Splenectomy and/or splenic irradiation have been used to manage symptomatic splenomegaly. Splenectomy has been associated with risk of leukemia transformation in some series, and splenic irradiation can result in severe myelosuppression. No medical therapy has been proven to prolong overall survival for these patients. Patients with an intact quality of life and no threatening hematologic abnormalities, such as erythrocytosis or thrombocytosis, have usually been considered to not require any therapy.

In vitro data suggests that cytokines elaborated by megakaryocytes stimulate human bone marrow fibroblasts to divide and secrete collagens. In patients with MF, increased levels of transforming growth factor-beta (TGF-beta) have been observed in circulating peripheral blood mononuclear cells (PBMC) of megakaryocytic lineage.

Increased levels of basic fibroblast growth factor (bFGF) have also been reported in patients with MF. Both TGF-beta and bFGF are members of multifunctional polypeptide families that regulate cell growth and differentiation. In addition to their potent fibrogenic activity, TGF-beta and bFGF regulate hematopoiesis by selective actions on primitive stem cells. Expression of TGF-beta in early CD34+ hematopoietic stem cells negatively regulated the cycle status; this effect could be abrogated by bFGF. In addition, bFGF has been shown to augment the activity of stem cell factor (SCF), interleukin-3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF), or erythropoietin on committed progenitor cells. Other cytokines/proteins that are dysregulated in MF include tumor necrosis factor-alpha (TNF-alpha) and angiogenic agents like vascular endothelial growth factor (VEGF).

2.2 Ruxolitinib

JAKs play an important role in signal transduction following cytokine and growth factor binding to their receptors. In addition, JAKs activate a number of downstream pathways implicated in the proliferation and survival of malignant cells including the STATs (signal transducers and activators of transcription), a family of important latent transcription factors. Aberrant activation of JAKs has been associated with increased malignant cell proliferation and survival in patients with Philadelphia chromosome negative MPD. The finding that peripheral blood from myeloproliferative disease (MPD) patients is capable of forming erythroid and megakaryocyte colonies in the absence of exogenous factors (which signal through JAKs) suggests that cells from these patients are intrinsically different than normal cells. Indeed, work from a number of laboratories led to the

identification of multiple somatic mutations in genes associated with cytokine and growth factor signaling. These include a mutation in the pseudo-kinase domain of JAK2V617F (amino acid 617, valine to phenylalanine) that results in constitutive activation of JAK2 and downstream STATs. This mutation was found in > 90% of all PV patients and in approximately 50% of all ET and MF patients. More recently, other mutations have been identified in MPD patients lacking the JAK2V617F mutation. For instance, additional activating mutations in JAK2, mutation in the thrombopoietin receptor (MPL), and calreticulin (CALR) mutations result in constitutive ligand-independent JAK-STAT activation. Importantly, ectopic expression of each of these mutant genes has been demonstrated to be sufficient to cause MPD-like syndromes in mice. Moreover, even in MPD patients lacking a confirmed JAK2 mutation, the detection of STAT activation suggests dysregulated JAK activity. In fact, regardless of the mutational status of JAK2, the malignant cells expectedly retain their responsiveness to JAK activating cytokines and/or growth factors; hence, they may benefit from JAK inhibition. These findings, in addition to the limited life span of these patients and lack of beneficial therapies for the treatment of PMF and post-PV/ET MF, clearly support the use of JAK inhibitor in these diseases. Ruxolitinib is a JAK1/2 inhibitor that is currently FDA approved for the treatment of patients with intermediate and high risk MF.

Ruxolitinib, has been shown to produce durable clinical benefits in terms of spleen volume reduction and improvement in constitutional symptoms with an acceptable toxicity profile[1,2]. Based on the results of two phase III studies (COMFORT-I, COMFORT-II)[3,4] showing improvement in spleen size, symptom control and improvement in quality of life, ruxolitinib was approved for use in patients with intermediate or high-risk myelofibrosis by the FDA. There is also evidence of a benefit in overall survival (OS) in patients treated with ruxolitinib. An OS advantage (HR=0.58; 95% CI, 0.39-0.85, p=0.005) in treated patients compared to historical controls has been reported[5]. In addition, an analysis of the COMFORT-I study confirmed that patients treated with ruxolitinib had an OS advantage compared with placebo (HR=0.58, 95% CI, 0.36-0.95, p-0.03) with a median of 102 weeks of follow up[6]. Follow-up analysis of the COMFORT-II trial has also demonstrated an OS advantage for patients treated with ruxolitinib[7].

2.3 Pracinostat

Abnormal epigenetic silencing of important regulatory genes has been described in cancer, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The reduction of acetylation status of histones is one mechanism for this silencing. Inhibitors of histone deacetylases (HDACs) have been extensively studied in both hematologic (‘liquid’) and solid tumors. Histone deacetylases are enzymes involved in the remodeling of chromatin, and therefore, have a key role in the epigenetic regulation of gene expression. There are currently two US Food and Drug Administration (FDA) approved HDAC inhibitors, one oral and one injectable, for the treatment of T-cell lymphoma.

The investigational agent pracinostat is a rationally designed, potent, oral, pan-HDAC (including Class I, II, and IV isoforms) inhibitor with predictable pharmacokinetic (PK) properties. The IC_{50}, across a broad range of human cancers in *in vitro* cytotoxicity assays, ranged from 0.1 to 1.5 µM, with the lowest values noted in leukemia and lymphoma cell lines. Synergistic interactions have been observed with multiple cytotoxic and targeted anti-cancer therapeutics; notably the combination index with azacitidine ranged from 0.44 to 0.55.

Clinical Experience

Pracinostat has been tested in more than 200 patients in multiple single agent and combination Phase I and pilot Phase II clinical trials and has been found to be generally well-tolerated at doses up to 60 mg given three times per week (e.g., Monday, Wednesday, Friday) for 3 weeks on an every-4-week schedule. The most common manageable side effects often associated with drugs of this class, including pracinostat, are fatigue, gastrointestinal (GI) effects (nausea, vomiting, diarrhea), and myelosuppression.

Pracinostat has demonstrated clinical evidence of both single-agent and combination clinical activity, including patients with advanced hematologic disorders. Data from nine patients in a pilot Phase II clinical trial of pracinostat in combination with azacitidine in heavily-treated patients with advanced MDS showed an encouraging overall response rate (ORR). This pilot study was conducted as an expansion cohort in the context of a Phase Ib trial of pracinostat in hematological malignancies to determine the efficacy and safety of the combination of pracinostat (60 mg orally every other day, 3 days a week, for 3 consecutive weeks) and azacitidine (75 mg/m^2 IV daily x 5 days every 3 to 6 weeks) given in 4-week cycles to patients with intermediate-2 (INT-2) or high risk MDS.

Baseline patient characteristics included the following: median age was 64 years (range, 22 to 73), white blood cells 2.4 x 10^9/dL (0.7 to 9.3), hemoglobin 10 g/dL (8.2 to 11), platelets 31 x 10^9/dL (14 to 269), and bone marrow blasts 7% (0% to 18%). Seven (78%) patients had therapy-related MDS with histories of prior chemotherapy/radiotherapy exposure (breast cancer [3], non-Hodgkin's lymphoma [2], breast and ovarian cancer [1], and melanoma [1]). Three patients had failed prior therapy: decitabine and haploidentical stem cell transplantation (N=1), lenalidomide (N=1), and decitabine and TXA-127 (N=1). Patients received a median of 4 cycles. All 9 patients were evaluable. The overall response rate (ORR; defined as complete remission [CR] + complete remission with incomplete blood count recovery [CRi] + partial response [PR]) was 8/9 (89%) and the CR+CRi rate was 7/9 (78%). Five (56%) patients achieved a complete cytogenetic response. Eight-week mortality was 0%. Only 1 (11%) patient died, and this was felt to be unrelated to study drug as it followed an allogeneic-stem cell transplantation. The median duration of response was 45 days (0 to 229), and the combination was generally well-tolerated. Reasons for discontinuation were: transition to allogeneic-stem cell transplantation (N=5), no pracinostat availability by Sponsor (N=2), no response (N=1), and progression to AML (N=1). All toxicities were Grade 1 or 2, with the most frequent being fatigue and nausea (56% each). Based on this data, Pracinostat is currently being tested in several Phase 2

clinical trials in combination with azacitidine and decitabine for patients with advanced hematologic malignancies.

Experience with pracinostat in MF is limited to one small study of 22 patients. Of them, 18 (82%) carried the JAK2V617F mutation, with a median baseline allele burden of 59.66% (range 19.85–94.95%). Seven (31%) patients had abnormal cytogenetics at study entry, including 2 patients who had a complex karyotype. Most patients (n = 20) were symptomatic at study entry with a performance status of 0 (n = 1 [5%]), 1 (n = 16 [73%]), or 2 (n = 5 [22%]). The majority of patients entered the study having splenomegaly (n = 21 [95%]), with a median spleen size of 13 cm beneath the left costal margin (range 0–29 cm). Median follow-up was 5 months (range 1–11.3 months). Overall, 8 (36%) patients experienced clinical benefit from pracinostat therapy. Although when analyzed as a whole, the cohort of patients did not experience a significant reduction in spleen size, 6 (27%) patients experienced a reduction in splenomegaly (median reduction 3 cm, range 1–4 cm) (Fig. 1). However, no patient had a response that could be classified as splenomegaly clinical improvement by IWG criteria (i.e. spleen reduction of at least 50% from baseline). When only responders were considered, the reductions in spleen size from baseline were significant (p = 0.02). Two patients had anemia clinical improvement by IWG criteria, experiencing increments in their hemoglobin level from 9.1 g/dL at baseline to 11.1 g/dL and from 7.9 g/dL to 15.3 g/dL at last follow-up, respectively. Five (50%) of 10 patients with hepatomegaly reduced their liver size by a median of 3 cm (range 1–6 cm). In summary, although a significant number of patients exhibited signs of clinical activity to pracinostat, only 2 patients achieved a response according to IWG criteria (2 anemia responses as described). While all patients started pracinostat at 60 mg daily, 8 of them required at least one dose reduction to 50 mg and 1 patient required two dose reductions to 40 mg daily. Conversely, 6 patients had their dose of pracinostat increased to 80 mg daily due to lack of efficacy. Eight patients continued therapy at 60 mg daily. The most frequent side effect associated to pracinostat therapy was fatigue, which occurred in 20 (91%) patients, which was grade 1 in 17 and grade 2 in 3 patients. Other toxicities included pain (n = 5), peripheral edema (n = 4), and diarrhea (n = 3), all grade 1. Rates of grade 3–4 neutropenia, anemia, and thrombocytopenia were 13%, 0%, and 21%, respectively. No patient had died during the conduct of the study and 1 remained on the study receiving pracinostat at 50 mg daily. Twenty-one patients were off study due to lack of response (n = 9), disease progression (n = 6), patient's request (n = 2), unrelated medical problems (n = 3: surgery for aortic aneurism, prostate cancer, infection) and fatigue (n = 1).

2.4 Rationale for combining ruxolitinib and pracinostat as therapy for MF

The rationale for the use of histone modifiers in MF relies on the fact that both JAK2 as well as JAK2V617F can translocate to the cell nucleus and phosphorylate specific lysine residues at histone H3 (H3Y41). The affinity of the transcriptional repressor heterochromatin protein 1α (HP1α) for histone H3 depends on the phosphorylation status of H3Y41. Phosphorylation of H3Y41 decreases the affinity of H3 to HP1α. JAK2 inhibitors such as TG101209 or AT9283 abrogate nuclear H3Y41 phosphorylation,

which increases chromatin-bound HP1α in cells, thus leading to repression of HP1α-regulated genes such as lmo2. Of note, lmo2 is involved in leukemogenesis. Thus, the JAK2-H3Y41-HP1α pathway links JAK2 kinase activity to histone modifications, aberrant gene expression and leukemogenesis, thus providing the rationale for the use of agents that target histone modifications in combination with JAK2 inhibitors to more thoroughly suppress JAK2V617F signaling in JAK2-driven malignancies. Clinically, then, one might expect synergism of the two medication in terms of improving further the number of patients with clinically relevant reduction in organomegaly and MF-related systemic symptoms, possibly for a longer duration of time. In addition, it is possible that addition of an HDACi would bring additional benefits to patients, like improvement in blood cell count or improvement in bone marrow fibrosis.

3.0 BACKGROUND DRUG INFORMATION

3.1 Ruxolitinib (INCB018424) (Refer to Investigator's Brochure)

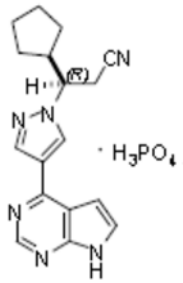
INCB018424 phosphate, referred to herein as INCB018424, is a substituted pyrrolopyrimidine compound that acts as a potent and selective inhibitor of the Janus kinase family of enzymes. INCB018424 is a novel, potent, and selective inhibitor of the JAKs with modest selectivity for JAK2. INCB018424 potently (IC50 values < 5 nM) inhibits JAKs, yet it does not significantly inhibit (<30% inhibition) a broad panel of 26 other kinases when tested at 200 nM (approximately 100 times the average IC50 value for JAK enzyme inhibition). Moreover, in cell-based assays relevant to the pathogenesis of MPDs, such as JAK-STAT signaling and the growth of cytokine-dependent lines, INCB018424 demonstrated excellent potency (IC50 values of 80-141 nM). This effect was not due to general cytotoxicity, because INCB018424 (up to 25 μM) had no significant effect on the growth of cytokine-independent cell lines transformed by the Bcr-Abl oncogene. In addition, INCB018424 inhibited JAK/STAT signaling and growth of a cell line expressing the JAK2 mutant variant (JAK2V617F) that has been implicated in the pathogenesis of the majority of Philadelphia chromosome negative MPD. Additional details as to the *in vitro* pharmacology of INCB018424 may be found in the Clinical Investigator's Brochure (CIB).

INCB018424 was evaluated in two mouse models where either a cytokine-dependent multiple myeloma cell line, INA-6, or a cell line, BaF3, engineered to express JAK2V617F was inoculated. The ability of INCB018424 to inhibit JAK pathway signaling as well as tumor cell survival and growth was assessed *in vivo*. *In vitro* cell biology experiments have demonstrated that the potency of INCB018424 is very similar between the cytokine-dependent INA-6 myeloma cells, with wild type JAKs, and the BaF3 cells expressing a clinically relevant mutant JAK2. As such, the *in vivo* studies described herein characterize the ability of INCB018424 to inhibit wildtype JAK2 (using the INA-6 xenograft model) and MPD-related mutant JAK2 (using a mouse model of splenomegaly driven by cells expressing the mutant JAK2V617F).

Treatment of mice with orally administered INCB018424 resulted in a dose-dependent suppression of STAT3 phosphorylation and tumor growth in the cytokine-dependent INA-6 xenograft model at doses ≥ 10 mg/kg BID. Moreover, oral administration of INCB018424 inhibited the dramatic splenomegaly in mice resulting from intravenous inoculation of the BaF3-JAK2V617F cells. Additional details as to the *in vivo* pharmacology of INCB018424 may be found in the Clinical Investigator's Brochure (CIB). In summary, pharmacological data obtained in both *in vitro* and *in vivo* model systems support the potential utility of orally administered INCB018424 in the treatment of malignancies, including MPD such as PMF and Post-PV/ET MF.

The chemical name of INCB018424 phosphate is (*R*)-3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate (Figure 1). INCB018424 phosphate has a molecular formula of C17H21N6O4P and a molecular weight of 404.36. INCB018424 phosphate drug substance is a white to off-white powder, and is referred to herein as ruxolitinib.

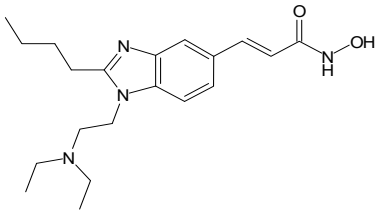
INCB018424 Phosphate Structural Formula



Ruxolitinib will be provided upon insurance approval through commercial supply.

3.2 Pracinostat (Refer to Investigator's Brochure)

Investigational Product



Structural formula: (free base)
Chemical name: (2*E*)-3-{2-butyl-1-[2-(diethylamino)ethyl]-1*H*-benzimidazol-5-yl}-*N*-hydroxyacrylamide hydrochloride
Common names: Pracinostat, SB939
Pharmacologic class: histone deacetylase (HDAC) inhibitor
Molecular mass: 358.38 (free base)
Salt Form: Hydrochloride
Molecular formula: $C_{20}H_{30}N_4O_2$•2HCl

Results from pharmacology studies showed that pracinostat inhibits predominantly Class I and II HDACs (K_i=16 to 240 nM). It effectively inhibits acetylation of histones and various other target proteins in a variety of human tumor cell lines at the same concentrations as it inhibits proliferation and promotes apoptosis (IC_{50}=0.1 to 1.5 µM). Lymphomas and tumors of hematological origin show the highest sensitivity.

The antitumor activity of orally administered pracinostat has been demonstrated in several xenograft mouse models of solid and hematological malignancies including colorectal cancer, ovarian cancer, prostate cancer, AML, and B cell lymphoma. In addition, antitumor activity has been shown in a transgenic murine model of early colorectal cancer. In all of these *in vivo* models, pracinostat was well tolerated (<20% body weight loss) at doses of 100 mg/kg for up to 21 consecutive days and demonstrated dose-dependent antitumor effects (statistically significant reductions in tumor growth compared with vehicle control). As observed in the *in vitro* cell studies, hematological malignancies are most sensitive to the anti-tumor effects of pracinostat.

Overall, results from nonclinical studies provided appropriate justification for the safety and efficacy of pracinostat in the proposed patient populations.

Drug Formulation, Availability, Administration and Toxicity Information

Study Medication

Investigational Product	Dosage Form and Strength	Manufacturer
Pracinostat	45 mg and 60 mg capsules	KP Pharmaceutical Technology, Inc.

Study Medication Labeling, Packaging, and Supply

Pracinostat will be supplied by Helsinn Healthcare SA in 2 strengths: 45 mg and 60 mg. Each 45 mg and 60 mg capsule contains 45 mg or 60 mg of pracinostat (as free base), respectively, and the following inactive ingredients: microcrystalline cellulose, USP/NF (Avicel PH102) and magnesium stearate, USP/NF. Pracinostat capsule size 1: 45 mg are flesh, opaque, hard gelatin capsules; 60 mg are Swedish orange and white, opaque, hard gelatin capsules.

All study medications must be kept in a secure place under appropriate storage conditions. Capsules are packaged in blister packs. Pracinostat capsules should be stored at controlled room temperature, 15°C to 30°C (59°F to 86°F).

Precautions and Risks Associated with the Study Medication Pracinostat

Hematologic: Treatment with pracinostat has been associated with cytopenias including anemia, thrombocytopenia, and neutropenia. The incidence of cytopenias may

increase with increasing doses of pracinostat. Subjects participating in clinical trials of pracinostat should be monitored closely for adverse hematologic affects. The effect of dose reduction on the amelioration of cytopenias has not been established; however prudent clinical management, including supportive care (appropriate use of transfusions and/or hematopoietic growth factors) and dose modification or discontinuation should be followed (see Section 5.1).

Gastrointestinal: Gastrointestinal disturbances, including nausea, vomiting, and diarrhea, have been reported in subjects treated with pracinostat. Standard antiemetic and antidiarrheal medications and appropriate supportive care should be used per normal clinical practice in subjects participating in clinical trials of pracinostat. Pre-existing nausea, vomiting, and diarrhea should be adequately controlled before beginning therapy.

QTc Prolongation/Cardiac: Asymptomatic QTc interval prolongation has been observed during clinical trials of pracinostat. Subjects with a prolonged QTc interval at Screening will not be permitted to participate in clinical trials of pracinostat. Hypokalemia or hypomagnesemia should be corrected prior to pracinostat administration and consideration should be given to monitoring potassium and magnesium in symptomatic subjects (e.g., subjects with nausea, vomiting, diarrhea, fluid imbalance, or cardiac symptoms).

General Signs and Symptoms: Fatigue has been the most common AE reported in subjects receiving pracinostat and has frequently prompted dose reduction in subjects receiving >60 mg/day. Anorexia has also been commonly reported. Subjects experiencing debilitating fatigue or anorexia with weight loss may benefit from dose reduction or interruption of pracinostat treatment.

Please refer to the IB for detailed information on the risks associated with the use of pracinostat.

4.0 PATIENT ELIGIBILITY CRITERIA

Inclusion criteria

1. Diagnosis of MF (either primary or post essential thrombocythemia/polycythemia vera) requiring therapy, including those previously treated and relapsed or refractory, or if newly diagnosed, with intermediate-1 or -2 or high risk according to International Prognostic Scoring System (IPSS).
2. Palpable splenomegaly of more than or equal to 5 cm below left costal margin on physical exam
3. Understanding and voluntary signing an IRB-approved informed consent form.
4. Age ≥ 18 years at the time of signing the informed consent.
5. Disease-free of other malignancies.
6. ECOG performance status 0 to 2.

7. Negative pregnancy test in females of childbearing potential (FCBP)† Male patients with female partners of child-bearing potential and female patients of childbearing potential are required to use two forms of acceptable contraception, including one barrier method, during their participation in the study and for 30 days following last dose. Acceptable forms of contraception include 1 highly effective method such as an intrauterine device (IUD), hormonal (birth control pills, injections, or implants), tubal ligation, or partner's vasectomy and at least 1 additional approved barrier method such as a latex condom, diaphragm, or cervical cap. Female patients of childbearing potential must not be breast-feeding or planning to breast feed and must have a negative pregnancy test ≤7 days before first study treatment.
8. QTcF interval ≤470 msec
9. Normal serum potassium and magnesium levels
10. Adequate organ function as demonstrated by the following:
    - Direct bilirubin $\leq$ 2.0 mg/dL
    - Serum creatinine $\leq$ 2.0 mg/dL.
    - SGPT $\leq$ 3 x upper limit of normal (unless considered to be related to MF or patient has known history of Gilberts)
11. Platelets >/= 50 x 10^9/L
12. ANC >/= 1 x 10^9/L

Exclusion Criteria

1. Prior therapy with a JAK inhibitor (other than ruxolitinib for less than 3 months duration and currently on it) or HDACi. Patients that are currently on ruxolitinib for less than 3 months of therapy are eligible.
2. Use of any other standard or experimental therapy within 14 days of starting study therapy.
3. Lack of recovery from all toxicity from previous therapy to grade 1 or baseline.
4. Suspected pregnancy, pregnant or lactating females.
5. Any condition, including the presence of laboratory abnormalities, which in the opinion of the treating physician places the subject at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study.
6. Known positive for HIV or infectious hepatitis, type A, B or C.
7. Patients with gastrointestinal (GI) tract disease, causing the inability to take oral medication, malabsorption syndrome, a requirement for intravenous (IV) alimentation, prior surgical procedures affecting absorption, uncontrolled inflammatory GI disease (e.g., Crohn's disease, ulcerative colitis)
8. Cardiopulmonary function criteria:
    - Current unstable arrhythmia requiring treatment
    - History of symptomatic congestive heart failure (New York Heart Association [NYHA] Class III or IV)
    - History of myocardial infarction within 6 months of enrollment

† A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

- Current unstable angina
- Family history of long QT syndrome

5.0 TREATMENT PLAN

On this study 4 weeks of therapy is considered one cycle of therapy. Ruxolitinib and pracinostat will be given orally in an outpatient setting. Ruxolitinib will be given continuously daily in 28-day cycles. Patients will receive ruxolitinib alone for the first 3 months, and then pracinostat will be added. Ruxolitinib provides maximum benefit usually within 3 months, therefore we add another agent to ruxolitinib after those initial 3 months, in order not to compromise benefit of ruxolitinib with some new side effect from additional medication (pracinostat in this case). Starting dose of ruxolitinib will be based on patients' platelet count, as per standard practice: 15 mg twice a day (BID) for patients with baseline platelet counts of 100–200 × 10^9/L and 20 mg BID for those with baseline platelet counts >200 × 10^9/L. For patients with platelet counts of 50–99 × 10^9/L, gradual up-titration from a starting dose of 5 mg BID is recommended. Dose of ruxolitinib may be increased or decreased at the discretion of the treating physician prior to initiation of pracinostat (see section 5.1). Patients who started ruxolitinib before enrollment into this study, will have the time they have been on ruxolitinib therapy counted toward the 3 cycles of ruxolitinib monotherapy, as per protocol. When a dosage change of the Ruxolitinib is indicated (except for holding therapy due to toxicity) patients will continue taking their previously prescribed dosing level until the new supply of Ruxolitinib is available for use by the patient.

Pracinostat is supplied as 45 and 60 mg capsules and will be self-administered (by the patient) one-time each day, 3 days a week for 3 weeks with doses approximately 48 hours apart, followed by one week of rest. Study medication will be taken approximately 30 minutes before a meal (e.g. breakfast) or $\geq$2 hours after a meal at approximately the same time each of the 3 days weekly (e.g., Monday, Wednesday, and Friday). Treatment cycles will be repeated every 28 days, unless delayed due to toxicity. Pracinostat should be taken with a glass of water; pracinostat is not to be taken with grapefruit or any other juice. Patients should be instructed to swallow the capsules whole and to not chew or crush them. If vomiting occurs, no attempt should be made to replace the vomited dose. Nicotine may alter metabolism of pracinostat such that potential efficacy may be decreased and therefore we will encourage smoking cessation while on study.

If the patient forgets to take his/her dose before noon, then the dose should be withheld that day and restarted the next dosing day in order to ensure approximately 48 hours between each dose. For example, if a patient misses a dose on Monday, the dose can be restarted on Tuesday, and the other doses would be taken on Thursday and Saturday.

Dosage of either drug for cycles can be reduced, increased or delayed based on the assessment of efficacy (lack of it) or due to adverse events, if any occur. Guidelines for reducing or increasing dosages are described in Section 5.1. Delays of a maximum of 8 weeks are allowed from scheduled next cycle.

Subjects will be asked to maintain a diary to record drug administration. Subjects will be asked to bring any unused study drug to the research center at their next visit. Research personnel will count and record the number of used and unused study drug capsules/tablets at each visit and reconcile with the subject diary. The study coordinator will question the patient regarding adherence to the dosing regimen, record the number of capsules/tablets and strengths returned, the date returned and determine treatment compliance before dispensing new medication to the study patient. Compliance below 80% will require counseling of the patient by study site personnel. Attempt will be made to provide an adequate treatment period of at least 6 months unless significant toxicity observed, to account for delayed time to response observed with biologic agents. Patients experiencing clinical benefit will continue therapy for 4 years unless progression of disease or toxicity warranting discontinuation of therapy is observed.

5.1 Dose Modification

Non-hematologic toxicity:
If drug-related grade 3 or 4 non-hematologic toxicity is attributable to one or both of the drugs, dose interruption of the drug(s) causing the toxicity, as assessed by the treating physician, is mandatory. Patient who experience grade 3 drug related non-hematological toxicity may be given a subsequent course one dose level below the previous course, but the patient must have recovered to grade </=1 or baseline before institution of the next course. If a patient has drug-related grade 4 non-hematologic toxicity, he/she may receive a subsequent courses at one reduced dose level after resolution of toxicity to grade </=1, only if approved by the Principal Investigator based on the clinical significance of the toxicity and only if patient has had derived a benefit from the therapy. The dose of therapeutic agents can be decreased at the discretion of treating physician, for chronic grade 2 non-hematologic toxicity. In specific circumstances, after discussion with the Principal Investigator, one of the two medications can be discontinued for safety. Documentation of the reason(s) study drug is discontinued is required. Other dose modifications may be considered as clinically indicated with documentation and approval of the PI.

Hematologic toxicity:
Ruxolitinib may be administered and dose adjusted as per standard practice (see Tables below). Reductions in dosing for therapy related anemia can be done at the discretion of the investigator

Ruxolitinib dose modifications recommended for patients with starting platelet count of at least 100 × 10^9/L*

	Dose at time of decline in platelet count					**Maximum dose based on platelet count after prior treatment interruption or dose reduction**
	25 mg BID	**20 mg BID**	**15 mg BID**	**10 mg BID**	**5 mg BID**	
Current platelet count	**New dose to be used**					

≥125 × 10^9/L	No change	No change	No change	No change	No change	20 mg BID
100 to <125 × 10^9/L	20 mg BID	15 mg BID	No change	No change	No change	15 mg BID
75 to <100 × 10^9/L	10 mg BID	10 mg BID	10 mg BID	No change	No change	10 mg BID for 2 weeks; if stable, may increase to 15 mg BID
50 to <75 × 10^9/L	5 mg BID	5 mg BID	5 mg BID	5 mg BID	No change	5 mg BID for 2 weeks; if stable, may increase to 10 mg BID
<50 × 10^9/L	Hold	Hold	Hold	Hold	Hold	Continue holding

* Starting ruxolitinib doses of 15 mg BID for patients with platelet counts of 100 to 200 × 10^9/L and 20 mg BID for those with a platelet count >200 × 10^9/L. Recommended dose modifications based on US prescribing information.
For insufficient response, doses may be increased in 5-mg BID increments to a maximum of 25 mg BID, provided that platelet and neutrophil counts are adequate.
BID: Twice daily

Ruxolitinib dose modifications recommended for patients with a starting platelet count of at least 50 × 10^9/L but less than 100 × 10^9/L*

Current platelet count	**Dosing recommendation**
<25 × 10^9/L	Interrupt treatment
25 to <35 × 10^9/L AND <20% decrease during the prior 4 weeks	Decrease dose by 5 mg QD or maintain the current dose if it is 5 mg QD
25 to <35 × 10^9/L AND ≥20% decrease during prior 4 weeks	Decrease dose by 5 mg BID or use 5 mg QD if the current dose is 5 mg BID or QD
≥40 × 10^9/L AND ≤20% decrease during prior 4 weeks, ANC >1 × 10^9/L, AND no dose reductions or treatment interruptions for AE or hematologic toxicity during the prior 4 weeks	Increase dose by increments of 5 mg QD to a maximum of 10 mg BID if response is insufficient

* Starting ruxolitinib dose of 5 mg BID. Recommended dose modifications based on US prescribing information.
See full prescribing information for a complete description of US Food and Drug Administration FDA-approved dosing of ruxolitinib in patients with intermediate or high-risk MF.
AE: Adverse event; ANC: Absolute neutrophil count; BID: Twice daily; QD: Once daily.

While a patient is on both medications concurrently, dosing of pracinostat as well as ruxolitinib will be discontinued for platelet counts </= 25X 10^9/L OR for ANC </= 0.5 x10^9/L. Patients who experience platelet count </= 25X 10^9/L or ANC </= 0.5 x10^9/L

Dose Level	Pracinostat
Level 0	60 mg
Dose Level -1	45 mg

during a cycle of therapy, will have a dose reduction of pracinostat by one dose level for subsequent cycles (see Table below).

5.2 Initiation of a new cycle of pracinostat and ruxolitinib

A new course of pracinostat may begin on the scheduled Day 1 of a new cycle if:

- The ANC is >/= 0.5 X 10^9/L
- The platelet count is > 25x 10^9/L,
- Any drug-related grade 3-4 adverse event that may have occurred has resolved to ≤ grade 1 severity or baseline
- Normal potassium and magnesium serum levels (hypokalemia or hypomagnesemia should be corrected prior to pracinostat administration)
- QTcF interval <500 msec (reversible causes of QTc prolongation should be corrected, e.g. hypomagnesemia and hypokalemia, and ECG repeated. Provided that the patient is asymptomatic and that QTc decreases below 500 msec, pracinostat administration may continue).

If these conditions are not met on Day 1 of a new cycle, the subject will continue ruxolitinib alone (see guidelines for restarting/continuing ruxolitinib in the next paragraph) and be evaluated weekly and a new cycle of pracinostat will not be initiated until the toxicity has resolved as described above. If pracinostat dosing was interrupted during the previous cycle or if the new cycle is delayed due to toxicity newly encountered on the scheduled Day 1, then the new cycle of pracinostat will be started when the criteria above are met AND with at least a one-level dose reduction.

Treatment with ruxolitinib may restart/continue at 5mg QD so long as ANC> 0.5x10^9/L AND platelets > 25x10^9/L (with approval of Principal Investigator, and documented) or at 5mg BID so long as ANC>/= 1 x10^9/L AND platelets > 50x10^9/L while waiting to initiate a new cycle of pracinostat. If the toxicity has not resolved as described above within 8 weeks of the scheduled start date for the new cycle of pracinostat, the patient may discontinue treatment with pracinostat and continue therapy with ruxolitinib alone. In unusual circumstances this rule may be modified by the Principal Investigator if felt to be in the best interest of the patient (for example, responding patient with neutropenia or low platelets, that resolve past 8 weeks may be restarted on therapy); such case must be fully documented.

5.3 Concomitant Therapy

Supportive measures consistent with optimal patient care may be given throughout the study. Concomitant medications will be captured in the medical record and will not be recorded on the case report form.

- Therapies considered necessary for the patient's well-being may be administered at the discretion of the investigator. These therapies include, but are not limited to antibiotics, analgesics, antihistamines, or other medications. Guidance for growth factors and transfusions of red blood cells, platelets, or fresh frozen plasma are described below:
- Packed red blood cells (RBCs): Transfusions of 2 units of packed RBCs should be considered for a decline in hemoglobin to <8 g/dl or symptoms of cardiovascular compromise. The following transfusion thresholds and guidelines are recommended for study participants:
    - hemoglobin <8 g/dl, 2 units of packed RBCs; hemoglobin <7, three units of packed RBCs
    - Platelets: Six to 10 units of random donor platelets or one cytopheresis unit of single donor platelets should be administered to all subjects with signs of hemostatic failure (i.e., bleeding or petechiae) or life-threatening thrombocytopenia (i.e., platelet count < 10,000/μ L)
    - Growth factor use with epoetin, darbepoetin, or granulocyte colony-stimulating factor must be terminated at least 14 days before initiation of study treatment and may not be used as part of supportive therapy while the patient is on study, except if in patients' best interest after approval of PI (e.g. neutropenic fever)
    - Patients should not receive androgens or steroids for treatment of their anemia or thrombocytopenia as part of supportive therapy
        - Patients may receive hydrocortisone prophylactically to prevent transfusion reactions but chronic systemic steroid (above 5mg/day) are not allowed.
- Over-the-counter products such as herbal preparations or dietary supplements with the exception of multivitamins, Ensure, and similar nutritional supplements are not allowed throughout the trial. Patients should stop using these herbal medications at least 7 days prior to the first dose of study medication.

Prohibited medications

Drug interaction studies with pracinostat are ongoing. Medications that have possible risk of prolonging the QT interval and/or Torsades de Pointes (listed below) may have an unacceptable risk of co-administration with pracinostat and are prohibited during . courses in which pracinostat is administered. The following website was used as a guide: http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm#. All concomitant medications that the subject is receiving both prior to enrollment and during study

conduct must be carefully reviewed and monitored. Subjects should avoid grapefruit juice and Seville oranges.

Prohibited drugs: Drugs that Have a Risk of Prolonging the QT Interval and/or Inducing Torsades de Pointes

Albuterol (salbutamol)
Alfuzosin
Amantadine
Amiodarone
Amisulpride
Amitriptyline
Amoxapine
Amphetamine
Anagrelide
Apomorphine
Arformoterol
Aripiprazole
Arsenic trioxide
Astemizole (Off US mkt)
Atazanavir
Atomoxetine
Azithromycin
Bedaquiline
Bepridil (Off US mkt)
Bortezomib
Bosutinib
Chloral hydrate
Chloroquine
Chlorpromazine
Ciprofloxacin
Cisapride (Off US mkt)
Citalopram
Clarithromycin
Clomipramine
Clozapine
Cocaine
Crizotinib
Dabrafenib
Dasatinib
Desipramine
Dexmedetomidine
Dexmethylphenidate
Dextroamphetamine (d-Amphetamine)
Dihydroartemisinin+piperaquine
Diphenhydramine (doses up to 25 mg per day allowed)
Disopyramide
Dobutamine
Dofetilide
Dolasetron

Domperidone
Dopamine
Doxepin
Dronedarone
Droperidol
Ephedrine
Epinephrine (Adrenaline)
Eribulin
Erythromycin
Escitalopram
Famotidine
Felbamate
Fenfluramine
Fingolimod
Flecainide
Fluconazole
Fluoxetine
Formoterol
Foscarnet
Fosphenytoin
Furosemide (Frusemide)
Galantamine
Gatifloxacin
Gemifloxacin
Granisetron
Grepafloxacin
Halofantrine
Haloperidol
Hydrochlorothiazide
Ibutilide
Iloperidone
Imipramine (melipramine)
Indapamide
Isoproterenol
Isradipine
Itraconazole
Ivabradine
Ketoconazole
Lapatinib
Levalbuterol (levsalbutamol)
Levofloxacin
Levomethadyl
Lisdexamfetamine
Lithium
Mesoridazine

Metaproterenol
Methadone
Methamphetamine (methamfetamine)
Methylphenidate
Metronidazole
Midodrine
Mifepristone
Mirabegron
Mirtazapine
Moexipril/HCTZ
Moxifloxacin
Nelfinavir
Nicardipine
Nilotinib
Norepinephrine (noradrenaline)
Norfloxacin
Nortriptyline
Ofloxacin
Olanzapine
Ondansetron
Oxytocin
Paliperidone
Pantoprazole
Paroxetine
Pasireotide
Pazopanib
Pentamidine
Perflutren lipid microspheres
Phentermine
Phenylephrine
Phenylpropanolamine
Pimozide
Pipamperone
Posaconazole
Probucol
Procainamide
Promethazine
Protriptyline
Pseudoephedrine
Quetiapine
Quinidine
Quinine sulfate
Ranolazine
Rilpivirine
Risperidone

Ritodrine
Ritonavir
Roxithromycin
Salmeterol
Saquinavir
Sertindole
Sertraline
Sevoflurane
Sibutramine
Solifenacin
Sorafenib
Sotalol
Sparfloxacin
Sulpiride
Sunitinib
Tacrolimus
Tamoxifen
Telaprevir
Telavancin
Telithromycin
Terbutaline
Terfenadine
Tetrabenazine
Thioridazine
Tizanidine
Tolterodine
Toremifene
Trazodone
Trimethoprim-Sulfa
Trimipramine
Vandetanib
Vardenafil
Vemurafenib
Venlafaxine
Voriconazole
Vorinostat
Ziprasidone

5.4 Correlative studies

Before initiation of therapy with pracinostat (C4D1), after one cycle of pracinostat therapy (C5D1), after 3 cycles (C7D1), and then every 3 cycles (C10, C13, C16,…) while on the study, peripheral blood will be collected if possible 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). Bone marrow aspirate will be collected every 6 cycles if possible (5ml purple top tube).

5.5 Pretreatment Evaluation

- History and physical exam including vital signs, body weight and height, and spleen and liver measurements. This also includes a transfusion history for 3 months prior to day 1 and concomitant medication notation (within 14 days of study day 1)
- CBC and differential, electrolytes (Na, K, Cl, Mg, HCO3), creatinine, uric acid, BUN, glucose, total and direct bilirubin, and SGPT, PT/PTT, INR (within 14 days of study day 1).
- Bone marrow biopsy and aspirate (within 1 month of study day 1, or within 3 months of study day 1 if patient was not on any therapy).
- ECG (within 14 days of study day 1)
- Pregnancy test (for FCBP only) within 7 days of study day 1
- Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) questionnaire

5.6 Evaluation During Study

- Directed history and physical examination including vital signs, body weight, and spleen and liver measurements once each cycle on cycles 2-7, then once every 3 cycles (C10, C13, C16, …). This also includes a transfusion history for previous cycle of therapy and concomitant medication notation. Spleen and liver measurements will be obtained by the physicians or physician assistants during the clinic visit and documented in the medical record.
- CBC and differential, electrolytes (Na, K, Cl, Mg, HCO3), creatinine, BUN, glucose, total bilirubin, and SGPT every 2 weeks for 6 cycles, then CBC with differential every cycle and electrolytes (Na, K, Cl, Mg, HCO3), creatinine, BUN, glucose, total bilirubin, and SGPT every 3 cycles and as deemed appropriate while on therapy. Therapy also includes the first 3 months of ruxolitinib alone.
- ECG will be performed prior to the first cycle of pracinostat (C4), then once during cycles 5 and 6, then every 3 cycles starting with cycle 10 (specifically, ECG should be performed approximately 90 minutes post pracinostat dosing on the day of visit to MD Anderson)
- Bone marrow aspiration and biopsy after cycles 6 (+/- 10 days) and 12 (+/- 10 days), and if deemed appropriate by the investigator, every 6 cycles after cycle 12.
- Response assessment after cycle 3 and cycle 6, then every 6 cycles. Assessment may not include bone marrow biopsy on all occasions.
- Collect any unused study drug and review patient study drug dosing diary for compliance during each cycle for the first 7 cycles then every 3 cycles.
- Continuous assessment of AEs
- Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) questionnaire during each cycle for the first 7 cycles then every 3 cycles.

Every effort will be made to adhere to the schedule of events and all protocol requirements within the +/- 10 day window. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory assessments completed outside of schedule, occasional missed required research samples such as PK or PD. All dose adjustments will be made according to the protocol unless discussed with the PI and do not exceed the dosages described in the protocol.

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 to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care
- 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.
- 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, 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 hospitalizations.
- 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 each cycle for the first 6 cycles then every 3 cycles as long as on the study for evaluation.

The patient will be contacted by the PI and his staff 30 days after discontinuation of protocol +/-14 days to assess for AE’s, unless the patient receives further MF-directed treatment.

5.7 Criteria for Removal from the Study

Treatment with study drugs is to be discontinued when any of the following occurs:

- Lack of therapeutic effect (any effort should be made to provide therapy to the patient in a safe way for at least 3-6 cycles for proper assessment of potential efficacy)
- Adverse event(s) that, in the judgment of the Investigator, may cause severe or permanent harm or which rule out continuation of study drug.
- Withdrawal of consent

- Lost to follow up
- Death
- Suspected pregnancy

6.0 CRITERIA FOR RESPONSE

Best overall response will be categorized according to the International Working Group Criteria[11]:

Complete remission (CR): Requires all of the following in the absence of both transfusion and growth factor support:

- Bone marrow: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF[†] and
- Peripheral blood: Hemoglobin ≥10 g/dL and <UNL; neutrophil count ≥ 1.0 × 10^9/L and <UNL;
- Platelet count ≥100 × 10^9/L and <UNL; <2% immature myeloid cells[‡] and
- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH

Partial remission (PR):

- Peripheral blood: Hemoglobin ≥10 g/dL and <UNL; neutrophil count ≥1 × 10^9/L and <UNL; platelet count ≥100 × 10^9/L and <UNL; <2% immature myeloid cells and

- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH or

- Bone marrow: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF[†], and peripheral blood: Hemoglobin ≥8.5 but <10 g/dL and <UNL; neutrophil count ≥1 × 10^9/L and <UNL; platelet count ≥50, but <100 × 10^9/L and <UNL; <2% immature myeloid cells[‡] and
- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH

Clinical improvement (CI): The achievement of anemia, spleen or symptoms response without progressive disease or increase in severity of anemia, thrombocytopenia, or neutropenia[§]

Anemia response	Transfusion-independent patients: a ≥2.0 g/dL increase in hemoglobin level[‖] Transfusion-dependent patients: becoming transfusion-independent[¶]
Spleen response[#]	A baseline splenomegaly that is palpable at 5-10 cm, below the LCM, becomes not palpable[**] or

	A baseline splenomegaly that is palpable at >10 cm, below the LCM, decreases by ≥50%**
	A baseline splenomegaly that is palpable at <5 cm, below the LCM, is not eligible for spleen response
Symptoms response	A ≥50% reduction in the MPN-SAF TSS††

Progressive disease:

- Appearance of a new splenomegaly that is palpable at least 5 cm below the LCM or
- A ≥100% increase in palpable distance, below LCM, for baseline splenomegaly of 5-10 cm or
- A 50% increase in palpable distance, below LCM, for baseline splenomegaly of >10 cm or
- Leukemic transformation confirmed by a bone marrow blast count of ≥20% or
- A peripheral blood blast content of ≥20% associated with an absolute blast count of ≥1 × 10(9)/L that lasts for at least 2 weeks

Stable disease: Belonging to none of the above listed response categories

Relapse:

- No longer meeting criteria for at least CI after achieving CR, PR, or CI, or
- Loss of anemia response persisting for at least 1 month or
- Loss of spleen response persisting for at least 1 month
- Recommendations for assessing treatment-induced cytogenetic and molecular changes.

Cytogenetic remission	At least 10 metaphases must be analyzed for cytogenetic response evaluation and requires confirmation by repeat testing within 6 months window CR: eradication of a preexisting abnormality PR: ≥50% reduction in abnormal metaphases (partial response applies only to patients with at least ten abnormal metaphases at baseline)
Molecular remission	Molecular response evaluation must be analyzed in peripheral blood granulocytes and

	requires confirmation by repeat testing within 6 months window CR: Eradication of a pre-existing abnormality PR: ≥50% decrease in allele burden (partial response applies only to patients with at least 20% mutant allele burden at baseline)
Cytogenetic/molecular relapse	Re-emergence of a pre-existing cytogenetic or molecular abnormality that is confirmed by repeat testing

EMH, extramedullary hematopoiesis (no evidence of EMH implies the absence of pathology- or imaging study-proven nonhepatosplenic EMH); LCM, left costal margin; UNL, upper normal limit.

[*]Baseline and post treatment bone marrow slides are to be interpreted at one sitting by a central review process. Cytogenetic and molecular responses are not required for CR assignment.

[†]Grading of MF is according to the European classification: Thiele et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90:1128. It is underscored that the consensus definition of a CR bone marrow is to be used only in those patients in which all other criteria are met, including resolution of leukoerythroblastosis. It should also be noted that it was a particularly difficult task for the working group to reach a consensus regarding what represents a complete histologic remission.

[‡]Immature myeloid cells constitute blasts + promyelocytes + myelocytes + metamyelocytes + nucleated red blood cells. In splenectomized patients, <5% immature myeloid cells is allowed.

[§]See above for definitions of anemia response, spleen response, and progressive disease. Increase in severity of anemia constitutes the occurrence of new transfusion dependency or a ≥20 g/L decrease in hemoglobin level from pretreatment baseline that lasts for at least 12 weeks. Increase in severity of thrombocytopenia or neutropenia is defined as a 2-grade decline, from pretreatment baseline, in platelet count or absolute neutrophil count, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. In addition, assignment to CI requires a minimum platelet count of ≥25 000 × 10(9)/L and absolute neutrophil count of ≥0.5 × 10(9)/L.

[‖]Applicable only to patients with baseline hemoglobin of <100 g/L. In patients not meeting the strict criteria for transfusion dependency at the time of study enrollment (see as follows), but have received transfusions within the previous month, the pretransfusion hemoglobin level should be used as the baseline.

[¶]Transfusion dependency before study enrollment is defined as transfusions of at least 6 units of packed red blood cells (PRBC), in the 12 weeks prior to study enrollment, for a hemoglobin level of <85 g/L, in the absence of bleeding or treatment-induced anemia. In addition, the most recent transfusion episode must have occurred in the 28 days prior to study enrollment. Response in transfusion-dependent patients requires absence of any PRBC transfusions during any consecutive "rolling" 12-week interval during the treatment phase, capped by a hemoglobin level of ≥85 g/L.

[#]In splenectomized patients, palpable hepatomegaly is substituted with the same measurement strategy.

[**]Spleen or liver responses must be confirmed by imaging studies where a ≥35% reduction in spleen volume, as assessed by MRI or CT, is required. Furthermore, a ≥35% volume reduction in the spleen or liver, by MRI or CT, constitutes a response regardless of what is reported with physical examination.

[††]Symptoms are evaluated by the MPN-SAF TSS.17 The MPN-SAF TSS is assessed by the patients themselves and this includes fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pain, abdominal discomfort, weight loss, and fevers. Scoring is from 0 (absent/as good as it can be) to 10 (worst imaginable/as bad as it can be) for each item. The MPN-SAF TSS is the summation of all the individual scores (0-100 scale). Symptoms response requires ≥50% reduction in the MPN-SAF TSS.

[##]Progressive disease assignment for splenomegaly requires confirmation by MRI or computed tomography showing a ≥25% increase in spleen volume from baseline. Baseline values for both physical examination and imaging studies refer to pretreatment baseline and not to posttreatment measurements.

7.0 REGULATORY AND REPORTING REQUIREMENTS

7.1 Adverse Event Reporting for MD Anderson-Sponsored IND Protocols

All adverse events that are possible, probably or definitely related to the study treatment will be captured on the case report form. Any grade 3 or higher adverse event that is considered unrelated or unlikely related to the study treatment will also be recorded. Adverse events will be defined using NCI CTCAE version 4.0.

All adverse events occurring between the time the subject is consented and begins study drug will be considered a baseline event and recorded. Baseline adverse events will be recorded in the case report form in prior medical history. All baseline adverse events that increase in severity during the course of treatment will be captured as an adverse event in the case report form.

Any adverse event that occurs during the study period (up through 30 days after the last day drug is administered) will be followed until clinical recovery is complete and laboratory tests have returned to normal, until progression of the event has been stabilized, or until the PI determines there has been acceptable resolution of the event.

7.2 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 as per “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 IRB, 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 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.

7.2 Expedited reporting by investigator to Helsinn Healthcare SA

Serious adverse events (SAE) are defined above. The investigator will inform Helsinn Healthcare SA of any SAE within 24 hours of being aware of the event via email and/or fax.

All pregnancies will also be reported.

The following SAEs are not subject to expedited reporting, but will be included in the annual report via the SAE log.

a. Infection or cytopenias leading to hospitalization or prolongation of hospitalization; disease progression leading to death; life-threating AE, hospitalization or prolongation of hospitalization, or disability.

Adverse events will be documented in the medical record and entered into the case report form. PDMS/CORe will be used as the electronic case report form for this protocol. 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. This form must be completed and supplied to Helsinn Healthcare SA 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 SAE reporting form.

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject or the female partner of a male subject occurring while the subject is on study drug, or within 30 days of the subject's last dose of study drug, are considered immediately reportable events. Study drug is to be discontinued immediately in a female patient and the patient instructed to return any unused portion of the study drug to the investigator(s). The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Helsinn Healthcare SA immediately by phone and facsimile using the MDACC AE report form. The female should be referred to an obstetrician-gynecologist experienced in reproductive toxicity for further evaluation and counseling.

8.0 STATISTICAL CONSIDERATIONS

This is a single-center phase II trial designed to assess the effect of ruxolitinib in combination with pracinostat in subjects with Primary, Post Polycythemia Vera, or Post Essential Thrombocythemia Myelofibrosis (PMF, post-PV MF, or post-ET MF).

The primary objective is to assess efficacy in terms of the objective response rate (i.e., complete and partial response, and clinical improvement by IWG-MRT) in patients with MF. Secondary objective is to determine the safety of the combination therapy in this population.

Primary Endpoint is the objective response rate (ORR), defined as a clinical improvement (CI), partial remission (PR), and complete remission (CR) according to the International Working Group (IWG) Criteria. All subjects meeting the eligibility criteria that have signed a consent form and have begun treatment will be evaluable for response.

A total of 25 patients will be enrolled from MDACC and patients will be registered through the Clinical Oncology Research System (CORE). The estimated accrual rate is 2-3 patients per month. Initially patients will receive ruxolitinib alone for the first 3 cycles, where 1 cycle is 28 days (i.e., 4 weeks). Then, starting from cycle #4, patients will receive the combination therapy of ruxolitinib with pracinostat.

The method of Thall, Simon and Estey [1995] will be used for futility and toxicity monitoring for this study. For futility monitoring, the primary endpoint of interest is the objective response (OR) rate at the end of 6 treatment cycles. Patients who drop off the study early due to any reason will be counted as failures (i.e., non-responders). The target response rate is 50% and the following futility stopping rule will be applied prob{p(OR)<0.5)>0.95, where p(OR) denotes the objective response rate, with a beta (1,1) prior distribution. That is, the treatment will be stopped if during the study we determine that there is more than 95% chance that the OR rate is less than 50%.

For toxicity monitoring, we denote the probability of toxicity by p (T), where toxicity is defined as Grade 3-4 clinically relevant non-hematologic toxicity or a serious adverse event that is at least possibly related to the study drug (Common Terminology Criteria for Adverse Events CTCAE version 4.0). We assume as a priori, p (T) ~ beta (0.4, 1.6). We will stop treating patients if Pr (p (T)> 0.20 | data) >0.87. That is, we will stop the trial for new patient enrollment if at any time during the study we determine that there is more than 87% chance that the toxicity rate is more than 20%. The OR rate and the toxicity will be monitored simultaneously using Bayesian stopping boundaries (Table 1) calculated based on beta-binomial distribution. Independence is assumed between OR and toxicity. Patients will be monitored in cohort size of 5, starting from the 5th patient. Multc Lean Desktop (version 2.1) was used to generate the futility/toxicity stopping boundaries and the operating characteristics table (Table 2).

Table 1: Stopping boundaries for OR and toxicity

Number of patients	Stop the trial if	Stop the trial if

evaluated	there are this many OR patients	there are this many patients with toxicities
5	0	3-5
10	0-2	4-10
15	0-4	6-15
20	0-6	7-20

Table 2: Operating characteristics of the Bayesian futility and toxicity monitoring

True OR Rate	True toxicity Rate	Prob (stop the trial early)	Average sample size
0.3	0.1	0.69	15.7
	0.2	0.74	14.6
	0.3	0.84	12.4
	0.4	0.94	9.8
0.4	0.1	0.35	20.3
	0.2	0.46	18.6
	0.3	0.67	15.1
	0.4	0.88	11.3
0.5	0.1	0.13	23.1
	0.2	0.27	21.1
	0.3	0.56	16.8
	0.4	0.83	12.1
0.6	0.1	0.04	24.3
	0.2	0.2	22.1
	0.3	0.52	17.5
	0.4	0.82	12.5

Statistical Analysis Plan

Summary statistics will be provided for continuous variables. Frequency tables will be used to summarize categorical variables. The objective response rate (ORR) will be estimated along with the Bayesian 95% credible interval. Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v4.0 whenever possible. We will follow standard reporting guidelines for adverse events. Safety data will be summarized by category, severity and frequency. The proportion of patients with AEs will be estimated, along with the Bayesian 95% credible intervals.

Duration of response: Duration of response is defined as the date at which the subject's objective status is first noted to be a CR, PR or CI to the date of progression (no longer meeting criteria for either CR, PR or CI) is documented (if one has occurred). Patients who continue to respond as of the data cut-off date will be censored as of the date of their last assessment documenting continued response.

Time to response: The time to response is defined as the time from study registration to the first date at which the subject's objective status was classified as a response (CR, PR or CI). In subjects who do not achieve a response, time to response will be censored at the subject's last evaluation date.

The distribution for each of these event-time variables (duration of response and time to response) will be estimated by Kaplan-Meier curves.

Anemia: Descriptive statistics will be used to explore improvements in anemia and transfusion dependence. The following outcomes will be summarized: mean changes in hemoglobin at monthly intervals. The proportion of transfusion independent patients not requiring transfusions on study who experience an increase of 2 g/dL in their hemoglobin relative to baseline, the proportion of transfusion dependent patients (defined as requiring a transfusion of 2 units PRBCs monthly for 3 months (12 weeks) prior to starting the trial) who become transfusion independent (not requiring a transfusion of PRBCs over a period of 3 months (12 weeks) while on study), the proportion of transfusion dependent patients who become transfusion independent and have a 1 g/dL increase in hemoglobin relative to baseline, and the proportion of transfusion independent patients requiring a transfusion while on study.

JAK2V617F Allele burden: Change in JAK2V617 allele burden from baseline to each visit where it is measured.

Correlative Studies: Descriptive statistics will be applied to the analysis of these findings.

9.0 REFERENCES

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