A Phase II Open-label Study of Combined Ruxolitinib and Enasidenib in Patients With Accelerated/Blast-phase Myeloproliferative Neoplasm or Chronic-phase Myelofibrosis With an IDH2 Mutation
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TABLE OF CONTENTS

LIST OF TABLES

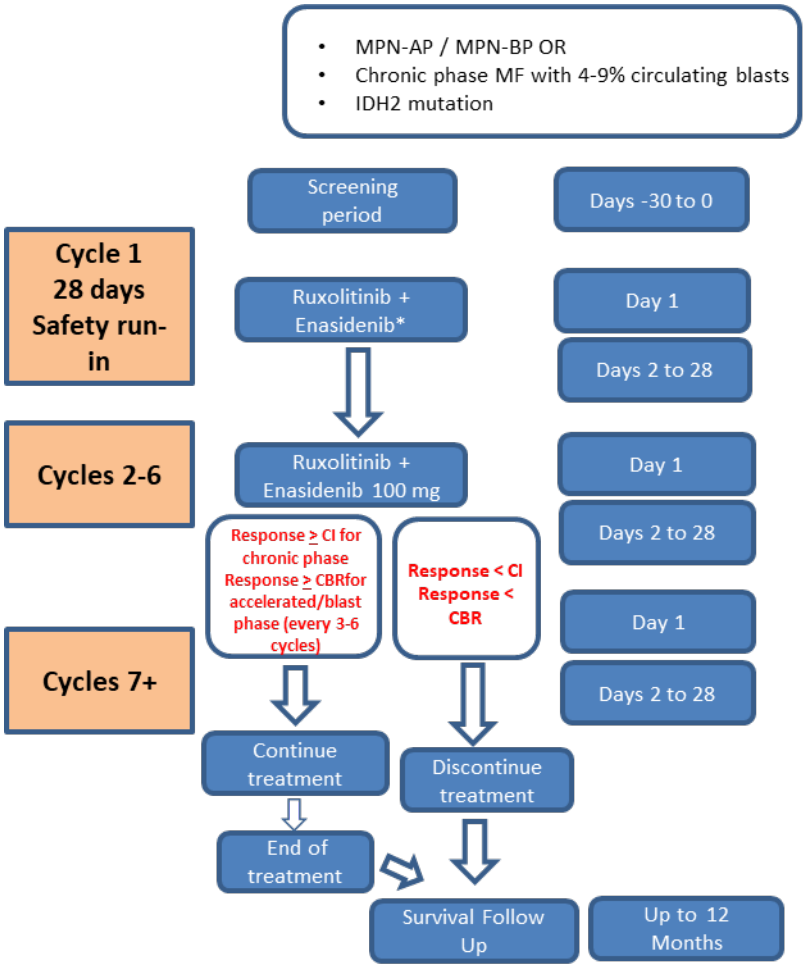
2. LIST OF ABBREVIATIONS AND TERMS

AE	adverse event
AESI	adverse event of special interest
ACS	acute coronary syndrome
ALT	alanine aminotransferase (SGPT)
AML	acute myelogenous leukemia
ASCT	allogeneic stem cell transplantation
AST	aspartate aminotransferase (SGOT)
AUC	area under the plasma concentration-time curve
BCRP	Breast Cancer Resistance Protein
BID	twice a day
BM	bone marrow
BMF	bone marrow failure
BUN	blood urea nitrogen
C	Cycle
CBC	complete blood count
CBR	complete blast response
CFR	Code of Federal Regulations
CI	clinical improvement
CK	creatine kinase
Cmax	maximum (peak) concentration
CRF	case report form
CR	complete response
Cri	complete response with incomplete recovery of counts
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events (Version 5.0)
C#D#	Cycle (number) Day (number)
D, d	day
DEHP	di(2-ethylhexyl)phthalate
EC	Ethics Committee
ECG	electrocardiogram
ECHO	echocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
EOS	end of study

2. LIST OF ABBREVIATIONS AND TERMS

EOT	end of treatment
ET	essential thrombocythemia
FCBP	Female of child bearing potential
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GI	gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonization
IMRA	Interval Milestone Response Assessment
IND	Investigational New Drug
IP	intraperitoneal
IRB	Institutional Review Board
IV	intravenous
IWG/ELN	International Working Group/European Leukemia Net
IWG-MRT	International Working Group for Myelofibrosis Research and treatment
LVEF	left ventricular ejection fraction
MF	myelofibrosis
MPN	myeloproliferative neoplasms
MPN-RC	Myeloproliferative Neoplasms Research Consortium
MRI	magnetic resonance imaging
MUGA	multi-grade acquisition
NaCl	sodium chloride
ORR	overall response rate
OS	overall survival
PB	peripheral blood
PBR	partial blast response
PD	progressive disease
PI	Principal Investigator
PK	pharmacokinetic
PMF	primary myelofibrosis
PO	orally, by mouth
PPHS	Program for the Protection of Human Subjects
PR	partial response
PV	polycythemia vera
QD	once a day
QT	ECG interval between onset of QRS complex to end of the T wave (QT interval)
QTc	QT interval corrected for heart rate
RBC	red blood cell
SAE	serious adverse event
SAF	symptoms assessment form
SUSAR	suspected unexpected serious adverse reaction
Tmax	time to peak plasma concentration
TSS	total symptoms score
ULN	upper limit of normal
UPR/UPIRSO	Unanticipated problem involving risk to subjects or others
US	United States
Vd	volume of distribution
Vol	volume
WBC	white blood cell

3. STUDY SCHEMA



Eligible patients with signed consent who have completed all requisite screening tests and procedures are to start Cycle 1 Day 1 within 30 days of signing consent.

All adverse events captured during the first 28 days of treatment with Ruxolitinib and Enasidenib will be used to assess safety and tolerability in the first 6 patients on study.

Cycles will be a minimum of 28 days.

Following cycle 6, response will be assessed by IWG/ELN consensus criteria and patients will continue treatment if response was deemed a CR/CRi/PR or CBR for MPN-AP and MPN-BP patients. For chronic phase MF, response will include CR/PR and clinical improvement.

Patients will continue to be followed post treatment for long term survival every 3 months for up to 12 months by telephone encounter or medical record review to determine the following variables: alive versus deceased and type of current therapy.

*Ruxolitinib dosing based on platelet counts; Enasidenib 100 mg for MPN-AP or MPN-BP and 50 mg for chronic phase MF for cycle 1

4. STUDY SUMMARY

Title	A Phase II Open-label Study of Combined Ruxolitinib and Enasidenib (AG-221) in Patients with Accelerated/Blast-phase Myeloproliferative Neoplasm or Chronic-phase Myelofibrosis with an IDH2 Mutation
Short Title	*MPN-RC 119-The IDH2 Study*
Protocol Number	TBD
Phase	Phase II
Methodology	Open-label
Study Duration	6 months; if response is met, subjects may enter the extension phase indefinitely.
Study Center(s)	Icahn School of Medicine at Mount Sinai, Mays Cancer Center at University of Texas San Antonio, Memorial Sloan-Kettering Cancer Center, Mayo Clinic-Arizona, Wake Forest Baptist Health, University of Kansas Cancer Center, Moffitt Cancer Center, University of Michigan, Cleveland Clinic Taussig Cancer Center Institute, Princess Margaret Cancer Centre, Cedars-Sinai Medical Center
Objectives	**Primary Objectives** • To estimate the efficacy of ruxolitinib and enasidenib combination therapy in patients with accelerated-phase and blast-phase disease. Efficacy will be defined as the proportion of patients who achieve a best leukemia response of: complete response (CR), Partial Response (PR), complete response with incomplete recovery of counts (CRi), as assessed by the modified 2013 International Working Group (IWG) Response Criteria. **Secondary Objectives** • To assess the efficacy of the combination of ruxolitinib with enasidenib in patients with accelerated-phase and blast-phase disease using complete (CBR) and partial blast response (PBR). • To assess the efficacy (CR, PR, Clinical Improvement) of the combination of ruxolitinib with enasidenib in patients with PMF, post-PV MF, or post-ET MF in chronic phase and 4-9% blasts as assessed by the modified 2013 International Working Group (IWG) Response Criteria • To determine the safety of the combination of ruxolitinib and enasidenib based on the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE). • To assess the impact of the combination of ruxolitinib with enasidenib on the overall survival (OS) of advanced phase MPN patients **Exploratory Objectives** • To determine the impact of the combination of ruxolitinib with enasidenib in patients with PMF, post-PV MF, or post-ET MF, on patient quality of life as assessed by Myelofibrosis Symptom Assessment Form (MF-SAFv4.0). • To evaluate the pharmacodynamics of ruxolitinib and enasidenib when combined in this population. • To evaluate the effects of combined therapy on gene expression and global methylation status. • To explore changes in mutant allele burden and clonal architecture of disease.
Number of Subjects	32

Diagnosis and Main Inclusion Criteria	1. Subjects must be ≥ 18 years at the time of signing the Informed Consent Form (ICF). 2. Understanding and voluntary signing an IRB-approved informed consent form. 3. Diagnosis of: a. Accelerated-phase (≥ 10% blasts in PB or BM) or blast-phase (≥ 20% blasts in PB or BM) myeloproliferative neoplasm (with history of prior myelofibrosis, polycythemia vera, or essential thrombocythemia) b. Previously treated patients with myelofibrosis with persistent disease or progressive disease (persistent or progressive splenomegaly, leukocytosis, anemia, or thrombocytopenia) with intermediate-1 or greater risk disease according to 2013 International Working Group (IWG) criteria, and 4-9% circulating blasts. 4. Demonstration of an IDH2 mutation 5. Platelet count ≥75,000X10^9/L for chronic phase myelofibrosis patients 6. Prior therapy with either ruxolitinib or enasidenib is permitted, but not a combination of ruxolitinib and enasidenib. 7. Patients with chronic phase myelofibrosis on ruxolitinib must be on the drug for at least 3 months and on a stable dose for at least one month. 8. Subjects must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. ECOG 3 status will be allowed if attributable to MPN. 9. Patients must have adequate organ function as demonstrated by the following: a. Direct bilirubin ≤ 2.0mg/dL, unless due to Gilbert's disease or current elevations in direct bilirubin associated with existing enasidenib use. b. Serum creatinine < 2.0 mg/dL. c. ALT and AST ≤ 3 x upper limit of normal (unless transaminitis is considered to be related to MF). 10. Females of childbearing potential (FCBP) must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL within 10 – 14 days prior to starting enasidenib and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 4 weeks before she starts taking enasidenib. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a condom during sexual contact with a female of child bearing potential even if they have had a successful vasectomy. All patients must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure. 11. All study participants must be able to swallow oral medication. 12. Ability to adhere to the study visit schedule and all protocol requirements.
Study Product(s), Dose, Route, Regimen	Ruxolitinib and enasidenib will be given orally in 28-day cycles. Ruxolitinib dose will be given per protocol, enasidenib will be given at 100 mg daily on enrollment for patients with accelerated and blast phase myelofibrosis regardless of platelet count. Patients with chronic phase myelofibrosis will receive enasidenib at starting dose of 50mg daily for cycle 1. The dose of enasidenib will be increased to 100mg for cycle 2 if the platelet count stays above 50,000 at the end of cycle 1 (Table 3a) and no other therapy emergent SAE of grade ≥2 developed (except grade 2 hyperbilirubinemia that is acceptable for dose escalation).

Duration of administration	Duration of treatment anticipated to be at least 6 months. Responders will continue therapy indefinitely unless progression of disease occurs, toxicity warranting discontinuation of therapy is observed, or at the discretion of the treating physician.
Statistical Methodology	The primary endpoint of this study is best overall response, defined as partial response or better (CR+PR+Cri), and will be defined as the best response by Cycle 6 of combination therapy. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response. Any evaluable patient not meeting the definition for response will be deemed as having a non- response. The largest overall response rate where the proposed combination treatment regimen would be considered ineffective in this patient population is 5%, and the smallest response rate that would warrant subsequent studies with the proposed regimen is 25%. This is based on retrospective analysis applying the 2013 IWG Response Criteria to patients treated with ruxolitinib. A Simon's two-stage minimax design will be employed to test the null hypothesis that the true overall response rate in this patient population is at most 5%.

6. SCIENTIFIC BACKGROUND AND RATIONALE

6.1 DISEASE BACKGROUND

Myeloproliferative neoplasms

Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are each clonal stem cell malignancies each of which are classified as Philadelphia chromosome negative myeloproliferative neoplasms (MPN) by the World Health Organization (WHO)[2]. A portion of patients with PV and ET can evolve to a clinical picture which resembles PMF (post- PV/ET MF). Post-PV/ET MF and PMF, collectively referred to as MF, have similar clinical phenotypes with a median age at diagnosis of 65 years[3, 3,4]. MF is characterized by severe constitutional symptoms, progressive hepatosplenomegaly, peripheral blood cytopenias often requiring transfusional support and an increased propensity for transformation to a clinical picture resembling acute leukemia, termed myelofibrosis in blast phase (MF-BP)[5]. Like chronic myeloid leukemia, the blast phase of the MPN is nearly always preceded by an accelerated phase (MF-AP). Patients with more than 10% blasts in the peripheral blood or bone marrow have a median survival of less than 12 months rendering such patients candidates for aggressive therapy and placing MF-AP squarely on the continuum of progression to blast phase (4.1). Approximately 10-20% of MF patients will transform to MF-BP during the first decade following diagnosis with a median overall survival at the time of transformation of 2.6 months[4,6].In addition, a recent publication reports that patient with 5-9% of blasts (≥ 4-9% PB or ≥ 5-9% BM) have similar inferior outcome as patients with ≥ 10% blasts as compare to patient with <4% circulating blasts[7].

The choice of appropriate treatment options for MF patients is currently based on the prognosis of the individual patient[8]. Several prognostic scoring systems that utilize various clinical parameters including age, leukocyte count, hemoglobin level, platelet count, monocyte count, peripheral blast count, presence of splenomegaly, constitutional symptoms and degree of bone marrow fibrosis (BMF) are helpful in predicting patient outcome[4,9-11]. Patients under the age of 65 without significant co-morbidities and intermediate/high risk disease by Lille classification or int-2/high risk by IWG-MRT PSS are considered candidates for reduced intensity conditioning stem cell transplant (RIC-SCT). Allogeneic stem cell transplant is the only modality that offers the potential for cure, although the risk of transplant related mortality (TRM) and the morbidity and mortality associated with graft versus host disease (GVHD) are considerable[12]. Patients older than 65 or with competing co-morbidities are considered not to be candidates for RIC-ASCT but rather candidates for therapies such as interferon-alfa, immunomodulatory agents (IMiDs) JAK2-TKIs or histone deacetylase inhibitors (HDACI). MF patients that either fail current experimental therapies or are ineligible due to limiting cytopenias often have no alternative treatment options other than agents including hydroxyurea, melphalan, thalidomide, lenalidomide and danazol that achieve response rates of 20-50%, but have not been shown to alter the natural progression of disease[13,14].

Activating mutations of the Janus Kinase 2 receptor tyrosine kinase (JAKV617F), the thrombopoietin receptor (MPL W515L/K) and Calreticulin (CALR)have been identified in many patients with MPNs. Approximately 50-60% of patients with MF carry the JAK2 mutations, 20-25% CALR mutations and 5% MPL mutations[15-20]. Mutations in additional Sex Combs-Like 1 (ASXL1), Ten-Eleven translocation-2 (TET2), serine/arginine-rich splicing factor 2 (SRSF2), U2AF1, IDH and others have also been discovered in MPN patients and can coexist independently of JAK2V617F[21-24].The prognostic significance of these mutations remains uncertain. Some of the mutation are considered high risk mutations and confer worse OS[25,26]. Ruxolitinib (Small molecule inhibitor of JAK2) has been shown to produce durable clinical benefits in terms of spleen volume reduction and improvement in constitutional symptoms with an acceptable toxicity profile[27,28]. Based on the results of two phase III studies (COMFORT-I, COMFORT-II)[27,28]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[29].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[30].Follow-up analysis of the COMFORT-II trial has also demonstrated an OS advantage for patients treated with ruxolitinib[31].

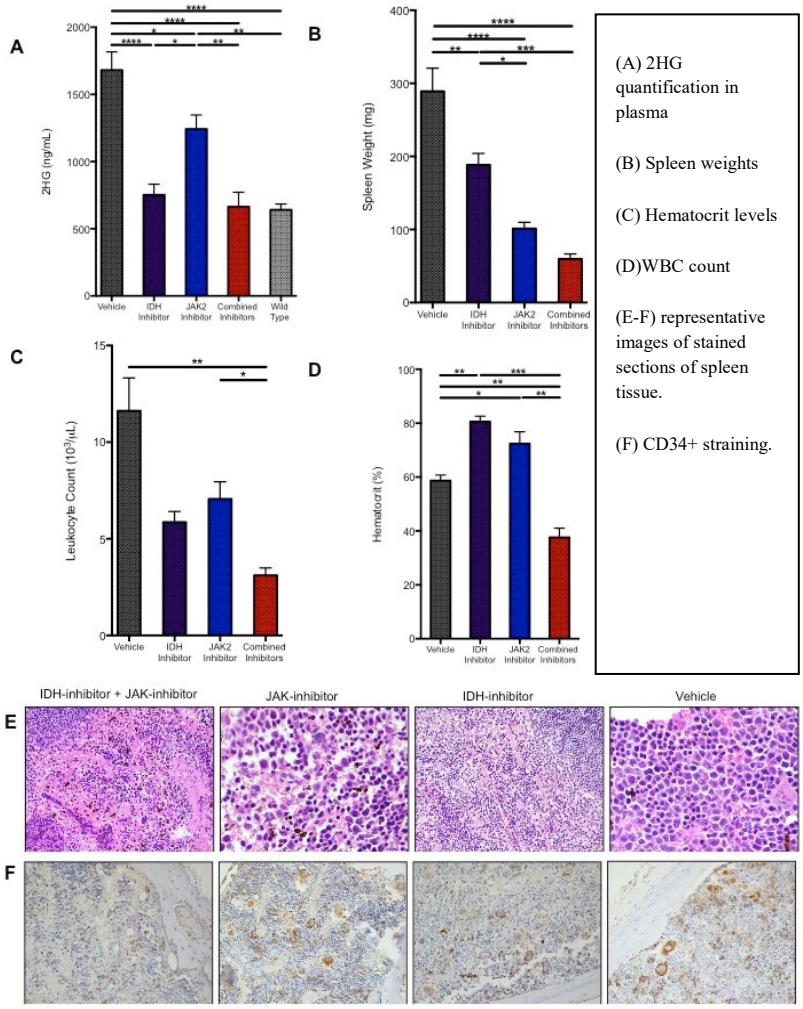
In comparison to de novo AML, MPN-BP is highly resistant to standard induction chemotherapy and responses, if achieved, are difficult to maintain. A retrospective review of 2333 myelofibrosis patients from Mayo clinic identified 91 patients who met WHO criteria for MF-BP[6]. The median overall survival of the entire group was 2.6 months after leukemic transformation with a median survival of 3.9 months in those patients receiving AML-like induction therapy. No patient achieved a complete response (CR) with induction therapy. In a retrospective study of 74 patients with BCR-ABL negative MPN-BP at the MD Anderson Cancer Center, the best outcomes were seen in those receiving allogeneic stem cell transplants either upfront or immediately after achieving a response to induction therapy[32]. The survival was the same (6-7 months) for those patients that received either standard AML induction-like therapy or low dose therapies such as gemtuzumab, azacytidine or decitabine. In this review, a CR/CRi rate of 46% was obtained with induction therapy but the median time to relapse was only 5 months. The prognosis for MPN-BP is dismal with present therapeutic options and more effective therapies are required.

IDH2 Mutations in MPN: The presence of IDH mutations has prognostic significance in patients with MPNs. IDH mutations are associated with an increased risk of leukemic transformation. Co-occurring mutations in *IDH1/2* and *JAK2V617F*[33]are associated with significantly reduced leukemia-free survival[25]in MF patients. Finally, *IDH1/2*mutations are among the most frequently encountered events in MPNs that have progressed to AML[33,34]. These clinical observations suggest, as is the case with TET2 mutations, that IDH mutations alter the disease biology of MPNs. Moreover, a recent meta-analysis has showed that the presence of IDH mutation is associated with worse survival in patients with myelofibrosis[35].

Preclinical Data: Development and characterization of a *JaK2*V617F-*Idh2*R140Q murine model of MPN: To create a faithful model of phenotypic cooperativity between*IDH2*and*JAK2* mutations, conditional knock-in *Idh2*R140Q mice were crossed with *Jak2*V617F and Mx1-cre. Intraperitoneal injections of polyI:polyC were used to excise and induce expression of both mutant alleles in hematopoietic cells. Mice expressing combined *Idh2*R140Q and *Jak2*V617F demonstrated splenomegaly, leukocytosis and polycythemia similar to that of *Jak2*V617F mice, at timed sacrifices at approximately 100 days. Histologically, these mice demonstrated disrupted splenic architecture with blast-like cells, not seen in *Jak2*V617F mice. Bone marrow cytospins demonstrated abnormal myeloid elements with a strongly elevated M:E ratio. These data indicate that combined mutant mice have a lethal myeloproliferative neoplasm with histological pre-leukemic features. Competitive transplants demonstrated that the presence of the *Idh2* mutant improved the competitive capacity of the donor bone marrow regardless of *Jak2* mutant status. Transplant recipients developed a phenotype very similar to primary mice including polycythemia and thrombocytosis. Notably, leukocytosis was greater in recipients of *Idh2*R140Q-*Jak2*V617F marrow in comparison to *Jak2*V617F marrow. Flow cytometric analysis of *Idh2*R140Q-*Jak2*V617F primary mice demonstrated expansion of the lineage negative Sca+ cKit+ (LSK) population. Preclinical evaluation of combined JAK1/2 inhibition and IDH2 inhibition: To test the therapeutic efficacy of combined JAK1/2 inhibition and mutant IDH2 inhibition, *Idh2*R140Q-*Jak2*V617F mice were treated with ruxolitinib (60mg/kg) and enasidenib (100mg /kg) twice daily by gavage for 21-28 days. Primary recipients of *Idh2*R140Q-*Jak2*V617F bone marrow treated with enasidenib showed reduction of plasma 2-hydroxyglutarate (an oncometabolite product of mutant IDH2) to the level of wild type mice (**Figure 1A**). Monotherapy with either enasidenib or ruxolitinib reduced splenomegaly, while combined treatment completely resolved splenomegaly (**Figure 1B**). Combined therapy with enasidenib and ruxolitinib normalized polycythemia and leukocytosis to a level not seen with monotherapy (**Figure 1C,D**). In the spleen, cells identified morphologically as blasts are no longer present in single AG221 or combined treatment with AG221, but not ruxolitinib, resulted in reduction of CD34 immunohistochemical staining in cells with megakaryocyte morphology (**Figure 1E, F**), transplant assay was used to examine the effect of therapy with inhibitors on donor chimerism. In

comparing peripheral blood from individual mice before and after treatment, we observed that mice treated with either enasidenib or with combined enasidenib and ruxolitinib showed significant reductions in mutant donor chimerism; in contrast, mice treated with either ruxolitinib or vehicle showed significant expansion of the proportion of mutant derived clones. These effects were not seen in mice transplanted with *Jak2*V617F-mutant cells demonstrating specificity for IDH2-mutant MPN cells.

Figure 1: Treatment of *Idh2*R140Q *Jak2*V617F combined mutant mice with combined JAK2 and IDH2 inhibitor:



6.2 RUXOLITNIB

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 malignant cell 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 Myelofibrosis with Myeloid Metaplasia (MMM) patients. More recently, other mutations have been identified in MPD patients lacking the JAK2V617F mutation. For instance, additional activating mutations in JAK2, as well as a mutation in the thrombopoietin receptor (MPL) and calreticulin (CALR) result in constitutive ligand- independent JAK activation. Importantly, ectopic expression of each of these mutant genes has been demonstrated to be sufficient to cause MPN-like syndromes in mice. Moreover, even in MPN 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 a 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 (as well as PV patients with inadequate response to or are intolerant of hydroxyurea). Ruxolitinib is a substituted pyrrolopyrimidine compound that acts as a potent and selective inhibitor of the Janus kinase family of enzymes. Ruxolitinib is a novel, potent, and selective inhibitor of the JAKs with modest selectivity for JAK2. Ruxolitinib 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, ruxolitinib demonstrated excellent potency (IC50 values of 80-141 nM). This effect was not due to general cytotoxicity, because ruxolitinib (up to 25 μM) had no significant effect on the growth of cytokine-independent cell lines transformed by the Bcr-Abl oncogene. In addition, ruxolitinib 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 MPN. Additional details as to the *in* vitro pharmacology of ruxolitinib may be found in the Clinical Investigator's Brochure (IB). Ruxolitinib was evaluated in two mouse models where either a cytokine-dependent multiple myeloma cell line, INA-6, oracell line, BaF3, engineered to express JAK2V617F was inoculated. The ability of ruxolitinib 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 ruxolitinib 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 ruxolitinib to inhibit wild type 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 ruxolitinib resulted in a dose-dependent suppression of STAT3 phosphorylation and tumor growth in the cytokine-dependent INA-6 xenograft model at doses ≥ 10mg/kg BID. Moreover, oral administration of ruxolitinib inhibited the dramatic splenomegaly in mice resulting from intravenous inoculation of the BaF3-JAK2V617F cells. Additional details as to the *in vivo* pharmacology of ruxolitinib 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 ruxolitinib in the treatment of malignancies, including MPD such as PMF and Post-PV/ET MF.

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

6.2.1 CLINICAL EXPERIENCE IN MYELOFIBROSIS WITH RUXOLITINIB

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[27,28]. Based on the results of two Phase III studies (COMFORT-I, COMFORT-II)[27,28] 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[29]. 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 followup[30]. Follow-up analysis of the COMFORT-II trial has also demonstrated an OS advantage for patients treated with ruxolitinib[31].

6.3 Enasidenib (AG-221)

The IDH enzymes play critical roles in catalyzing the oxidative decarboxylation of isocitrate to produce carbon dioxide (CO2) and alpha-ketoglutarate (α-KG) enzyme in the citric acid cycle. Of the two best characterized isoforms, IDH1 and IDH2, mutations in IDH2 have been found in approximately 15% of subjects with AML[36,37]. The mutated proteins have a gain-of-function, neomorphic activity, catalyzing the reduction of α-KG to 2-HG while lacking the ability to synthesize isocitrate[38]. The excess accumulation of 2-HG leads to inhibition of dioxygenase dependent enzymes such as ten-eleven translocation (TET) and Jumonji which are responsible for demethylating DNA and histones, respectively, the consequence of which results in an altered epigenetic state and impaired cellular differentiation[39,40].

Recurring mutations in IDH2 may be important for AML pathogenesis or disease progression[41]. Retrospective clinical data have revealed that the presence of IDH1 mutations is associated with a worse prognosis in younger cytogenetically-normal AML subjects and subjects with IDH2 mutations had a significantly lower complete response rate[37]. In a retrospective study of 398 subjects < 60 years of age who were diagnosed with AML and randomly assigned to receive induction therapy with high-dose or standard dose daunorubicin, IDH2 mutations were in general associated with an improved overall survival[42]. Prospective clinical studies will help further understand the impact mutations, co-mutations, and/or karyotype status have on disease outcomes in the presence of an IDH mutation.

Enasidenib is a first-in-class, selective, potent inhibitor of the neomorphic activity of IDH2 mutant enzyme and has been shown to suppress 2-HG production in enzymatic, cell-based, and in vivo systems. Pharmacology studies support that suppression of 2-HG levels by enasidenib results in alterations of cellular downstream markers, leading to a release in the block of tumor cell differentiation.

Enasidenib has rapid oral absorption, low to medium total body plasma clearance, and low to high volume of distribution. The half-lives range from 2.5 to 5.4 hours, and oral bioavailability was approximately 40% to 50% (free base) in toxicology species. Enasidenib is metabolized by multiple cytochromes (CYPs) and uridine diphosphate (UDP)-glucuronosyl transferases (UGTs). Enasidenib is a direct inhibitor for CYP2C8,2C9, 2C19 and 2D6, as well as UDP-glucuronosyl transferase 1 family, polypeptide A1 (UGT1A1). The N-dealkylated metabolite, AGI-16903 is also an inhibitor of CYP1A2, 2C8 and 2C9. Enasidenib and AGI-16903 are weak inducers of CYP3A4. Preliminary results indicate that enasidenib increased 4β-hydroxycholesterol level in subjects, indicating CYP3A induction in patients. Enasidenib is not a substrate, but is a potent inhibitor of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). In vitro, enasidenib is an inhibitor of organic anion transporter (OAT) 1, OATP1B1, OATP1B3, and organic cation transporter (OCT) 2, while AGI-16903 is an inhibitor of BCRP, OAT1, OAT3, OATP1B1, and OCT2. AGI-16903 is also a substrate of P-gp and BCRP. Clinical relevance of such in vitro interactions will be evaluated in humans as needed.

The toxicity profile of enasidenib has been evaluated in repeat dose oral toxicity studies of up to 90 days in Sprague-Dawley rats, and cynomolgus monkeys and for 7 days in beagle dogs, and in the bacterial reverse mutation (Ames) and mammalian chromosomal aberration assays in vitro. Enasidenib was neither mutagenic nor clastogenic. Dose-limiting toxicities in rats after 28 days of dosing were

multifactorial and included gastrointestinal tract atrophy and erosions, lymphoid atrophy, degeneration, and necrosis, bone marrow hypocellularity and necrosis, skeletal muscle degeneration and necrosis, pancreas and kidney epithelial vacuolation, and adrenal cortical hemorrhage and necrosis. After 90 days of dosing, non-dosing limiting target organ toxicities in rats were limited to testes/epididymides and pancreas. In monkeys, ulcerative inflammation in the large intestine was determined as the dose-limiting toxicity. No changes in cardiovascular functions were noted in monkeys; however coronary artery lesion (periarteritis) was observed at the dose associated with dose-limiting toxicity indicated above. Findings in large intestine and coronary artery in monkeys were observed following 28 days of dosing. Additional target organ toxicities secondary to decreased body weight and food consumption in monkeys included femur/tibia (either dysplasia or decreased thickness of physis), bone marrow (decreased cellularity), thymus (atrophy/involution), liver (cytoplasmic rarefaction) and pancreas (acinar cell degranulation). In dogs, there were enasidenib dose-related prolonged heart rate-corrected QT (QTc) intervals, hypotension and increased heart rate. These functional cardiovascular effects of enasidenib were the likely underlying causes of coronary artery lesions in dogs.

As of 15 Apr 2016, data are available for 330 subjects from Study AG221-C-001 who have been administered enasidenib across 13 dosing cohorts up to a 650 mg daily dose, including both BID and QD dosing regimens. Study AG221-C-002, a food effect study in healthy volunteers is complete. Study AG-221-CP-001, a pharmacokinetic (PK) and safety study in healthy male Japanese and Caucasian adult subjects, and Study AG-221-CP-002, a PK study to evaluate the metabolism and excretion and determine absolute bioavailability of enasidenib in healthy male adult subjects, are also complete. Study AG221-C-003, a Phase 1/2 dose escalation study, evaluated enasidenib at doses ≥ 100 mg daily in subjects with advanced solid tumors with an IDH2 mutation, including glioma, and in subjects with AITL with an IDH2 mutation. Doses up to 650 mg were well-tolerated. The expansion phase of AG221-C-003 was canceled based on the Sponsor's decision to focus on the relapsed refractory AML indication and not for any safety reason. Ongoing studies include a Phase 3 randomized study in subjects with R/R AML with IDH2 mutation (AG-221-AML-004), a Phase 1b/2 study in subjects with newly-diagnosed AML (AG-221-AML-005), and a Phase 1 study in subjects with newly diagnosed AML sponsored by Agios in collaboration with Celgene (AG120-221-C-001). Study AG-221-C-005 is evaluating oral AG-120 plus subcutaneous (SC) azacitidine and oral enasidenib plus SC azacitidine in subjects with newly diagnosed AML with IDH1 or IDH2 mutation, respectively, who are not candidates to receive intensive induction chemotherapy. Study AG120-221-C-001 is evaluating AG-120 or enasidenib in combination with induction and consolidation therapy in subjects with newly diagnosed AML with an IDH1 and/or IDH2 mutation.

Data from Study AG221-C-001 showed that enasidenib was well-tolerated at total daily doses up to 650 mg. The MTD was not reached in the dose escalation part of the AG221-C-001 study. Based on pharmacodynamic and efficacy data, the 100 mg daily dose was chosen for further development. No meaningful differences in nature, incidence, or severity of the Adverse Events (AE) have been observed between subjects with R/R AML, previously untreated AML, or MDS. As of 15 Apr 2016, 93 deaths have been reported in Study AG221-C-001 either on study or within 28 days of the last dose of study treatment. Deaths were attributed to complications of the underlying condition or disease progression, with an exception of 1 subject who experienced pericardial effusion complicated by fatal cardiac tamponade. Retrospective analysis of this case suggests that pericardial effusion was a likely sign of isocitrate dehydrogenase-differentiation syndrome (IDH-DS). Serious adverse events (SAEs) reported in ≥ 5% of the study participants included febrile neutropenia in 79 (23.9%), pneumonia in 47 (14.2%), sepsis in 39 (11.8%), pyrexia in 28 (8.5%), leukocytosis in 27 (8.2%), IDH-DS in 24 (7.3%), lung infection in 21 (6.4%), acute renal failure in 18 (5.5%), tumor lysis syndrome (TLS) in 18 (5.5%), and fatigue in 17 (5.2%) subjects.

The safety and tolerability of enasidenib has been evaluated in more than 440 subjects enrolled in clinical studies of subjects with hematologic malignancies or solid tumors and single-dose pharmacology studies in healthy volunteers, as of the data cutoff date for this IB (15 Apr 2016 for Study AG221-C-001 and 01 Jul 2016 for all other ongoing clinical studies). In clinical studies, enasidenib overall was generally well tolerated, with a relatively low incidence of discontinuations due to AEs.

Specific adverse drug reactions (ADRs) to enasidenib were identified. Events of IDH-DS (similar to a syndrome observed with retinoic acid in acute promyelocytic leukemia), noninfectious leukocytosis, TLS, GI disturbances (such as nausea, diarrhea, vomiting, and associated dysgeusia and decreased appetite), and hyperbilirubinemia have been observed in subjects with hematologic malignancies. Gastrointestinal disturbances and hyperbilirubinemia have been also observed in subjects with solid tumors. IDH-DS and noninfectious leukocytosis are associated with enasidenib mechanism of action and generally decrease in incidence with time.

Preliminary analysis of PK data in Study AG221-C-001 (as of 15 Apr 2016) from subjects with advanced hematologic malignancies up to the 650 mg QD dose level demonstrated high plasma exposures, a long mean plasma half-life of enasidenib after a single dose, high drug accumulation after multiple doses, and relatively high PK variability. The mean time to Cmax (Tmax) ranged from approximately 1 to 24 hours when administered as a single dose 3 days before study treatment began. In a subset of IDH2 mutated AML subjects, correlation of drug exposure, 2-HG levels, and IDH2 mutation site was evaluated. A comparison of PK data between the 2 groups (R140Q and R172K) at the enasidenib 100-mg dose level indicated that the average steady state exposure level (AUC0-10 or Cmax) was similar. Enasidenib treatment decreased 2-HG levels in R140Q and R172K patients. Preliminary analysis showed no clear correlation between 2-HG suppression and clinical response to enasidenib.

As of 15 Apr 2016 in Study AG221-C-001, the overall response rate (ORR) (defined as complete response [CR] + CR with incomplete neutrophil recovery [CRi] + morphologic CR with incomplete platelet recovery [CRp] + partial response [PR] + marrow CR [mCR] + morphologic leukemia free state [MLFS]) among the 113 subjects in the dose escalation phase was 45.1% with a median time to first response of 1.9 months and a median time to best response of 3.7 months. Twenty-four of the 113 subjects (21.2%) achieved a best response of CR, with a median time to CR of 3.8 months. Responses occurred in all dosing groups (30 mg BID to 650 mg QD). Of the 176 subjects with R/R AML in the combined Phase 1 part of the study, the ORR was 40.3%, with a median duration of response of 5.8 months. Thirty-four of the 176 subjects (19.3%) achieved a CR, with a median duration of CR of 8.8 months. The ORR, CR, and median duration of response were similar in the subset of subjects who received the 100 mg daily dose compared with the total R/R AML population. Of the 71 responders among subjects with R/R AML, the median time to first response was 1.9 months and the median time to best response was 3.7 months. The median times to first response and best response were similar in the subset of subjects who received the 100 mg daily dose. At the time of data cutoff, median event-free survival for subjects with R/R AML was 6.4 months (95% CI = 5.4, 7.5) and median overall survival was 9.3 months (95% CI = 8.2, 10.9).

This study[43] led to the Food and Drug Administration approval to enasidenib for the treatment of adult patients with relapsed or refractory acute myeloid leukemia with IDH2 mutation in August 2017.

Please refer to the current version of the enasidenib IB for detailed information concerning the available pharmacology, toxicology, drug metabolism, clinical studies, and AE profiles of enasidenib.

7. STUDY OBJECTIVES

Ruxolitinib (also known as INCB018424), a JAK1/2 inhibitor, and enasidenib (AG-221, an IDH2 inhibitor) are effective and tolerable treatments for patients with myelofibrosis (MF) and acute myeloid leukemia (AML), respectively. We hypothesize that the combination of these agents in patients with MPN with an IDH2 mutation will improve the overall clinical response to therapy without causing excessive toxicity.

7.1 PRIMARY OBJECTIVES

- To estimate the efficacy of ruxolitinib and enasidenib combination therapy in patients with accelerated-phase and blast-phase disease. Efficacy will be defined as the proportion of patients who achieve a best leukemia response of: complete response (CR), Partial Response (PR),

complete response with incomplete recovery of counts (Cri), as assessed by the modified 2013 International Working Group (IWG) Response Criteria.

7.2 SECONDARY OBJECTIVES

- To assess the efficacy of the combination of ruxolitinib with enasidenib in patients with accelerated-phase and blast-phase disease using complete (CBR) and partial blast response (PBR).
- To assess the efficacy (CR, PR, Clinical Improvement) of the combination of ruxolitinib with enasidenib in patients with PMF, post-PV MF, or post-ET MF in chronic phase and 4-9% blasts as assessed by the modified 2013 International Working Group (IWG) Response Criteria
- To determine the safety of the combination of ruxolitinib and enasidenib based on the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE).
- To assess the impact of the combination of ruxolitinib with enasidenib on the overall survival of advanced phase MPN patients.

7.3 EXPLORATORY OBJECTIVES

- To determine the impact of the combination of ruxolitinib with enasidenib in patients with PMF, post-PV MF, or post-ET MF, on patient quality of life as assessed by the Myelofibrosis Symptom Assessment Form (MF-SAFv4.0).
- To evaluate the pharmacodynamics of ruxolitinib and enasidenib when combined in this population.
- To evaluate the effects of combined therapy on gene expression and global methylation status.
- To explore changes in mutant allele burden and clonal architecture of disease.

8.0 PATIENT ELIGIBILITY

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and not meet any of the exclusion criteria to be registered to the study by the Central Office. Study treatment may not begin until a subject is registered.

8.1 INCLUSION CRITERIA

1. Willing and able to adhere to the study visit schedule and all protocol requirements
2. Subjects must be ≥ 18 years at the time of signing the Informed Consent Form (ICF).
3. Diagnosis of:

a. Accelerated-phase (≥ 10% blasts in PB or BM) or blast-phase (≥ 20% blasts in PB or BM) myeloproliferative neoplasm (with history of prior myelofibrosis, polycythemia vera, or essential thrombocythemia)

b. Previously treated patients with myelofibrosis with persistent disease or progressive disease (persistent or progressive splenomegaly, leukocytosis, anemia, or thrombocytopenia) with intermediate-1 or greater risk disease according to 2013 International Working Group (IWG) criteria, and 4-9% circulating blasts.

4. Demonstration of an IDH2 mutation.
5. Platelet count ≥ 75,000 X 10^9/L for chronic phase myelofibrosis patients.
6. Prior therapy with either ruxolitinib or enasidenib is permitted, but not a combination of ruxolitinib and enasidenib.
7. Patients with chronic phase myelofibrosis on ruxolitinib must be on the drug for at least 3 months and on a stable dose for at least one month.

8. Subjects must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. ECOG 3 status will be allowed if attributable to MPN.
9. Patients must have adequate organ function as demonstrated by the following:
   a. Direct bilirubin ≤ 2.0mg/dL, unless due to Gilbert's disease or current elevations in direct bilirubin associated with existing enasidenib use.
   b. Serum creatinine< 2.0 mg/dL.
   c. ALT and AST ≤ 3x upper limit of normal (unless transaminitis is considered to be related to MF).
10. Females of childbearing potential (FCBP) must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL **within 10 – 14 days prior** to starting enasidenib and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 4 weeks before she starts taking enasidenib. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a condom during sexual contact with a female of child bearing potential even if they have had a successful vasectomy. All patients must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure.
11. All study participants must be able to swallow oral medication.

8.2 EXCLUSION CRITERIA

1. Use of any other standard anti-neoplastic drug or growth factor (e.g., anagrelide, G-CSF, lenalidomide, thalidomide clofarabine) except hydroxyurea or experimental drugs, with the exception of ruxolitinib or enasidenib, less than 14 days or 5-half-lives, whichever is longer, prior to starting study therapy and/or lack of recovery from all toxicity (except for alopecia) from previous therapy to Grade 1 or better.
   a. Patients will be permitted to receive hydroxyurea while on study for up to a total of 3 cycles of combined therapy.
2. Known prior clinically relevant hypersensitivity reaction to ruxolitinib or enasidenib.
3. Prior therapy with enasidenib in combination with ruxolitinib.
4. Concurrent use of strong inducers of CYP3A4 (Rifampin, St. John's Wort, Carbamazepine, Phenytoin) and/or the following strong inhibitors of CYP3A4 (protease inhibitor containing HIV anti-retrovirals, cobicistat, clarithromycin, itraconazole, ketoconazole, nefazodone, and telithromycin) are prohibited. Also prohibited are CYP2C9 substrate medications that have a narrow therapeutic range: phenytoin and warfarin.
5. Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form, which places the subject at unacceptable risk if he/she were to participate in the study or which confounds the ability to interpret data from the study.
6. Lactating females.
7. Active uncontrolled infections.
8. Patients with active malignancy of other type than required for this study are not eligible with the exception of currently treated basal cell, squamous cell carcinoma of the skin, or carcinoma "in situ" of the cervix or breast. Patients with malignancies with indolent behavior such as prostate cancer treated with radiation or surgery can be enrolled in the study as long as they have a reasonable expectation to have been cured with the treatment modality received.
9. Subject has significant active cardiac disease within 6 months prior to the start of study treatment, including New York Heart Association (NYHA) Class III or IV congestive heart failure (Appendix G) acute coronary syndrome (ACS); and/or stroke; or left ventricular ejection fraction (LVEF) < 40% by echocardiogram (ECHO) or multi-gated acquisition (MUGA) scan obtained within 28 days prior to the start of study treatment.

10. QTc interval (Fridericia's correction [QTcF]) > 450 ms

All inclusion and exclusion criteria will be reviewed by the Investigator or qualified designee to ensure that the patient qualifies for the trial.

9.0 SUBJECT RECRUITING AND SCREENING

Patients will be recruited from the sites participating in the MPN-RC. It is expected that a maximum of 32 patients will be recruited to this study. The estimated accrual rate is 2-3 patients per month.

9.1 STUDY-WIDE RECRUITMENT METHODS

Potential research subjects will be identified by a member of the patient's treatment or research teams. If the Investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the Investigator/research staff of the study. The extent of the risks, benefits, toxicities/side effects, alternatives/options for treatment, financial costs/burdens, and the voluntary nature of the study will be explained to patients. All patients will be required to sign a written informed consent prior to being registered on this protocol. Every effort will be made to answer questions raised by patients and their families or advocates regarding the protocol and alternative therapies prior to asking a patient to sign the consent form.

Patients of all races, both male and female, will be accepted into the protocol.

10. TREATMENT DOSAGE AND ADMINISTRATION

Patients who are on ruxolitinib will continue their current dose. Patients who are not on ruxolitinib will receive ruxolitinib per Table 1 dosing based on platelet count (modified package insert dosing). Enasidenib will be given at 100 mg daily on enrollment for patients with accelerated and blast phase myelofibrosis regardless of platelet count (this will be deemed Cycle 1). Patients with chronic phase myelofibrosis will receive Enasidenib at starting dose of 50 mg daily for cycle 1. The dose of Enasidenib will be increased to 100 mg for cycle 2 if the platelet count stays above 50,000 at the end of cycle 1 (Table 3a) and no other therapy emergent SAE of grade $\geq$2 developed (except grade 2 hyperbilirubinemia that is acceptable for dose escalation).

Ruxolitinib and enasidenib will be given orally in an outpatient setting unless the patient is being seen inpatient for another reason. Ruxolitinib and enasidenib will be given orally in 28-day cycles.

Patients should fast 2 hours before and 1-hour post enasidenib. Ruxolitinib may be administered independent of fasting and may be given with enasidenib. All protocol mandated visits will be performed at one of the participating institutions. Patients will return to the treating institution at a minimum of weekly during Cycles 1 and 2, every 4 weeks for Cycles 3 and beyond. Additional clinic visits will be mandated according to the patient clinical condition and at the discretion of the treating provider. Patients will have an end of study visit for a safety follow-up at 30 days after their last dose of therapy.

The dosages of ruxolitinib and/or enasidenib can be reduced, based on assessment of adverse events, if any occur, as described below in Section 10.1. Missed or vomited doses of ruxolitinib or enasidenib will not be made up.

Twenty-eight days is considered one cycle of therapy. Attempts will be made to provide an adequate treatment period with the combination of at least 6 cycles, unless significant toxicity is observed, to account for delayed time to response observed with biologic agents. Responders will continue therapy indefinitely unless progression of disease occurs, toxicity warranting discontinuation of therapy is

observed, or at the discretion of the treating physician. Patients will have an end of study visit for a safety follow-up at 30 days after their last dose of therapy.

10.1 DOSE MODIFICATION FOR NON-HEMATOLOGICAL AE'S, CLINICALLY SIGNIFICANT IN THE OPINION OF THE TREATING PHYSICIAN

Based on available pharmacokinetic and drug metabolism data, ruxolitinib is mainly metabolized by CYP3A4 with additional contribution from CYP2C9. Enasidenib inhibits CYP2C9 and induces CYP3A4. Although no adjustment is recommended when ruxolitinib is co-administrated with CYP3A4 inducer, potential drug-drug interactions might occur. This study will be the first time ruxolitinib and enasidenib will be used in combination for this patient population. Therefore, the safety of the combination will be confirmed in the first six patients who accrue. If no more than one patient out of the initial six has a treatment-related Grade 3 or higher adverse event attributable to the study drugs, the combination therapy will be considered safe. If more than one patient out of the initial six has limiting toxicity, the trial will be suspended, and the safety data will be further reviewed.

If drug-related Grade 3 clinically relevant non-hematologic toxicity is attributable to one or both of the drugs (based on known adverse events profile), dose interruption of that particular drug(s) is mandatory. Patients who experience Grade 3 drug related clinically relevant 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 before initiation of the next course. If attributable to ruxolitinib, dosing modifications in Tables 2a and 2b should be utilized. If attributable to enasidenib, dosing modification in Table 3b should be utilized. If a patient has drug-related clinically relevant Grade 4 non-hematologic toxicity, he/she will be taken off study. The dose of therapeutic agents can be decreased, at the discretion of treating physician, for chronic Grade 2 non-hematologic toxicity. Additionally, ruxolitinib dosage may be adjusted if patients develop decline in Creatinine clearance (CrCl) as calculated by the Cockcroft- Gault equation as follows: Reduce ruxolitinib dose by one dose level for patients with moderate (CrCl 30-59 mL/min) or severe renal impairment (CrCl 15-29 mL/min) if the patient is receiving a ruxolitinib dose of 10 mg BID or higher. (Per FDA package insert for ruxolitinib).

Myelosuppression and cytopenias are expected outcomes of MF disease processes and MF treatments and per se will not constitute adverse events except as follows: hematologic toxicity will be considered Grade 4 neutropenia with fever that does not clinically resolve within 14 days in the setting of optimal interventions or Grade 4 thrombocytopenia with clinically-significant bleeding. Dose interruption of the drug to which the adverse event is attributable is required. Patients may be given a subsequent course one dose level below the previous course, but the patient must have recovered to grade ≤ baseline at study entry. The dose of therapeutic agents can be decreased, at the discretion of treating physician, for chronic Grade 3 hematologic toxicity.

10.1.1 QT PROLONGATION

QTc interval has been observed in dogs at relatively low doses of enasidenib. Prolonged QTc interval has not been seen in other animal species at high doses. Subjects who experience prolongation of the heart-rate corrected QT interval, Fridericia's correction (QTcF) to > 480 msec (Grade ≥ 2) while treated with enasidenib, should be promptly evaluated for causality of the QTc prolongation and managed according to the following guidelines:

- Levels of electrolytes (potassium, calcium, and magnesium) should be checked and supplementation given to correct any values outside the normal range.
- Concomitant medications should be reviewed and adjusted as appropriate for medication with known QT prolonging effects.

- If no other cause is identified and the Investigator believes it is appropriate, particularly if QTc remains elevated (after above measures have been implemented, or as determined by the Investigator), investigational product may be interrupted, and an ECG should be rechecked in approximately 1 week after the QTc prolongation was first observed, or more frequently as clinically indicated.
    - If QTc has recovered or improved and the Investigator believes it is safe to do so, re-challenge with enasidenib should be considered if held. ECGs should be conducted at least weekly (e.g., at every scheduled visit) for 2 weeks following QTc reduction ≤ 480 msec.
    - If Grade 2 (QTcF> 480 and ≤ 500 msec), the dose of enasidenib may be reduced to a dose approved by the Study Chair without interruption of dosing. The enasidenib dose may be re-escalated to the prior dose in ≥ 14 days after QT prolongation has decreased to ≤ Grade 1.
    - If Grade 3 (QTcF> 500 msec), when QTc prolongation is first observed, hospitalization for continuous cardiac monitoring and evaluation by a cardiologist should both be considered. Dosing with enasidenib will be interrupted. If QTc returns to within 30 ms of baseline or < 450 msec within 14 days, treatment may be resumed at a reduced dose. The enasidenib dose cannot be re-escalated following dose reduction for Grade 3 QTcF prolongation unless the prolongation was associated with an electrolyte abnormality or concomitant medication.
    - If Grade 4 (QTcF> 500 msec or > 60 msec change from baseline with torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia), subjects should be admitted to hospital when QTc prolongation is first observed for continuous cardiac monitoring and be discharged only after review by a cardiologist. Dosing with enasidenib should be permanently discontinued.

10.1.2 ABNORMAL LEVEL OF LIVER ENZYMES (HYPERBILIRUBINEMIA)

Subjects with elevations in ALT ≥ 3-fold ULN, subjects will be monitored using the algorithm:

- Repeat LFT (i.e., ALT, AST, total bilirubin, ALP, GGT) within < 3 days of the initial test. Follow-up 2-3 times weekly, and weekly when stable.
- Perform additional diagnostic follow up:
    - Focused medical history, including review of prior history of liver or biliary disorders, concurrent symptoms, review of all concomitant medications (e.g., acetaminophen-containing medications, over-the-counter or herbal medications, nutritional supplements) including any changes in medications, detailed review of alcohol use.
    - Hepatitis serology (anti-HAV, HBsAg, anti-HBs, anti-HB core, anti-HCV, HCV RNA, EBV and CMV screen), and autoantibodies (e.g., ANA, anti-smooth muscle antibody).
    - Complete physical examination.
    - Liver ultrasound and follow-up imaging as appropriate.
    - Additional evaluation as appropriate (INR, PT).

Hyperbilirubinemia generally decreased in frequency as enasidenib treatment continued. \

Table 1. Ruxolitinib initial dose for patients' baseline platelet count by cohort*

Cohort	Plt>100 X10^9/L	Plt 50-100 X10^9/L	Plt 20-50 X10^9/L	Plt<20 X10^9/L
Blast/Accelerated Phase	20 BID	15 BID	10 BID	5 BID
Chronic Phase	15 BID	10 BID**	NA	NA

*Patients already receiving Ruxolitinib will begin trial at the dose they were on prior to combination therapy.
**Chronic phase patients with platelet<75 will not be enrolled to the trial.

Table 2a. Ruxolitinib Dose Adjustment for patients with accelerated and blast phase MF for NON-HEMATOLOGICAL AEs*

Dose level	PATIENTS' BASELINE PLATELET COUNTS			
	Plt>100 X10^9/L	Plt 50-100 X10^9/L	Plt 20-50 X10^9/L	Plt<20 X10^9/L
+1	25 BID	20 BID	NA	NA
0	20 BID	15 BID	10 BID	5 BID
-1	15 BID	10 BID	5 BID	5QD
-2	10 BID	5 BID	5 QD	Stop treatment
-3	5 BID	5 QD	Stop treatment	
-4	5 QD	Stop treatment		
-5	Stop treatment			

* For patients with accelerated and blast phase MF, treatment will continue at the same dose regardless of platelet count.

Table 2b. Ruxolitinib Dose Adjustment for patients with chronic phase MF for thrombocytopenia or non-hematological AEs

Dose level		DOSE ADJUSTMENT FOR THROMBOCYTOPENIA		
		Plt>100 X10^9/L	Plt 50-100 X10^9/L	Plt 20-50 X10^9/L
DOSE ADJUSTMENT FOR NON-HEMATOLOGICAL AEs	+1	20 BID	15 BID	N/A
	0	15 BID	10 BID	5 BID*
	-1	10 BID	5 BID	5 QD
	-2	5 BID	5 QD	Stop treatment
	-3	5 QD	Stop treatment	
	-4	Stop treatment		

Table 3a. Enasidenib Dose Adjustment for thrombocytopenia

MF chronic phase Cycle 1	MF chronic phase Cycle 2 and above	MF chronic phase Cycle 2 and above	MF chronic phase Cycle 2 and above	MF accelerated phase and blast phase
Platelet >75X10^9/L	Platelet > 50X10^9/L	Platelet 35-50X10^9/L	Platelet<35X10^9/L	Any platelet count
50mg	100mg	50mg	Hold	100mg

Table 3b. Enasidenib Dose Adjustment for non-hematological AE

Dose Level	**Enasidenib**
-1	50 mg QD
0	100 mg QD

10.2 DOSE MODIFICATIONS FOR HEMATOLOGICAL AE'S

For subjects receiving ruxolitinib and enasidenib, ruxolitinib dosages will be adjusted as detailed in **Table 2a** and **Table 2b**.

For chronic phase MF, if platelets are reduced to < 20X10^9/L ruxolitinib should be stopped

Ruxolitinib can be rechallenged at 5mg BID when platelets recover to >20X10^9/L, and enasidenib could be added the subsequent cycle as long as platelets recover to >50X10^9/L.

Interrupt treatment for absolute neutrophil count (ANC) less than 0.5 X 10^9 /L. After recovery of ANC above 0.75 X 10^9 /L, dosing may be restarted; restart dosing at 5 mg per day lower than the dose prior to the treatment interruption.

For patients with accelerated and blast phase MF, treatment will continue at the same dose regardless of platelet count or ANC.

10.3 DOSE ESCALATIONS

Patients may be escalated to the next higher dose level of ruxolitinib as per dose levels **in Table 2a** in the event that a patient's platelet count increases, after Cycle 3 of combined therapy or in case of suboptimal response in the opinion if the investigator. Patients with chronic phase MF will escalate the dose of enasidenib from 50mg to 100 mg in cycle 2 and ongoing if platelet count stays above 50,000 at the end of cycle 1 (Table 3a) and no other therapy emergent SAE of grade ≥2 developed (except grade 2 hyperbilirubinemia that is permitted).

Dose of ruxolitinib and enasidenib cannot be simultaneously increased in the same cycle. Dose escalation is permitted if ***ALL*** of the following criteria are met:

1. No dose reductions due to toxicity were required in the prior cycle
2. No dose interruptions due to toxicity were required in the prior cycle
3. The prior cycle was not delayed due to toxicity
4. The current cycle is not delayed due to toxicity

5. ANC ≥ 1.0 x 10^9/L
6. The patient has sub-optimal benefit, defined as a failure to reduce palpable spleen length by at least 50% and/or suboptimal improvement in disease- related symptoms as assessed by the Investigator.
7. No development of new on set transfusion dependency judged to be due to therapy

10.4 CONCOMITANT MEDICATIONS/TREATMENTS

All supportive measures consistent with optimal subject care will be given throughout the study. Concomitant medications will be captured in the Case Report Form (CRF) beginning at Screening. Packed red blood cell or platelet transfusions are allowed when necessary. Growth factor use (including erythropoietin) is not allowed with the exception of the use of filgrastim (G-CSF) or pegfilgrastim, which is permitted ONLY when used to treat febrile neutropenia or in patients who have prolonged (one week or more) Grade 3-4 neutropenia, after approval from the Study Chair.

Concurrent use of strong inducers of CYP3A4 (Rifampin, St. John's Wort, carbamazepine, phenytoin) and/or the following strong inhibitors of CYP3A4 (protease inhibitor containing HIV anti-retrovirals, cobicistat, clarithromycin, itraconazole, ketoconazole, nefadozone, and telithromycin) are prohibited. Also prohibited are CYP2C9 substrate medications that have a narrow therapeutic range: phenytoin and warfarin.

The following medications that are known to prolong QT interval are prohibited: amiodarone, arsenic trioxide, astemizole, azithromycin, bepridil, chloroquine, chlorpromazine, cisapride, citalopram, clarithromycin, disopyramide, dofetilide, domperidone, droperidol, erythromycin, escitalopram, flecainide, halofantrine, haloperidol, ibutilide, levomethadyl, mesoridazine, methadone, moxifloxacin, pentamidine, pimozide, probucol, procainamide, quinidine, sevoflurane, sotalol, sparfloxacin, terfenadine, thioridazine, or vandetanib.

Concomitant use of other cytotoxic chemotherapeutic agents (e.g. anagrelide) or other experimental drug or therapy while the subject is on study is prohibited, except for hydroxyurea.

Patients will be permitted to receive hydroxyurea while on study for a total of 3 cycles of combined therapy to control leukocytosis and thrombocytosis at the discretion of the treating physician. Patients who enter study on hydroxyurea will be permitted to remain on hydroxyurea for a total of 3 cycles of combined therapy. Patients who continue to require hydroxyurea after 3 cycles will be removed from study.

Steroids are allowed for the treatment of IDH-Inhibitor Differentiation Syndrome (IDH-DS), if warranted, as standard of care. The use of chronic low-dose steroids to treat an underlying medical condition that is not a malignancy is permitted during the course of study treatment

For patient's requiring ant-fungal prophylaxis, intravenous micafungin is the preferred agent. If micafungin is not feasible, the oral antifungal of choice would be posaconazole barring other patient specific factors that would preclude use of posaconazole.

Given the solubility profile of enasidenib, the exposure can be much lower for subjects with elevated gastric pH. Thus antacids, H2 blockers, or proton pump inhibitors should be used only if medically necessary and with at least 4 hours of elapsed time after enasidenib administration.

As enasidenib is an inhibitor of UGT1A1, the metabolism of drugs that are substrates for UGT1A1, including ezetimibe, raloxifene and raltegravir, may be slowed, leading to increased exposure to these compounds. Therefore, subjects on these drugs should be switched to lower doses or alternate therapies, if possible, or otherwise monitored for AEs associated with the respective products and elevations in bilirubin levels.

10.5 IDH-INHIBITOR DIFFERENTIATION SYNDROME (IDH-DS)

Subjects treated with enasidenib may develop signs and symptoms of a IDH-Inhibitor Differentiation Syndrome (IDH-DS). This may include any of the following: unexplained fever, skin rash, leukocytosis, thrombocytosis, hypoxia, respiratory distress, interstitial pulmonary infiltrates, pleural and/or pericardial effusion, peripheral edema, weight gain, or clinical deterioration. No single sign or symptom may be considered per se as diagnostic of the syndrome. It is recommended that the prophylactic and therapeutic measures indicated below be undertaken at the earliest manifestations of suspected IDH-Inhibitor Differentiation Syndrome (IDH-DS):

- Enasidenib may be held at the Investigator's discretion, and must be held if severe symptoms persist after 48 hours of systemic steroid administration.
- Prompt initiation of hydroxyurea at a suggested dose of 2 to 3 g PO two or three times daily.
- Prompt administration of corticosteroids at a suggested dose of 10 mg of dexamethasone intravenously (IV) every 12 hours until the disappearance of symptoms and signs, and for a minimum of 3 days.
- Initiation of furosemide per local standard practice, if clinically required.
- Prompt initiation of leukapheresis, if clinically required.

Once the signs and symptoms resolve and the subject's clinical condition improves, enasidenib may be reinitiated. The dose of enasidenib at re-initiation is to be discussed with the Study Chair.

For more information See Appendix D.

10.6 NON-INFECTIOUS LEUKOCYTOSIS

Initiation of treatment with the differentiating agents may lead to rapid WBC expansion not associated with infectious process and not manifesting with the signs and symptoms of IDH-Inhibitor Differentiation Syndrome (IDH-DS) discussed above.

In subjects with elevated WBC, prompt initiation of hydroxyurea is suggested, as per standard local practices (e.g., dose of 2 to 3 g PO twice or three times daily for WBC > 30 x 109/L). In case of severe leukocytosis (WBC >100 x 109/L), use of leukapheresis may be appropriate. Subject should be regularly monitored for changes in WBC count and for new signs and symptoms of infection or IDH-Inhibitor Differentiation Syndrome (IDH-DS).

10.7 DISCONTINUATION OF RUXOLITINIB AND ENASIDENIB

Upon planned discontinuation of ruxolitinib it is recommended to taper off the drug over the course of 4-7 days and permissible to use prednisone daily during this period to blunt rebound of symptoms. Since patients will be on varying dosing regimens of ruxolitinib, investigators are urged to discuss the ruxolitinib taper plan with Study Chairs.

If a patient has to have ruxolitinib held abruptly for the occurrence of adverse events (e.g. major bleeding, significant thrombocytopenia, etc.), then the ruxolitinib may be stopped WITHOUT a taper and a pulse of prednisone may be utilized if deemed necessary by the Investigator.

Enasidenib may be interrupted or stopped without tapering.

10.8 EVALUATION DURING TREATMENT/INTERVENTION

Baseline evaluations are to be conducted within 28 days of the first study drug administration, unless otherwise indicated within the Schedule of Assessments outlined in **Table 4**. All scheduled visits will have a ±7 days window unless otherwise stated or indicated within the Schedule of Assessments.

11. WHEN AND HOW TO WITHDRAW SUBJECTS

In accordance with the Declaration of Helsinki and the guidelines of the country of the participating MPN-RC Clinical Study Center, each subject is free to withdraw from the study at any time. An Investigator also has the right to withdraw a subject from the study in the event of the patient suffering an intercurrent illness, adverse events, or other reasons concerning the health or well-being of the patient, or in the case of lack of cooperation by the patient. All serious adverse reactions need to be followed up until resolution and information returned to study coordinators.

Should a subject decide to withdraw after administration of study drug, or should the Investigator decide to withdraw the subject, all efforts should be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A final evaluation, the reason for, and the date of withdrawal must be recorded on the CRF. The last visit for each subject will be defined as a study discontinuation/ end-of-study visit, which will occur 30 (±7 days) days after time of treatment withdrawal.

Subjects with clinically significant abnormal laboratory values as determined by the Investigator or who have ongoing clinically significant treatment-related adverse events during their last scheduled clinical evaluation will be monitored and treated until resolution or stabilization is achieved; or, in the event that the subject's condition is not likely to improve because of disease progression, until the cause of the abnormal test result or adverse event can be determined.

11.1 DATA COLLECTION AND FOLLOW-UP FOR WITHDRAWN SUBJECTS

The reason for and date of withdrawal from study drug treatment; and the reason for and date of withdrawal from the study will be recorded on the case report form (CRF). If a subject withdraws consent, every attempt will be made to determine the reason. If the reason for withdrawal is an adverse event or a clinically significant abnormal laboratory test result, monitoring will continue until the event has resolved or stabilized, until the subject is referred to the care of a local health care professional, or until a determination of a cause unrelated to the study drug or study procedure is made. The specific event or test result(s) must be recorded on the CRF. All evaluations will be performed, according to the protocol, 30 days (±7 days) after the patient is withdrawn from treatment.

Patients will continue to be followed for long term survival every 3 months by telephone encounter or medical record review to determine the following variables: alive versus deceased and type of current therapy. Patients will be followed for this long term survival data until death, confirmed lost to follow up, or a maximum of 1 year after end of treatment.

A subject will be considered to be lost to follow-up if all of the following criteria have been met:

1. More than three phone calls to the subject are unanswered.
2. A next of kin is contacted and no information is available.
3. The referring physician is contacted and no information is available.
4. A telegram or certified letter is unanswered.

11.2 DURATION OF FOLLOW-UP

The patient will have an end of study visit at 30 days after discontinuation of the protocol treatment, with a window of ±7 days, to assess for AEs.

Patients will continue to be followed for long term survival every 3 months by telephone encounter or medical record review to determine the following variables: alive versus deceased and type of current therapy. Patients will be followed for this long term survival data until death, confirmed lost to follow up, or a maximum of 1 year after end of treatment.

11.3 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

Patients will be removed from therapy when any of the criteria listed in When and How to Withdraw Subjects section apply. Notify the Principal Investigator and document the reason for study removal and the date the patient was removed in the Case Report Form.

11.4 PATIENT ACCRUAL

Subjects are considered evaluable as long as they have signed informed consent, are eligible for participation and started study treatment. The maximum sample size in the Phase II (accelerated/blast-phase) is 20 evaluable patients. Allowing for enrollment of up to 2 additional patients to account for ineligibility, cancellation, major treatment violation, or other reasons , this study will enroll a maximum of 22 patients in the Phase II AP/BP cohort. The maximum sample size in the chronic phase MPN is 9 evaluable patients. Allowing for enrollment of up to 1 additional patient, this study will enroll a maximum of 10 patients in the descriptive chronic phase MPN cohort. Therefore, a maximum total of 32 (22 +10) patients will be enrolled in the trial.

12.0 STUDY TIMELINES

Baseline evaluations are to be conducted within 30 days of the first study drug administration, unless otherwise indicated within the Schedule of Assessments outlined in **Table 4**. All scheduled visits will have a ±7 days window unless otherwise stated or indicated within the Schedule of Assessments outlined below.

Ruxolitinib and enasidenib will be given orally in 28- day cycles. Attempts will be made to provide an adequate treatment period with the combination of at least 6 cycles, unless significant toxicity is observed, to account for delayed time to response observed with biologic agents. Interval Milestone Response Assessment (IMRA) can be performed at the beginning of any cycle after cycle 1 if specific milestones are achieved that will prompt such an evaluation. Specific milestones that will prompt assessment consist of clearance of blasts from peripheral blood in accelerated/blast phase patients, which will prompt bone marrow aspirate and biopsy. In addition progression of disease (as determined by an increase in blasts in the peripheral blood - defined as doubling of blasts % and achieving more than 10% in the chronic phase patients at least 7 days apart) will prompt IMRA. Responders will continue therapy indefinitely unless progression of disease occurs, toxicity warranting discontinuation of therapy is observed, or at the discretion of the treating physician. Patients will have an end of study visit for a safety follow-up at 30 days after their last dose of therapy.

13.0 STUDY ENDPOINTS

13.1 PRIMARY ENDPOINT

- The proportion of treated accelerated-phase and blast-phase MPN patients (primary cohort) that achieve a best response of either complete response (CR), Partial Response (PR), or complete response with incomplete recovery of counts (CRi), when treated with the combination of ruxolitinib with enasidenib within 6 cycles of combined therapy.

13.2 SECONDARY ENDPOINTS

- To assess the efficacy of the combination of ruxolitinib with enasidenib in patients with accelerated-phase and blast-phase disease using complete (CBR) and partial blast response (PBR).

- The proportion of treated patients with MF-CP and 4%-9% circulating blasts that achieve complete response (CR) Partial Response (PR), clinical improvement (CI) with the combination of ruxolitinib and enasidenib within 6 cycles of combined therapy.
- To assess the impact of the combination of ruxolitinib with enasidenib on the overall survival of advanced phase MPN patients

13.3 SAFETY ENDPOINTS

- The proportion of patients developing treatment-emergent hematological and non-hematological adverse events for the combination of ruxolitinib and enasidenib.

13.4 EXPLORATORY ENDPOINTS

- The proportion of patients who achieve improvement quality of life as assessed by the by the Myelofibrosis Symptom Assessment Form (MF-SAFv4.0) with the combination of ruxolitinib and enasidenib
- Assess the pharmacodynamics of ruxolitinib and enasidenib when combined in this population.
- Assess the effects of combined therapy on gene expression and global methylation status.
- Assess changes in mutant allele burden and clonal architecture of disease.

14.0 STUDY PROCEDURES

Study Design

This is a multicenter, Phase II trial designed to assess the effect of ruxolitinib and enasidenib combination in subjects with accelerated or blast-phase MPN and subjects with chronic phase Primary Myelofibrosis, Post Polycythemia Vera, or Post Essential Thrombocythemia Myelofibrosis (PMF, post-PV MF, or post-ET MF) with 4-9% blasts. Patients will receive ruxolitinib and enasidenib orally on days 1-28 of a 28-day cycle.

Specifically, a Phase II Simon min-max design will be conducted in accelerated or blast-phase MPN patients (20 patients) and a descriptive cohort of up to 9 chronic phase patients.

Intervention

Patients who meet eligibility criteria will be treated with ruxolitinib and enasidenib orally on days 1-28 of a 28-day cycle. Cycles will be continued until the patient wishes to be removed from the study, unacceptable toxicity develops, disease progression, treating physician recommends removal, or termination of study occurs.

14.1 SCREENING/BASELINE PROCEDURES

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed within 30 days prior to registration unless otherwise stated. The screening procedures include:

- Patient Informed Consent

  Before a patient undergoes screening, a medically qualified member of the Study Team who has been specifically trained in the implementation of this Protocol during the Site Initiation Visit or thereafter, such as the Site Principal Investigator, any of the co-Investigators, or a Clinical

Research Nurse must obtain documented consent from each potential patient prior to participating in a clinical trial.

Consent must be documented by the patient's dated signature or by the patient's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the patient before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the patient must receive the IRB/EC's approval/favorable opinion in advance of use. The patient or his/her legal representative should be informed in a timely manner if new information becomes available that may be relevant to the patient's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form that captures the patient's or legal representative's dated signature. The informed consent will adhere to IRB/EC requirements, applicable laws and regulations and Sponsor requirements.

- Demographics (age, gender, race, ethnicity)
- Medical History (A medical history will be obtained by the Investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the patient has enrolled in this study will be recorded separately and not listed as medical history).
- Prior Treatment Details (The Investigator, or qualified designee, will review all prior cancer treatments including systemic treatments, RT and surgeries)
- Bone marrow biopsy and aspirate with cytogenetics and molecular genetic profiling for presence of JAK2 (or MPL or CALR mutation status in the case that no JAK2 mutation is detected) as well as IDH2 mutation status. Bone marrow biopsy obtained within 84 days prior to screening in the absence of disease modifying therapy may be used for determining eligibility. Mutational profiling may be carried out at local laboratories and does not require central laboratory confirmation.
- Physical exam including vital signs, body weight and height, and spleen and liver measurements
- Transfusion history for 3 months prior to Day 1
- Review previous concomitant medications within 90 days of screening and all myeloproliferative neoplasms directed therapies since time of diagnosis
- Complete Blood Count (CBC) with differentials (includes platelet count)
- Chemistries (LDH, total and direct bilirubin, phosphorus, albumin, uric acid, Na, K, Cl, Mg, CO2, BUN, Cr, glucose (random), Ca, AST, ALT, Alk Phos, total protein)
- Lipid Panel
- Urinalysis
- Pregnancy test, urine or blood, with a sensitivity of at least 50 mIU/mL (for FCBP only)
- Eastern Cooperative Oncology Group (ECOG) performance status
- ECG
- Spleen volume assessment by CT scan or MRI
- Cardiac function assessment (LVEF) by ECHO or MUGA
- Review patient eligibility criteria

14.2 REGISTRATION PROCEDURES

Only MPN-RC approved centers may participate and register patients to this study.

To register a potential participant, complete the Registration Form for the trial located at the study REDCap link provided by the Mayo Statistical Data Center. This form confirms eligibility criteria and informed consent and contains basic demographic and clinical information. Once eligibility is confirmed and informed consent has been obtained, registration of a patient in the trial can occur. After completion of this form, a unique patient ID will be assigned by the system. An email notification will be sent to the MPN-RC Administrative and Statistical and Data Center core for each registered patient.

14.2.1 SITE REGISTRATION WITH MPN-RC

Before an MPN-RC institution can enroll patients, protocol specific regulatory documents must be submitted to the MPN-RC Central Coordinating Office via E-mail (PDF file preferred) or fax:

MPN-RC Central Coordinating Office
Phone: (212) 241-9138
Fax: (212) 876-5276
MPNRC_ClinicalTrialCentralOffice@mssm.edu

Required Protocol Specific Regulatory Documents (E-mailed by PDF format preferred):

1. Submit a copy of IRB approval for MPN-RC 119
2. Submit a copy of the IRB approved consent form for MPN-RC 119
3. Submit a copy of the CV of the investigational pharmacist and Pharmacy license
4. Submit a copy of the site's drug destruction policy
5. Submit a copy of a signed and dated CV and medical license of the Principal Investigator
6. Submit a copy of Form FDA 1572 as completed by the Principal Investigator

Before an MPN-RC institution can enroll patients, please contact the Clinical Central Office (e-mail: MPNRC_ClinicalTrialCentralOffice@mssm.edu), to ensure that a slot is available for enrollment prior to approaching a patient about possible participation in this trial.

This study uses the MPN-RC on-line Patient Registration system (https://redcap2.mayo.edu/redcap/). Registration will be accepted only through use of the MPN-RC database. Registration must occur prior to the initiation of therapy.

Confirm eligibility criteria and complete the on-line Registration Worksheet.

If the registering clinical research coordinator (CRC) requires assistance, he/she may consult the on-line help file located under the Help menu of the MPN-RC application or call the central office.

When the patient is registered to MPN-RC 119, a patient identification number will be generated. Registration will not be completed if eligibility requirements are not met for the selected trial.

The registering institution will receive a Confirmation of Registration email from the database that should be printed and included in the patient chart. If a registering institution needs to correct the registration form, contact the MPN-RC Statistics and Data Center at Mayo (DLARZmpnrcstats@mayo.edu) and the Clinical Central Office: E-mail: MPNRC_ClinicalTrialCentralOffice@mssm.edu.

Before Study Drug Shipment

Before Study Drug can be shipped to the MPN-RC center to begin accruing patients, the above regulatory documents are required to be emailed to the MPN-RC Central Office to the attention of the Clinical Trials Manager, as noted above.

14.3 INFORMED CONSENT

The patient must be aware of the neoplastic nature of his/her disease, as well as of the expected life expectancy at his/her stage of disease. The patient must willingly consent after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. BRANY Institutional Review Board (or other local ethics committees for non-US sites) approval of this protocol and of its consent form is required.

14.4 DATA MANAGEMENT

After registration of a patient, additional case report forms in the study database will be available for completion according to the Schedule of Events (Table 4). All clinical data and obtained specimens will be labeled with the patient ID assigned at registration for the entire duration of the subject's participation.

Data for all clinical variables will be stored in the REDCap database. Access to the REDCap database will be limited to MPN-RC investigators and designees; users at each site will only be able to view the records pertaining to subjects enrolled at their particular site.

15. TREATMENT PERIOD

The subject must be enrolled into the study, at the latest 30 days from signing the ICF, following Sponsor review of eligibility. The Treatment Period begins at Cycle 1 Day 1 and ends at the End-of-Treatment Visit. During the Treatment Period, all subjects will be closely monitored for safety. Toxicities (AEs and laboratory abnormalities) will be evaluated and graded according to the CTCAE. During this period the following assessments should occur:

- Bone marrow biopsy and aspirate with cytogenetics and molecular genetic profiling for presence of JAK2 (or MPL or CALR mutation status in the case that no JAK2 mutation is detected) as well as IDH2 mutation status. This is done at C4 D1 (for all accelerated patients, may be done at the discretion of the Investigator for chronic phase patients), C7 D1 (for all patients), C10 D1(for accelerated phase patients, may be done at the discretion of the Investigator for chronic phase patients), and every 6 cycles, thereafter (for all patients).
- Transfusion history will be collected on Day of each cycle
- Pregnancy Test
- A physical exam including a review of current medical conditions and medications
- Vital signs (heart rate, breathing rate, blood pressure, temperature, weight, and height)
- ECOG performance status
- Adverse events and concomitant medications
- Complete blood counts and serum chemistry
- Spleen volume assessment by CT/MRI at C7 D1, and C10 D1
- ECG, day 1 of each cycle until Cycle 7
- Research blood sample collection
- MF-SAFv4.0 questionnaire (paper or electronic)
- Enasidenib administration
- Medication diary collected and new one dispensed

15.1 END OF TREATMENT VISIT

The post-treatment period includes the time after the End of Treatment Visit until the End of Study Visit. This period is 30 days. The following assessments should occur:

- Bone marrow biopsy and aspirate with cytogenetics and molecular genetic profiling for presence of JAK2 (or MPL or CALR mutation status in the case that no JAK2 mutation is detected) as well as IDH2 mutation status. Mutational profiling may be carried out at local laboratories and does not require central laboratory confirmation.
- Physical exam
- Vital signs, body weight and height, and spleen and liver measurements
- Concomitant medications and adverse events
- CBC and differentials
- Serum chemistry
- ECG
- Spleen volume assessment by CT scan or MRI
- Research blood sample collection
- MF-SAFv4.0 questionnaire (paper or electronic)

15.2 END OF STUDY VISIT

The visit is the mandatory safety follow-up visit and should be conducted approximately 30 days after the end of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the End of Study Visit should be recorded.

Table 4. Schedule of Events

Procedure	Screening Period	Cycle1-2				Cycle 3-6	Cycle 7 and beyond	End of treatment	End of Study[11]	Survival Follow Up
		Day 1[5]	Day 8	Day 15	Day 22	Day 1	Day 1			
Informed Consent	X									
Medical History (including prior MF-directed therapies)	X									
Physical examination[1]	X	X		X		X	X	X	X	
Transfusion History	X[2]	X[2]				X	X	X	X	
Pregnancy test (for FCBP only)[3]	X	X	X	X	X	X	X	X	X	
CBC and differentials	X	X	X	X	X	X	X	X	X	
Serum Chemistry[4]	X	X	X	X	X	X	X	X	X	
Urinalysis	X									
12-LeadECG[14]	X	X	X	X	X	X	X	X	X	
Echo/MUGA	X									
Bone Marrow Aspirate and Biopsy[6,7]	X[6]					X[6,7]	X[6,7]		X[6]	
Biospecimen Collection– Peripheral blood	X[12]	X[12]				X[12]	X[12]		X[12]	
Adverse event evaluation		X		X		X	X	X	X	
Concomitant medications, therapies, and procedures evaluation	X	X		X		X	X	X	X	
Spleen Volume Assessment[8]	X						X[8]		X	
Response Assessment							X[9, 13]		X	

Nail Clippings	X									
Study drug dispensing[10]		X				X	X			
MF-SAFv4.0 Survey		X	X	X	X	X	X	X	X	
Survival Data[15]										X

1. Includes vital signs, weight, height and measurements of the spleen and liver. Height is only required at screening.
2. Subjects must have 3 months documented transfusion history prior to Day 1.
3. Pregnancy test, urine or blood, with a sensitivity of at least 50 mIU/mL, within 10 – 14 days prior to and again within 24 hours of starting enasidenib; then every week x 4, then monthly for all FCBP with regular menstrual cycles, or every 2 weeks if irregular cycles.
4. Includes sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, total bilirubin, alanine aminotransferase, , and glucose. Magnesium, , PT/PTT and INR need to only be done at screening. Lactate dehydrogenase uric Acid, phosphorus need to be checked day 1 of every cycle and in the EOS
5. If not done within 3 days of Day 1.
6. Bone Marrow Aspirate and Biopsy to be collected at baseline (within 84 days from screening is also permissible with permission from the Study Chair), Cycle 4 day 1 (at the discretion of the Investigator for chronic phase patients), Cycle 7 day 1, Cycle 10 day1(at the discretion of the Investigator for chronic phase patients), every 6 cycles, thereafter, and at the end of study visit. Peripheral blood draws may be used to perform JAK2 mutation testing if the bone marrow is not aspirable.
7. May be omitted if so decided by the treating physician. Should be done to confirm complete response and when clinically indicated.
8. CT scan or MRI to be performed at baseline, Cycle 7, Cycle 10, and at the end of study.
9. Response assessments (IWG-MRT criteria 2013 and ELN revised response criteria) should be after 6 cycles and every 3 - 6 cycles thereafter for the remainder of the study.
10. Collect pill diaries and any unused study drug to review for compliance prior to each cycle through cycle 24. After cycle 24, review patient drug diaries for ruxolitinib and enasidenib at each protocol visit.
11. The discontinuation visit will be considered as such on the date the patient is taken off treatment. The patient will have an end of study visit approximately 30 days after the end of trial treatment to assess for AEs.
12. Peripheral blood biomarker samples will be collected at screening, and day 1 of Cycle 1 -Cycle 7, every 3 cycles thereafter, and at the end of study, in conjunction with standard-of-care blood draws. Bone marrow aspirate samples will be collected whenever a bone marrow examination is scheduled to take place as designated in item 6 above.
13. Interval Milestone Response assessment can be performed at the beginning of any cycle after Cycle 1 if specific milestones are achieved as specified in study timeline.
14. ECGs done day 1 of each cycle until Cycle 7, ECGs should be done at EOS.\
15. Patients will continue to be followed for long term survival every 3 months by telephone encounter or medical record review to determine the following variables: alive versus deceased and type of current therapy. Patients will be followed for this long term survival data until death, confirmed lost to follow up, or a maximum of 1 year after end of treatment.

16. ADVERSE EVENTS

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or

death.

16.1 DEFINITIONS OF ADVERSE EVENT

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values, regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded rather than the individual signs or symptoms of the diagnosis or syndrome

16.2 SEVERITY OF ADVERSE EVENT

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events version 5.0 (CTCAE). The CTCAE is available at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (Grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (Grade 2): the event causes discomfort that affects normal daily activities.
- Severe (Grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (Grade 4): the patient was at risk of death at the time of the event.
- Fatal (Grade 5): the event caused death.

16.2.1 SERIOUS ADVERSE EVENTS (SAE)

An adverse event is considered serious if it results in ANY of the following outcomes:

- Results in death. If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- Is life-threatening. (The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect
- Is an important medical event

Events not considered SAEs are hospitalizations for:

1. A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
2. Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
3. The administration of blood or platelet transfusion as routine treatment of studied Indication. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.

4. A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
5. Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
6. A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.
7. An elective treatment of or an elective procedure for a pre-existing condition unrelated to the studied indication.
8. Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE page/screen of the CRF and the MEDWATCH form must be completed. For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to IP, action taken regarding IP, and outcome.

- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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 (21CFR312.32).

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 investigator.

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is not the same as "serious" which is based on subject/event outcome or action criteria associated with events that pose a threat to a subject's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

16.2.2 ADVERSE EVENTS OF SPECIAL INTEREST (AESI)

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring of patients and rapid communication by the Investigator to the Sponsor. An AESI may be serious or non-serious. The rapid reporting of AESIs allows ongoing surveillance of these events in order to characterize and understand them in association with the use of this investigational product.

AESIs for enasidenib include but are not limited to events with AESIs observed with enasidenib include those specified in the IB.

16.3 CAUSALITY

The Investigator must determine the relationship between the administration of IP and the occurrence of an AE/SAE as unrelated, possible, probable or definite as defined below.

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.

- Possible – The AE *may be related* to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

When a determination of possibly, probable or definite is used, the Investigator should also determine whether the suspected agent is ruxolitinib or enasidenib.

16.4 DURATION

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

16.5 ACTION TAKEN

The Investigator will report the action taken with IP as a result of an AE or SAE, as applicable (e.g., discontinuation, interruption of IP, as appropriate) and report if concomitant and/or additional treatments were given for the event.

16.6 OUTCOME

The Investigator will report the outcome of the event for both AEs and SAEs. All SAEs that have not resolved upon discontinuation of the subject's participation in the study must be followed until recovered (returned to baseline), recovered with sequelae, or death (due to the SAE).

16.7 OVERDOSE

Overdose, as defined for this protocol, refers to enasidenib and ruxolitinib dosing only (as applicable).

On a per dose basis, an overdose is defined as the following amount over the protocol-specified dose of enasidenib and ruxolitinib assigned to a given patient, regardless of any associated adverse events or sequelae - PO any amount over the protocol-specified dose. On a schedule or frequency basis, an overdose is defined as anything more frequent than the protocol required schedule or frequency. . Complete data about drug administration, including any overdose, regardless of whether the overdose was accidental or intentional, should be reported.

To date, doses of enasidenib up to 650 mg QD were well tolerated in clinical trials. No information is currently available regarding overdose with enasidenib. In the event of overdose with toxicity, supportive clinical care should be provided.

16.8 ABNORMAL LABORATORY VALUES

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of IP dose, or any other therapeutic intervention; or
- is judged to be of significant clinical importance, e.g., one that indicates a new disease process and/or organ toxicity, or is an exacerbation or worsening of an existing condition.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (e.g., record thrombocytopenia rather than decreased platelet).

17. REPORTING REQUIREMENTS FOR ADVERSE EVENTS

All adverse events must be recorded in the subject's medical records and on the Case Report Form from the time the subject signs informed consent until 30 days after the last dose of IP and those SAEs made known to the investigator at any time thereafter that are suspected of being related to IP. The onset and end dates, severity, duration, effect on study drug administration (e.g. discontinuation), relationship to study drug, and administration of any other drug(s) to treat this event will be recorded for each adverse event. The BRANY Institutional Review Board is charged with the responsibility of reviewing and maintaining records of all Serious Adverse Events occurring in patients who are participating in BRANY IRB approved research trials at BRANY sites. All SAEs must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form.

17. 1 STEPS TO DETERMINE IF AN ADVERSE EVENT REQUIRES EXPEDITED REPORTING (WITHIN 24 HOURS)

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events version 5.0 (CTCAE).

Step 2: Grade the adverse event using the NCI CTCAE v5.0.

Step 3: Determine whether the adverse event is related to the protocol therapy

Attribution categories are as follows:

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

  Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

17.2 EXPEDITED REPORTING

The Site must notify the Central Office and the local IRB within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug. A Serious Adverse Event (SAE) MEDWATCH form must be completed by the Investigator and emailed to the MPN-RC Central Office within 24 hours of learning of the SAE. The Site Investigator will print and keep a copy of this SAE form on file at the study site. Report serious adverse events by phone, email, or facsimile to the Data Monitor and Clinical Trials Manager:

MPN-RC Central Office Phone: (212) 241-4546
Fax: (212) 876-5276
Email: MPNRC_ClinicalTrialCentralOffice@mssm.edu

Central Office personnel is responsible for reporting all SAEs to Celgene via MedWatch to drugsafety@celgene.com.

At the time of the initial report, the following information should be provided:

- Study name and IND#
- Site name or site #
- Subject ID number
- A description of the event
- Date of onset
- Current status of patient
- Whether study treatment was discontinued
- CTCAE grade
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment (if related or not and if expected or not)

Within the following 48 hours, the Investigator must provide further information about the serious adverse event in the form of a written narrative. This should include an updated Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the Study Sponsor.

- The site must notify the central IRB, BRANY, within 5 business days of "any unanticipated problems involving risk to subjects or others" (UPR/UPIRSO). Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder. The IRB should only be notified of a SAE if it meets the definition of "possibly, probably or definitely" related to the study drug, and is an "unexpected" event.

  The following events meet the definition of UPR:

  - Any new information that indicates a new or increased risk, or safety issue (e.g., Interim Analysis, Safety Monitoring report, publication, updated Sponsor Safety report), that indicates an unexpected change to the risk/benefit ratio for the research.
  - An Investigator Brochure, package insert, or device labeling is revised to indicate an increase or magnitude of a previously known risk or describes a new risk.
  - Withdrawal, restriction, or modification of a marketed approval of a drug, device, or biologic used in research protocol
  - Protocol deviation or violation that harmed subjects or others or that indicated subjects or others might be at increased risk of harm.
  - Complaint of subject that indicates subjects or others might be at increased risk of harm or at risk of a new harm
  - Any breach in confidentiality that may involve risk to the subject or others.
  - Any harm experienced by a subject or other individual that in the opinion of the Investigator is unexpected and at least probably related to the research procedures.

For IND/IDE trials: The Regulatory Agencies (FDA) should be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

The MPN-RC Data Center and Regulatory Coordinator will receive reports of SAEs through the Central Office. The MPN-RC as Study Sponsor shall notify the Regulatory Agencies by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the Sponsor's original receipt of the information(1-800-332-0178) of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the Sponsor's original receipt of the information. All other unexpected, serious adverse events that are considered related to study treatment will be reported on a MedWatch form by the Study Sponsor to the Regulatory Agencies within 15 calendar days. If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the Study Sponsor will submit the adverse event in a written report to the FDA and Health Canada as soon as possible, but no later than 15 calendar days from the time the determination is made.

17.3 REPORTING GUIDELINES FOR PHARMACEUTICAL PARTNER

For the purpose of regulatory reporting, Celgene Drug Safety will determine the expectedness of events of being related to enasidenib based on the Investigator Brochure. In the United States, all suspected unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner in accordance with 21 CFR 312.32.

Serious adverse events (SAE) are defined above. The Central Office must inform Celgene in writing using a MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours. The initial report must be as complete as possible, including 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 report. A final report to document resolution of the SAE is required. The Celgene tracking number (XX-XX-XX- PI-#####) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

Drug Safety Contact Information:
Celgene Corporation
Global Drug Safety and Risk Management
86 Morris Avenue
Building S12
Summit, New Jersey 07901
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

For any Canadian sub-sites:
Celgene Inc.
Drug Safety Department
6755 Mississauga Road, Suite 600
Mississauga, ON L5N 7Y2
Telephone: 289-291-4838
Fax: 289-291-4820
Email: drugsafety-canada@celgene.com

17.4 MPN-RC REPORTING OF SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS (SUSARS) TO REGULATORY BODIES AND ETHICS COMMITTEES

All suspected adverse reactions related to an investigational medicinal product (the tested IMP) which occurs in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. These events should be reported by the individual investigators to the MPN-RC.

17.5 MPN-RC REPORTING OF SUSARS

MPN-RC, or designee, should report all the relevant safety information previously described to the concerned Regulatory Agencies. MPN-RC or designee shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects. All SUSAR reports will also be reported to the FDA and Health Canada.

17.6 TIMELINES FOR REPORTING

Fatal or life-threatening SUSARs

The MPN-RC should notify the Regulatory Agencies as soon as possible but no later than 7 calendar days after the Sponsor has first knowledge of the minimum criteria for expedited reporting. In each case relevant follow-up information should be sought and a report completed as soon as possible.

Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues must be reported to the Regulatory Agencies as soon as possible but no later than 15 calendar days after the Sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

17.7 HOW TO REPORT SAFETY EVENTS

Minimum criteria for initial expedited reporting of SUSARs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted by the Sponsor within the time limits as soon as the minimum following criteria are met:

a. a suspected investigational medicinal product,
b. an identifiable subject (e.g. study subject code number),
c. an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,
d. an identifiable reporting source, and, when available and applicable:
   i. a unique clinical trial identification number or the Sponsor's trial protocol code number)
   ii. a unique case identification (i.e. Sponsor's case identification number).

Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. The Sponsor should report further relevant information after receipt of

follow-up reports. In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

Routine Reporting

All other adverse events, such as those that are expected or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

18. DRUG INFORMATION

All study treatments will be administered on an outpatient basis. No investigational or commercial agents or therapies other than those described herein may be administered with the intent to treat the patient's malignancy while they are on this study.

18.1 RUXOLITINIB

Ruxolitnib is a JAK inhibitor approved for the treatment of patients with MF. It will be dispensed commercially, and is available as tablets formulated in 5, 10, 15 and 20 mg strengths. In the US, a 25 mg strength is also available. See the applicable product label (i.e., USPI, Canadian PM or the EMA SmPC) for more details.

18.1.1 SIDE EFFECTS

Reported toxicity of ruxolitinib includes the following:

- Hematologic adverse events include; anemia, thrombocytopenia and neutropenia, all of which are dose related effects.
- Non-hematologic adverse events include; bruising, dizziness, headache, raised ALT, raised AST, hypercholesterolemia, urinary tract infections, weight gain, flatulence, herpes zoster, increase in systolic blood pressure, constipation, infection, including Tuberculosis

18.2 ENASIDENIB

Enasidenib tablets are available in 50-, 100-, 150- and 200-mg free-base equivalent strength tablets for oral administration. Each tablet is formulated using excipients that are generally regarded as safe and are used in marketed drug products. Celgene Corporation will supply 50-mg and 100-mg tablets.

All tablets will be packaged in high density polyethylene (HDPE) bottles with a desiccant (silica gel) canister and child resistant closures with heat induction seal. All tablets should be swallowed whole and should not be broken or chewed.

Bottles of enasidenib tablets must be stored according to the package label. The storage area should be secure and have limited access. Enasidenib tablets will be monitored by the Sponsor for stability for the duration of the study.

18.2.1 SIDE EFFECTS

Reported specific adverse drug reactions of enasidenib includes the following:

- Hematologic adverse events include: neutropenia and leukocytosis
- Non-hematologic adverse events include: IDH-DS

TLS, GI disturbances (such as nausea, diarrhea, vomiting, and associated dysgeusia and decreased appetite), and hyperbilirubinemia.

18.2.2 STUDY DRUG PACKAGING AND LABELING

Enasidenib tablets will be supplied in high density polyethylene (HDPE) bottles with a desiccant (silica gel) canister, a polyester coil, and child resistant closures with heat induction seal. Packaging and labeling will be prepared to meet all regulatory requirements.

18.2.3 STUDY DRUG STORAGE

Bottles of enasidenib tablets must be stored according to the package label.

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

18.2.4 RECEIVING DRUG SUPPLY, RECEIPT AND STORAGE

Drug will be distributed from Celegene to each participating site. Tablets are to be stored according to guidance in drug label insert.

18.2.5 RETURN OR DESTRUCTION OF STUDY DRUG

Destruction of any remaining study drug will occur according to Mount Sinai Investigational Drug Services' standard operating procedures.

18.3 RISKS TO SUBJECTS

Adverse events listed in current investigational brochures research medications:

Both ruxolitinib and enasidenib have known side effects profile in patients with myelofibrosis and AML respectively. Please refer to to the investigator brochures for detailed listings.

Known potential side effects from **Ruxolitinb** in humans include the following:

Hematologic: Anemia (72% to 96%; Grade 3: ≤34%; Grade 4: ≤ 11%), Thrombocytopenia (27% to 70%; Grade 3: 5% to 9%; Grade 4: ≤4%), Neutropenia (3% to 19%; Grade 3: 5%; Grade 4: ≤2%) all of which are dose-related effects.

Non-hematologic: Dizziness (15% to 18%), Headache (15% to 16%), Fatigue (15%), Insomnia (12%), Weakness (7%), Bruise (23%), Epistaxis (6%), Pruritus (14%), Increased serum cholesterol (17% to 35%), Hypertriglyceridemia (15%). Diarrhea (15%), Abdominal pain (15%), Constipation (8%), Nausea (6%), Flatulence (5%), Vomiting. Increased serum ALT (25%; Grade 3: <1%), Increased serum AST (17% to 23%). Muscle spasm (12%); Weight gain (≤7%). Dyspnea (13%), Edema (8%), Hypertension (<6%). Infection including: Urinary tract infection (≤9%), Herpes zoster (2% to 6%), Nasopharyngitis (9%), Cough (8%).

Known potential side effects from **Enasidenib** in humans include the following:

Non-hematologic: IDH-DS (14%), Noninfectious leukocytosis (8.2%), TLS (5.5%), GI disturbances Nausea (50%), Diarrhea (43%), Decreased appetite (34%), Vomiting (34%), Dysgeusia (12%), Hyperbilirubinemia (81%). In addition, Febrile neutropenia (23.9%), Pneumonia (14.2%), Sepsis (11.8%), Pyrexia (8.5%), Lung infection (6.4%), Acute renal failure (5.5%), and Fatigue (5.2%)

Based on available pharmacokinetic and drug metabolism data, there are no anticipated major adverse reactions that may manifest secondary to drug-drug interactions when enasidenib is utilized concomitantly with ruxolitinib; however, there is a potential for unforeseeable adverse events with the combination.

18.3.1 REPRODUCTIVE RISKS

Patients should not become pregnant or father a baby while on this study. Women should not breastfeed a baby while on this study. Females of childbearing potential must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 4 weeks before she starts taking study medications (enasidenib and ruxolitinib). Females of childbearing potential must also agree to ongoing pregnancy testing. Men must agree to use a condom during sexual contact with a female of child bearing potential even if they have had a successful vasectomy. All patients must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure.

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on IP, or within (4 months), are considered immediately reportable events. IP is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form. The female subject may be referred to an obstetrician-gynecologist (not necessarily one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

Male Subjects

If a female partner of a male subject taking investigational product becomes pregnant while the male subject is on study treatment or within 4 months of the male subject's last study treatment, the male subject taking IP should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

CORRELATIVES/ SPECIAL STUDIES

Submission of samples for correlative studies is mandatory. The correlative biomarker studies will evaluate a series of biomarkers at baseline (at study entry), and during treatment at each study visit, and either at the time of relapse/progression or at termination of study (that is off study drug for any reason). Please see Table 5 for various time points for collection of samples.

19.1 SHIPPING INSTRUCTIONS

Each site will provide the necessary shipping materials to package.Blood and bone marrow samples will be shipped at room temperature on the day of collection. All shipments must be sent by overnight courier for delivery before 10 AM or morning delivery the next day. Nail may be sent with the ambient shipment on the day of collection. Slides may be batched and shipped every 3 months. In general shipments cannot be received on Saturday. For special arrangements, contact Dr. Weinberg prior to collection of tissue and shipment.

Rona Singer Weinberg, Ph. D
New York Blood Center
310 East 67th Street, Room 2-44
New York, New York 10065
Phone: (212) 570-3488
Fax: (212) 570-3495
rweinberg@nybc.org
Laboratory Phone: (212) 570-3412
Laboratory Fax: (212) 570-3495
MPDLab@nybc.org

SAMPLES ARE ACCEPTED MONDAY-THURSDAY 9AM TO 5PM; FRIDAY 9AM TO 1PM.

NOTE:SAMPLES CANNOT BE RECEIVED ON SATURDAY OR SUNDAY. CALL DR. WEINBERG BEFORE COLLECTING SAMPLES IF SPECIAL ARRANGEMENTS NEED TO BE MADE.

Include a de-identified copy of the institutional bone marrow aspiration and biopsy report with your shipment to the NYBC in New York. This report must include MPN-RC patient ID number, differential cell counts on the marrow aspirate, if performed. Contact the central office with any questions.

19.2 BONE MARROW BIOPSY SLIDES GUIDELINES

The following samples are to be collected at screening, at baseline, at Cycle 4 Day 1 (at the discretion of the Investigator for chronic phase patients), at Cycle 7 Day 1, Cycle 10 Day 1 (at the discretion of the Investigator for chronic phase patients), after every 6 cycles thereafter, and at termination of study for any reason. Biopsies should be done to confirm complete response and when clinically indicated. The following bone marrow aspiration and biopsy specimens must be obtained and submitted to Dr. Rona Weinberg at the New York Blood Center:

- three (3) air-dried, unstained bone marrow aspirate slides (if available)
- four (4) unstained peripheral blood films (thin smears)
- two (2) air dried unstained bone marrow biopsy touch preps for patients with no aspirate sample (these are made with 8 touches per slide – 4 horizontal touches in 2 rows on the slide)

Additionally:

- three (3) unstained paraffin-fixed bone marrow biopsy slides each containing at least 2 sections
- one (1) stained H & E biopsy slide
- one (1) biopsy slide stained for iron
- one (1) biopsy slide stained with the silver for reticulin fibrosis stain should be submitted for confirmatory cytologic and cytochemical studies. These should be submitted together with the final institutional pathology, cytochemistry, and immuno-phenotyping reports (if possible)

Bone marrow biopsy should be repeated if relapse occurs or transformation to MDS, or acute leukemia is suspected.

There must be an adequate amount of marrow biopsy specimen in each smear. The above requirements are considered minimal.

Identify each slide with the subject's patient ID number, protocol number, time point, and type of sample.

The histopathology slides may be batch shipped every 3 Months to Dr. Weinberg at the NY Blood Center.

Identify each slide with the patient's MPN-RC patient ID number, protocol number, time point, and type of sample.

Include a de-identified copy of the institutional bone marrow aspiration and biopsy report with your shipment to the NYBC in New York. This report must include MPN-RC patient ID number, differential cell counts on the marrow aspirate, if performed.

19.3 BONE MARROW ASPIRATE GUIDELINES

Bone marrow aspirate samples will be collected for histopathology, biomarkers, and cytogenetics/FISH. For biomarkers and cytogenetics, obtain a total of 3-7 mL of aspirated bone marrow. Apirate samples collected for biomarkers will be sent to the NYBC on the day of collection. Aspirate samples collected for cytogenetics/FISH will be sent to each site's local labs for analysis. Samples are to be divided as follows:

- 2-5 mL of bone marrow aspirate in a green top, heparinized tube (BD catalog number 366480)
- 1-2 mL of bone marrow aspirate for Cytogenetics

In the event that a biopsy does not produce aspirate, additional perpipheral blood samples must be sent for cytogenetic/FISH analysis.

19.4 PERIPHERAL BLOOD: (USE STERILE TECHNIQUE)

Peripheral blood samples will be collected at screening, and day 1 of Cycle 1-Cycle 7, every 3 cycles, and at the end of study, in conjunction with standard-of-care blood draws. The total amount to be collected should not exceed 25cc (approximately 5 teaspoons). Peripheral blood samples must be shipped ambient on the day of collection.

- 25 mL of blood: three ACD (yellow top) tubes (BD catalogue number 364606; 8.5 mL tubes)

19.5 NAIL CLIPPINGS

Two to ten nail clippings are to be obtained from each patient during the initial entry onto the protocol. Clippings are to be placed in a paper envelope and the envelope is to be sealed. The sealed envelope maybe stored and shipped at room temperature.

19.6 CYTOGENETIC ANALYSIS

Diagnostic and follow up cytogenetics will be performed by local institutional laboratories. All karyotypes will be centrally reviewed (see below). Samples are to be sent for cytogenetic analysis at screening, at cycle 4 day1, cycle 7 day 1, cycle 10 day 1, every 6 cycles thereafter, and at termination of study for any reason. JPEG or TIFF images must be uploaded into the MPN-RC REDCap database for each cytogenetic assessment. To ensure uniformity of cytogenetic preparations all participating local laboratories must adhere to the following requirements established by the central karyotype review co-Investigator (Dr. Vesna Najfeld).

19.6.1 GENERAL INCLUSION CRITERIA FOR CONVENTIONAL CYTOGENETICS

1. Banding level 300 or more and structural aberrations are accepted at 400 or higher banding level or confirmed by FISH.
2. Minimum requirements for baseline assessments: From each patient, bone marrow (PV, ET) and or unstimulated PB (PMF) specimen must be set up into two, preferably three, different cultures (direct, 24 hrs. with and without marrow max media). Every effort should be made to obtain the growth of cells that are cytogenetically abnormal.
3. 20 metaphase evaluated from two or more cell cultures or from marrow max culture only.
4. Only G-banding of chromosomes is acceptable. Other banding methods are not acceptable.
5. Analysis of cell at 300 band resolution, or low-quality banding, irrespective of the findings, to be complemented with FISH screening for subtle aberrations, including del(20)(q11q13) with D20S108 probe.
6. ISCN 2009 must be used to describe the karyotype.
7. Aberrations classified as clonal should be present in at least 2 cells by G-banding or confirmed by FISH.
8. Unusual or novel findings should be fully characterized by FISH using appropriate probes.
9. Three karyotypes (and metaphase cells) from each patient must be uploaded electronically into the MPN-RC REDCap database to document the stated cytogenetic diagnosis. The karyotypes are uploaded either as TIFF or JPEG files.

FISH studies, IF POSSIBLE and warranted in an individual case

1. Case normal by G banding, if possible, ought to be screened for cryptic changes by interphase FISH using a MPN panel of 12 probes: CEP1(1q12)/1q21, 5p15.2/5q31(EGR1), CEP7/7q31, CEP8, CEP9/9p21, RB1 at 13q14, P53/CEP17, 20q12/D20S108.
2. FISH studies are performed on BM or PB on interphase cells processed directly (no culture) using Abbott Molecular FISH probes. A minimum of 200 cells at baseline should be scored by two individuals. Image of FISH results should be uploaded as JPEG or TIFF file into the MPN-RC database.

Central Karyotype Review: Vesna Najfeld, Ph.D., Director, Tumor Cytogenetics, Icahn School of Medicine, NY.

One of the **QC indicators** is a proper entry and submission of cytogenetic forms and karyotypes by the institutional cytogenetic laboratories. If the local laboratory fails two consecutive specimens (poor quality of chromosome banding, missed abnormality, wrong ISCN nomenclature) or lack of entering cytogenetic results into MPN- RC, the laboratory will be informed, and the third failure will result in placing the local laboratory on probation and the institutional PI will be informed. If required, either the central lab in NY or other MPN-RC approved cytogenetic lab will perform the studies from the institution whose cytogenetic lab is placed on probation.

For any questions please call Joe Tripodi PhD, Cytogenetic Research Coordinator, Mount Sinai Medical Center: **212 241-8801** or Dr. Vesna Najfeld at **212 241-8801.**

19.7 SPECIMEN BANKING

Subject samples collected for this study will be retained at New York Blood Center. Specimens will be stored indefinitely or until they are used up. If future use is denied or withdrawn by the subject, best efforts will be made to stop any additional studies and to destroy the specimens.

The MPN-RC Consortium's Tissue Distribution Committee will be responsible for reviewing and approving requests for clinical specimen from potential research collaborators outside of the

Consortium. Collaborators will be required to complete an agreement (a Material Transfer Agreement or recharge agreement) that states specimens will only be released for use in disclosed research.

The specimens, DNA, and their derivatives may have significant therapeutic or commercial value. The Informed Consent form contains this information and informs the subject that there is the potential for financial gain by the Investigator or a collaborating researcher or entity. Genetic testing is a plan for future research.

The following information obtained from the subject's medical record may be provided to research collaborators when specimens are made available:

- Diagnosis
- Collection time in relation to study treatment
- Clinical outcome – if available
- Demographic data

Sampling times may be adjusted according to early trial results in order to optimize evaluation. No additional samples will be collected without formal amendment to this protocol. Detailed procedures for collection, handling, and shipment of samples will be provided in the study Laboratory Manual.

Samples will be sent to special laboratories at the New York Blood Center for careful study and analysis where they will be store using a unique code. The code linking the sample will be stored at Mount Sinai and known only to members of the research team at the Central Coordinating Office.

Table 5. Correlative Studies Sample Collection Schedule

	Sample		Baseline	Cycle 1-6 Day 1	Cycle 4, 7, 10, day 1 and every 3 cycles[4]	End of study[5]
Bone Marrow	**Aspirate** Histopathology[1]	Three (3) air dried unstained slides	X		X	X
	Aspirate Cytogenetics[2]	At local institution, upload data and karyotypes to the website[1]. If no aspirate, provide peripheral blood karyotypes.	X		X	X
	Aspirate Biomarkers[3]	2 to 5 mL of bone marrow aspirate in a green top, heparinized tube (BD catalog #366480)	X		X	X
	Biopsy Histopathology[1]	One (1) H&E stain	X		X	X
		One (1) Iron stain	X		X	X
		One (1) Silver impregnated reticulin stain	X		X	X
		Three (3) Unstained paraffin-fixed slides each with at least 2 sections	X		X	X

	Sample		Baseline	Cycle 1-6 Day 1	Cycle 4, 7, 10, day 1 and every 3 cycles[4]	End of study[5]
		If no aspirate, two (2)air dried unstained touch preps, 8 touches/slide, 2 horizontal	X		X	X
Peripheral Blood	Histopathology[1]	Four (4) Unstained peripheral blood films, thin smears	X		X	X
	Cytogenetics[2]	If no BM aspirate karyotypic analysis[1]	X		X	X
	Biomarkers[3]	Three (3) ACD: yellow (BD# 364606; 8.5 mL)	X	X	X	X
Other	Nails[3]	2 to 10 clippings (pre-treatment, at enrollment only)	X			

1. All slides MUST BE LABELED properly. Labels are to include the patient's ID number, protocol number, time of study (e.g., baseline), type of specimen (e.g., BM biopsy, BM aspirate, blood), type of stain (e.g., Iron, H& E). For unstained slides, use a pencil to label the slides. All slides are to be sent to Dr. Rona Weinberg at the NYBC in a labeled slide box. The label on the box is to include the patient's ID, protocol number, and time of study.
2. Cytogenetic analyses are performed as a standard of care. Karyotypic analysis of unstimulated bone marrow after direct 24 and 48 hours, if available, or peripheral blood shall be performed. Karyotypic analysis using G-banding technology is required.
3. **All tissue samples must be registered via the MPN-RC on-line Patient Registration system (**https://redcap2.mayo.edu/redcap/ **the day they are collected and prior to shipment.** All tubes, and envelopes (nails) must be labeled with patient's ID number, protocol number, time of study (e.g. baseline), type of specimen (e.g. BM biopsy, BM aspirate, blood) and date of collection.
4. BMB is optional for cycles 4 and 10 at the discretion of the Investigator for chronic phase patients
5. At relapse/progression or termination of study drug (that is off study drug for any reason).

Fresh blood and bone marrow are to be stored at ambient temperature and must be shipped on the day that they are collected.

Nails are to be stored at ambient temperature and may be shipped with the fresh specimens or subsequently.

All slides are to be shipped to the tissue bank laboratory in a labeled slide box. The label on the box is to include the patient's ID, protocol number, and time point of study. Slides may be shipped in batches together with the local institution's histopathology report, every 3 months. All slides are shipped to Dr. Rona Singer Weinberg at the address above.

20. STATISTICAL CONSIDERATIONS

STUDY DESIGN/STUDY ENDPOINTS

This is a Phase II design to evaluate the efficacy of ruxolitinib and enasidenib combination therapy for accelerated or blast-phase MPN patients with IDH2 mutations. The study will also include a descriptive cohort of up to 9 evaluable patients with chronic phase MPN and will utilize a Gehan design in this cohort. This study will be the first time ruxolitinib and enasidenib will be used in combination for this patient population. Therefore, the safety of the combination will be confirmed in the first six patients

who are enrolled in the study. If no more than one patient out of the initial six has a treatment-related Grade 3 or higher adverse event attributable to the study drugs, the combination therapy will be considered safe. If more than one patient out of the initial six has a limiting toxicity the trial will be suspended, and the safety data will be further reviewed.

20.1 ACCELERATED / BLAST-PHASE COHORT

Primary endpoint: The primary endpoint of this study is best overall response, defined as partial response or better (CR+PR+Cri), and will be defined as the best response within 6 cyclesof combination therapy. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response. Any evaluable patient not meeting the definition for response will be deemed as having a non- response.

Design: The largest overall response rate where the proposed combination treatment regimen would be considered ineffective in this patient population is 5%, and the smallest response rate that would warrant subsequent studies with the proposed regimen is 25%. This is based on retrospective analysis applying the 2013 IWG Response Criteria to patients treated with ruxolitinib[1]. A Simon's two-stage minimax design will be employed to test the null hypothesis that the true overall response rate in this patient population is at most 5%.

Decision Rule: Enter 13 patients into the study. If 0 responses are observed in the first 13 patients (Stage 1), we may consider this regimen ineffective in the patient population and the trial will close due to a lack of efficacy. If 1 or more responses are observed in the first 13 evaluable patients, we will proceed to Stage 2 and enroll an additional 7 patients onto the study for a total of 20 patients. After completion of both stages, if 3 or more responses out of the 20 patients are observed, the combination therapy will be considered promising for further investigation.

20.1.2 POWER AND SIGNIFICANCE LEVEL / PRECISION

Assuming that the number of overall response is binomially distributed, the significance level is <0.10 and the probability of declaring that this regimen warrants further studies (i.e., statistical power) under various response proportions can be tabulated as a function of the true response proportion as shown in the following table.

Table 6. Statistical Power

Accelerated / Blast-Phase MPN					
If the true response rate is…	0.05	0.10	0.15	0.20	0.25
Then the probability of early termination of the study is …	0.51	0.25	0.12	0.05	0.02
Then the probability of declaring that the regimen warrants further studies is…	0.07	0.32	0.59	0.79	0.90

20.2 CHRONIC MPN PHASE

Primary endpoint: The primary endpoint of this study is best overall response, defined as clinical improvement or better (CI+PR+CR) according to IWG-ELN criteria, and will be defined as the best response within 6 cycles of combination therapy. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response. Any evaluable patient not meeting the definition for response will be deemed as having a non- response.

Design: Based on a Gehan design, observation of at least 1 response out of 9 evaluable patients would indicate activity in this cohort. This design has 90% power if the true response rate is 25%.

21. SAMPLE SIZE AND ACCRUAL

The maximum sample size in the Phase II (accelerated/blast-phase) is 20 evaluable patients. Allowing for enrollment of up to 2 additional patients as replacements for unevaluable patients (ineligibility, cancellation, major treatment violation, or other reasons) , this study will enroll a maximum of 22 patients in the Phase II AP/BP cohort. The maximum sample size in the chronic phase MPN is 9 evaluable patients. Allowing for enrollment of up to 1 additional patient as replacement for unevaluable patients, this study will enroll a maximum of 10 patients in the descriptive chronic phase MPN cohort. Therefore, a maximum total of 32 (22 +10) patients will be enrolled in the trial.

The anticipated accrual rate is approximately 2-3 patients per month. Therefore, the accrual period for this study is expected to be 15 months. The primary analysis can begin approximately 21 months after the trial begins, i.e., as soon as the last patient has been observed for 6 cycles.

22. DATA ANALYSIS PLANS

22.1 PRIMARY ENDPOINT

Overall response rate (ORR): The overall response rate will be estimated by the number of patients that attain a response (defined above per cohort) by 6 cycles of combination therapy divided by the total number of treated eligible patients. A two-sided 95% confidence interval for the true overall response rate will be calculated according the exact binomial method.

22.2 SECONDARY ENDPOINTS

To additionally assess the efficacy of the combination of ruxolitinib with enasidenib in patients with accelerated or blast-phase disease after 6 cycles of combined therapy using complete (CBR) or partial blast response (PBR). CBR and PBR will be defined according to Appendix A. This will be calculated similarly to the primary endpoint for the MPN-AP/BP cohort.

To assess the efficacy of the combination of ruxolitinib with enasidenib in patients with PMF, post-PV MF, or post-ET MF in chronic phase and 4-9% blasts as assessed by the modified 2013 International Working Group (IWG) Response Criteria, after 6 months of combined therapy. This will be calculated similarly to the primary endpoint for the MPN-AP/BP cohort.

Adverse events: All eligible patients that have been initiated treatment will be included in the calculation of adverse event rate(s). The maximum grade for each type of adverse event will be tabulated for each patient, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration. Adverse events will be summarized overall and by cohort.

Overall Survival (OS) will be defined as the time from first treatment with the ruxolitinib/enasidenib combination to death by any cause. Patients will be considered censored at the last known date alive, if death is not documented. OS will be estimated using the Kaplan-Meier method.

22.3 EXPLORATORY/CORRELATIVE ENDPOINTS

- Patient-reported symptoms and quality of MF-SAFv4.0.

The MF-SAF v4.0 will be scored using published scoring algorithms. Patient-reported symptoms and quality of life (QOL) will be described at each time point using the mean, 95%

confidence interval, median, and range. Changes from baseline will also be described using the mean, 95% confidence interval, median and range, and assessed using paired t-tests or Wilcoxon signed-rank tests as appropriate. Graphical procedures will include plots of average values over time. .

- Biomarkers:

Biomarkers will be described at each time point using the mean, 95% confidence interval, median, and range if continuous, or frequency and relative frequency if binary. Continuous changes from baseline will be described using the mean, 95% confidence interval, median and range, and assessed using paired t-tests or Wilcoxon signed-rank tests as appropriate. Binary changes from baseline will be described using frequencies and relative frequencies and assessed using McNemar's tests as appropriate. Biomarker associations with response (IWG/ELN and/or bone marrow) will be primarily descriptive in nature given the small sample size.

23. STUDY MANAGEMENT

23.1 CONFLICT OF INTEREST

Any research personnel who has a conflict of interest with this study (patent ownership, intellectual property, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must declare their conflict of interest to the appropriate institutional review bodies. Local institutional conflict of interest policies will be followed for all research personnel associated with the research project.

23.2 INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL AND CONSENT

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB must approve the consent form and protocol.

In obtaining and documenting informed consent, the Investigator should comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the Investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion. A copy of the signed consent form should be provided to the patient.

24. DATA MANAGEMENT AND MONITORING/AUDITING

24.1 DATA MONITORING COMMITTEE/DATA SAFETY MONITORING BOARD (DMC/DSMB)

This trial will be monitored by the MPN-RC Data Safety Monitoring Board according to the established Charter.

An External Data and Safety Monitoring Board has been established. All members have experience and expertise in clinical trials. DSMB members are not directly involved in any phase of MPN-RC clinical

trials and they have no major financial or intellectual conflict of interest that would prevent them from objectively reviewing the interim data and providing advice to the Trials Steering Committees and the Clinical Advisory Group. They function independently of all other individuals, processes, and progress to ensure study integrity, monitor patient safety (providing quarterly safety reports), evaluate the results of interim analysis to assess efficacy, and make recommendations about protocol amendments and early termination to the Trials Steering Committees. The External Data Safety and Monitoring Board must meet at least two times a year.

24.2 MEDICAL MONITORING

It is the responsibility of the local institutional Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

24.3 MONITORING PLAN AND PERIOD OF OBSERVATION

The MPN-RC will monitor study progress on an on-going basis; this will include electronic and telephone correspondence between the central coordinating office and with individual investigators and/or their designees at other sites.

The Sponsor or the Sponsor designees, "study monitors," will monitor the study according to a predetermined monitoring plan (centrally and with on-site visits). The monitoring visit will provide the Sponsor with opportunity to:

- Evaluate the progress of the study
- Verify the accuracy and completeness of CRFs
- Assure that all protocol requirements, applicable laws and/or regulations, and Investigator's obligations are being fulfilled
- Resolve any inconsistencies in the study records

25. REGULATORY REQUIREMENTS

See the Regulatory Requirements section for a listing of required regulatory documents. All protocol amendments will be generated through and distributed by the MPN-RC Central Coordinating Office, which will also maintain records of IRB approval, amendments, SAEs, and annual reviews.

25.1 ADHERENCE TO THE PROTOCOL

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

25.2 EMERGENCY MODIFICATIONS

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

25.3 OTHER REPORTABLE NEW INFORMATION AND PROTOCOL DEVIATIONS/VIOLATIONS

In accordance with BRANY IRB requirements, the following information must be reported within five (5) business days:

- Non-compliance with federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance.
- Failure to follow the protocol due to the action or inaction of the Investigator or research staff.
- Breach of confidentiality
- Premature suspension or termination of the research by the Sponsor or Investigator.

25.4 AMENDMENTS TO THE PROTOCOL

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

25.5 RECORD RETENTION

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the Study Investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

26. OBLIGATIONS OF INVESTIGATORS

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including Sub-Investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

26.1 GOOD CLINICAL PRACTICE

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator should be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents should be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

26.2 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments should be submitted to a properly constituted independent EC or IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study should be made in writing to the Investigator and a copy of this decision should be provided to the MPN-RC Central Office before commencement of this study. The Investigator should provide a list of EC/IRB members and their affiliate to the MPN-RC Central Office.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form should be submitted with the protocol for review and approval by the EC/IRB for the study. The consent of a subject, using the EC/IRB-approved consent form, must be obtained before a subject is allowed to participate. This consent form must be signed by the subject or legally acceptable surrogate, and the Investigator obtaining the consent.

26.3 PATIENT INFORMATION AND INFORMED CONSENT

After the study has been fully explained, written informed consent should be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent should comply with ICH-GCP and all applicable regulatory requirement(s).

26.4 PATIENT CONFIDENTIALITY

Information about study subjects should be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

The patient has the right to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the Investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

26.5 PROTOCOL COMPLIANCE

The Investigator should conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies). Changes to the protocol will require approval of the MPN-RC and written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The MPN-RC will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations. Any departures from the protocol must be fully documented in the source documents.

26.6 MANAGEMENT OF INFORMATION

The MPN-RC at Mount Sinai will maintain sponsorship authority at the Icahn School of Medicine at Mount Sinai School and all other participating sites.

Eligibility criteria will be confirmed using the electronic CRF. All inclusion and exclusion criteria are listed; the Investigator is obliged to fill in all items. Subjects are enrolled in the study only if he/she fulfills all inclusion and no exclusion criteria.

IRB approval for each site will be submitted to the MPN-RC Central Coordinating Office via e-mail (PDF file preferred) or fax:

MPN-RC Central Coordinating Office
Clinical Trials Manager
MPN-RC Central Office
Phone: (212) 241-4546
Fax: (212) 876-5276
Email: MPNRC_ClinicalTrialCentralOffice@mssm.edu
Office hours: Monday through Friday 9AM to 5PM EST/EDT

Upon receipt of these documents and confirmation of certification of the Study Investigators, the site will be granted access to the online registration system and case report forms.

In addition, all protocol amendments will be generated through and distributed by the MPN-RC Central Coordinating Office, which will also maintain records of IRB approval, amendments, SAEs, and annual reviews.

Serious adverse event reporting is detailed in the Reporting Requirement section of this protocol.

Protocol deviations will be reported to the MPN-RC Data Monitor and CTM:

CTM: Lonette Sandy
Tel: (212) 241-4546
Email: lonette.sandy@mssm.edu
Office hours: Monday through Friday 9AM to 5PM (EST)

Data Monitor: Melissa Nashawati
Tel: (210) 450-3955
Email: nashawati@uthscsa.edu
Office hours: Monday through Friday 8:00AM to 5:00 PM (CT)

The MPN-RC Principal Investigators will be notified as soon as possible (and within 7 days) of reporting and will be responsible for granting approval.

The MPN-RC will monitor study progress on an on-going basis; this will include electronic and telephone correspondence between the central coordinating office and with individual investigators at other sites.

26.7 DRUG ACCOUNTABILITY

Accountability for the study drug at all study sites is the responsibility of the site Principal Investigator. The responsible Investigator at each participating center will ensure that the study drug is used only in accordance with this protocol, drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers. Conditions of the excursion will be evaluated on a case by case basis and a memo indicating continued use or discontinuation of the drug product involved will be communicated.

26.8 SOURCE DOCUMENTS

Source data includes all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

26.9 CASE REPORT FORMS (CRF)

The study CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. The CRFs may be found online at the MPN-RC website at (https://redcap2.mayo.edu/redcap/). The CRF Form Submission schedule is listed below:

Table 7. CRF Form Submission Schedule

MPN-RC #119 Form#	MPN-RC #119 FORM NAME	CRF SUBMISSION SCHEDULE
00	Recruitment Form	At Registration
01	Baseline	After Registration
02	Bone Marrow Report	At required time points specified in Table 5.
03	Cytogenetic	At required time points specified in Table 5.
04	Symptom Assessment Package	At required time points specified in Table 4.
05	Drug Dosage form	At required time points specified in Table 4.
06	Adverse Event form	At occurrence of Adverse Event
07	Cycle 1-2 Day 1 CRF	At Cycle 1-2 Day 1
08	Cycle 1-2 Day 8 CRF	At Cycle 1-2 Day 8
09	Cycle 1-2 Day 15 CRF	At Cycle 1-2 Day 15
10	Cycle 1-2 Day 22 CRF	At Cycle 1-2 Day 22
11	Cycle 3+ everyDay 1 CRF	At Cycle 3+ Day 1

MPN-RC #119 Form#	MPN-RC #119 FORM NAME	CRF SUBMISSION SCHEDULE
12	Off Treatment	At time of treatment withdrawal
13	Off Study	At time of off study, 30 days post treatment withdrawal

26.10 PREMATURE CLOSURE OF THE STUDY

This study may be prematurely terminated, if in the opinion of the MPN-RC Trial Steering Committee there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the Investigator by the terminating party.

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

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the study drug

26.11 RECORD RETENTION

It is the Investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents must be retained at least 2 years following completion of the last follow-up on patients on active study. These documents should be retained for a longer period if required by an agreement with the Sponsor. In such an instance, it is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

26.12 DATA MANAGEMENT

Study data will be recorded and managed using the Research Electronic Data Capture (REDCap) system developed by Vanderbilt University. Research Electronic Data Capture is a secure, web-based application designed to support data capture for research studies, providing:1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

After IRB approval and site training, REDCap database access will be given to approved and trained individuals at each site. Each user will have a unique username/password assigned by the REDCap administrator at Mayo Clinic (where the MPN-RC Statistical and Data Center Core is located).

26.13 QUALITY ASSURANCE

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the

study team for discussion and action. Monthly teleconferences including the Principal Investigators and/or their designees from all sites will be held to expedite the reviews of toxicity and efficacy data.

27. WITHDRAWAL OF SUBJECTS

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

- Lack of any response by 6 cycles of combined enasidenib and ruxolitinib therapy (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
- Toxicity requiring dose modification/treatment suspension, without recovery to baseline, in a patient with less than a CRi
- Death

The patient will have an end of study visit at 30 days after discontinuation of the protocol treatment ±7 days to assess for AEs.

28. BREACH OF CONFIDENTIALITY

One of the risks is release of information from health or research records in a way that violates privacy rights. The investigators will protect records so that name, address, phone number, and any other information that identifies the participant will be kept private. It will be stated to the participant that the chance that this information will be given to an unauthorized individual without the participant's permission is very small.

29. POTENTIAL BENEFITS TO SUBJECTS

We hypothesize that the combination of ruxolitinib and enasidenib in patients with MPN both in accelerate/blasts-phase as well as with high risk chronic phase myelofibrosis with an IDH2 mutation will improve the overall clinical response to therapy including complete response, partial response, complete and partial blasts response, symptomatic improvement and improvement in quality of life.

30. SHARING OF RESULTS WITH SUBJECTS

Results of tests performed during the study will be shared with the subject. Results of biomarker correlates will not be shared.

31. PRIOR APPROVALS

Approval to commence research will be obtained initially at Mount Sinai through the usual protocol approval pathway including the Disease Focus Group, Resource Allocation and Evaluation, Protocol Review and Monitoring Committees. Once this is complete, the protocol will be submitted for an IND to the Food and Drug Administration. The IND-approved protocol will then be submitted to the central SMART IRB (BRANY) for ethical and regulatory evaluation. Once this is IRB approved the final contract (with budget) will be executed and a Site Initiation Visit (SIV) will be scheduled. This will then allow for Mount Sinai to activate the protocol and start enrollment. The BRANY approved protocol will be concurrently submitted for internal review at the other MPN-RC participating sites. SIVs will

be scheduled at each site once the protocol is approved locally. This will be coordinated throughout the MPN-RC central office within Project 4.

32. ECONOMIC BURDEN TO SUBJECTS

There will be no additional costs to the subject to participate in the study beside the standard of care costs. Research related per patient costs will be covered by the MPN-RC and will be determined by Medicare Analysis review (MCA).

33. CONSENT PROCESS

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of each site. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the Investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form. The consent process should be documented in the patients' medical records.

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34.1 APPENDIX A: RESPONSE CRITERIA

Complete Response	As per 2013 IWG-MRT and ELN Criteria
Partial Response	As per 2013 IWG-MRT and ELN Criteria
Clinical Improvement (CI)	As per 2013 IWG-MRT and ELN Criteria
Anemia Response	As per 2013 IWG-MRT and ELN Criteria
Spleen Response	As per 2013 IWG-MRT and ELN Criteria
Symptom Response	As per 2013 IWG-MRT and ELN Criteria
Progressive Disease	After at least 3 cycles of combined therapy: • New splenomegaly that is palpable at least 5cm below the LCM • a ≥100% increase in palpable distance, below LCM, for baseline splenomegaly of 5-10cm or • a 50% increase in palpable distance, below LCM, for baseline splenomegaly of > 10cm • Progression from chronic-phase disease (< 10% blasts) to blast-phase (≥ 20% blasts) disease on two separate CBCs within 28 days • For patients in accelerated of blast phase disease at baseline, a doubling of the absolute blast count
Stable Disease	As per 2013 IWG-MRT and ELN Criteria
Cytogenetic Remission	As per 2013 IWG-MRT and ELN Criteria
Molecular Remission	• Complete Molecular remission: eradication of *IDH2* and *JAK2/MPL/CALR* mutations as assessed by both next-generation sequencing and digital PCR assays. • Partial Molecular remission: 50% reduction in mutant *IDH2* and *JAK2/MPL/CALR* allele burden as assessed by next-generation sequencing
Cytogenetic/Molecular Relapse	As per 2013 IWG-MRT and ELN Criteria
Complete Blast Response	Absence of circulating peripheral blood blast count with residual features of chronic-phase MPN maintained for at least 28 consecutive days.
Partial Blast Response	Reduction to < 4% blast count in peripheral blood with residual features of chronic-phase MPN maintained for at least 28 consecutive days.

34.2 APPENDIX B: IWG-MRT AND ELN RESPONSE CRITERIA

Response categories	Required criteria (for all response categories, benefit must last for ≥12 wk to qualify as a response)
CR	Bone marrow:* Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF† and Peripheral blood: Hemoglobin ≥100 g/L 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
PR	Peripheral blood: Hemoglobin ≥100 g/L 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 ≥85 but <100 g/L 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 ≥20 g/L 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 A spleen response requires confirmation by MRI or computed tomography showing ≥35% spleen volume reduction
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 posttreatment 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 my 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.

34.3 APPENDIX C: MEDICATIONS KNOWN TO PROLONG THE QT INTERVAL

amiodarone	citalopram	Escitalopram	methadone	sevoflurane
astemizole	clarithromycin	Flecainide	moxifloxacin	sotalol
azithromycin	disopyramide	Halofantrine	pentamidine	sparfloxacin
bepridil	dofetilide	Haloperidol	pimozide	terfenadine
chloroquine	domperidone	Ibutilide	probucol	thioridazine
chlorpromazine	droperidol	Levomethadyl	procainamide	
cisapride	erythromycin	Mesoridazine	quinidine	

34.4 APPENDIX D: GUIDANCE IDH-INHIBITOR DIFFERENTIATION SYNDROME (IDH-DS) GUIDANCE FOR MANAGEMENT OF DIFFERENTIATION SYNDROME IN SUBJECTS WITH HEMATOLOGIC MALIGNANCIES TREATEDWITH IDH-INHIBITORS

The participating investigators/health care professionals are responsible for the appropriate evaluation and management of the safety related issues in subjects enrolled in the study. The approach outlined below for identifying and managing Differentiation Syndrome in patients treated with IDH inhibitors should be considered as guidance and therefore management of each case should be individualized, as appropriate.

Introduction to IDH inhibitors Differentiation Syndrome

The isocitrate dehydrogenase (IDH) mutation, found in up to 15% of subjects with AML[36,37], leads to excess accumulation of 2-hydroxyglutarate (2-HG) that causes inhibition of dioxygenase dependent enzymes responsible for demethylation of DNA and histones, and consequently resulting in impaired bone marrow differentiation[39,40].

Direct inhibition of mutated protein activity by IDH inhibitor is intended to block abnormal production of the oncogenic metabolite 2-HG and impact clinical outcome through a variety of mechanisms that include restoration of differentiation of the malignant cells.

In patients with hematologic malignancies, initiation of treatment with the differentiating agents, such as IDH inhibitors, and associated maturation of aberrant bone marrow, may alter the balance of the expression of adhesion molecules and chemoattractants (cytokines) that could mediate tissue infiltration by maturing blood cells[44], associated with signs and symptoms of Differentiation Syndrome (DS).

Signs and symptoms could include fever, dyspnea, edema/ weight gain, increased serum creatinine and, in some cases, clinical features consistent with the acute respiratory distress syndrome with associated bilateral pulmonary infiltrates, and pulmonary or pericardial effusions. Increases in white blood cell (WBC) count concurrent to DS has been observed, but by itself do not substantiate the syndrome.

There is no pathognomonic clinical sign or laboratory test to diagnose DS. In some instances, clinical presentation of DScan be confounded by other medical condition(s), such as concurrent infections or heart failure. Consequently, diagnosis of DS is made by excluding other potential causes, rather than based on a specific set of signs and symptoms.

To improve diagnostic and treatment practices for management of DS associated with treatment with IDH-inhibitors (IDH DS), data for adverse events meeting diagnostic criteria for DS will be reviewed and assessed by a Differentiation Syndrome Review Committee.

Clinical Assessment of Subjects for signs and symptoms of IDH-inhibitors differentiation syndrome

Due to the heterogeneity of clinical symptoms and differences in adverse events reporting practices (e.g., symptoms of suspected IDH DS reported in place of DS diagnosis), exact incidence of Differentiation Syndrome in subjects treated with IDH-inhibitors has not been determined. In the clinical trial conducted for Idhifa's approval, at least 14 percent of patients experienced differentiation syndrome

As it has been reported by the Study Investigators, IDH DS is more likely to manifest within 10 days to 5 months after the start of treatment, or after re-initiation of the study drug following prolonged treatment interruption (e.g., >3 weeks) in subjects who are not in complete remission. In some cases, symptoms re-appeared after cessation of treatment for the initial manifestation of IDH DS.

Signs and Symptoms

In clinical trials with IDH inhibitors, DS has been described in two categories of severity:

Severe IDH-Differentiation Syndrome

Based on presence of any of the below listed factors, with, or without symptoms of moderate severity IDH DS

- New or worsened progressive dyspnea; hypoxia with increasing demands for supplemental oxygen; respiratory distress, without definitive etiology or refractory to treatment for the initially suspected cause
- Radiologic evidence of new or worsened bilateral pulmonary involvement (infiltrates or opacities) with or without presence of infection, refractory to treatment with anti-infectives (antibiotics, antivirals, antifungals) that was not attributed to another cause
- Radiologic evidence of new or worsened pleural or pericardial effusion that has no definitive etiology or is refractory to treatment for the initially suspected cause.

Moderate Severity IDH Differentiation Syndrome

Based on presence of at least 2 of the below listed factors, in absence of signs of severe IDH DS

- Unexplained fever ≥38°C (100.4°F)
- New or worsened peripheral edema without definitive etiology, or rapid weight gain (e.g., > 5 kg/11 pounds)
- Rash of unknown origin
- New or worsened bone pain or arthralgia without other clear etiology
- Lymphadenopathy
- Increase in serum creatinine (e.g., >2-fold from baseline)

Conditions that may co-occur with differentiation syndrome

- Commonly co-occur: Leukocytosis in the absence of an infectious process
- Infrequently co-occur: Laboratory Tumor Lysis Syndrome (TLS), or TLS associated with increased serum creatinine level, in settings of rapidly increasing blood cell count (leukocytosis)
- Rarely co-occur: Coagulopathy in the setting of increased blood cell count

Management of subjects with suspected IDH inhibitors-differentiation syndrome

Conditions consistent with signs and symptoms of IDH DS but refractory to treatment for the initially suspected cause(s), or that worsen within the first 48 hours after treatment initiation, should be managed as potential IDH DS.

It is recommended that the measures indicated below are undertaken at the earliest manifestations of suspected IDH DS.

- Patients with severe or rapidly progressing IDH DS should be hospitalized for continued observation
- In case of uncertainty with the diagnosis, e.g. presence of less specific symptoms of moderate severity IDH DS, patients should be closely monitored, as condition may rapidly exacerbate
- Corticosteroids should be promptly initiated at a suggested dose 10 mg of dexamethasone IV every 12 hours until complete resolution of IDH DS, after which the dose can be progressively reduced in the next few days or weeks
- The study drug may be withheld at the investigator's discretion. Due to long half-life of the IDH-inhibitors, treatment interruption may not immediately reverse symptoms of IDH DS. Potential for drug-drug interaction between IDH inhibitor and treatments initiated for management of IDH DS should be considered, when making decisions about study drug treatment interruption. If interrupted, study drug treatment may be reinitiated at the original or reduced dose once the signs and symptoms resolve and the subject's clinical condition improves.

- If renal dysfunction or severe pulmonary symptoms requiring intubation or ventilator support persist for more than 48 hours after initiation of systemic corticosteroids, interrupt enasidenib treatment until signs and symptoms are no longer severe
- In subjects with elevated WBC, prompt initiation of hydroxyurea is suggested, as per standard local practices (e.g., dose of 2 to 3g PO twice or three times daily for WBC >30x10^9/L)
- In cases of severe leukocytosis, use of leukapheresis may be appropriate
- For substantial fluid accumulation, initiation of furosemide may be appropriate, as per local standard practice
- Pericardial effusion - a less common symptom of IDH DS - can be life threatening condition requiring urgent intervention
- Subjects with increasing serum creatinine levels should be evaluated for TLS
- Subjects experiencing rapid increase in peripheral blood cells should be monitored for disseminated intravascular coagulopathy and hemorrhages
- Imaging techniques such as standard or high-resolution CT or chest X-ray are useful in establishing a diagnosis of IDH DS. However, chest X-ray is less sensitive in detecting early radiological signs of IDH DS associated changes.

Immediate communication with the Study Co-Chairs is encouraged for guidance regarding treatment.

34.5 APPENDIX E: EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE SCORE RATINGS

At each clinic visit after the screening visit, the investigator will assess each subject's ECOG performance status according to the following scale:

0 Fully active, able to carry on all pre-disease performance without restriction

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house-work, office work

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

5 Dead

34.6 APPENDIX F: MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MF-SAF) V4.0

Instructions: The following questions refer to symptoms that you may experience as a result of your myelofibrosis. Please read through and complete the questions below. There are no right or wrong answers. Please select the answer that best applies to you.

1. During the past 7 days, how severe was your worst fatigue (weariness, tiredness)?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

2. During the past 7 days, how severe were your worst night sweats (or feeling hot or flushed)?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

3. During the past 7 days, how severe was your worst itching?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

4. During the past 7 days, how severe was your worst abdominal discomfort (feel pressure or bloating)?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

5. During the past 7 days, how severe was the worst pain under your ribs on your left side?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

6. During the past 7 days, what was the worst feeling of fullness you had after beginning to eat?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

7. During the past 7 days, how severe was your worst bone pain (not joint or arthritis pain)?

0 1 2 3 4 5 6 7 8 9 10

Absent Worst Imaginable

34.7 APPENDIX G: NEW YORK HEART ASSOCIATION (NYHA) CLASS III OR IV CONGESTIVE HEART FAILURE

Class	Patient Symptoms
I(Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
II(Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
III(Moderate)	Marked limitation of physical activity. Comfortable at rest, but less-than-ordinary activity causes fatigue, palpitation, or dyspnea.
IV(Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.
Source: Dolgin M, Fox AC, Devereaux RB. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston: Little Brown and Company; 1994 p.253-256.	

34.8 APPENDIX H: FDA MEDWATCH FORM

https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf