A Multicenter, Open-Label, Pilot Study of Alisertib (MLN8237), A Novel Inhibitor of Aurora Kinase A, in Adult Patients with Relapsed/Refractory Acute Megakaryoblastic Leukemia or Myelofibrosis (Including Primary and Post-Essential/Post-Polycythemic Myelofibrosis)

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LIST OF ABBREVIATIONS

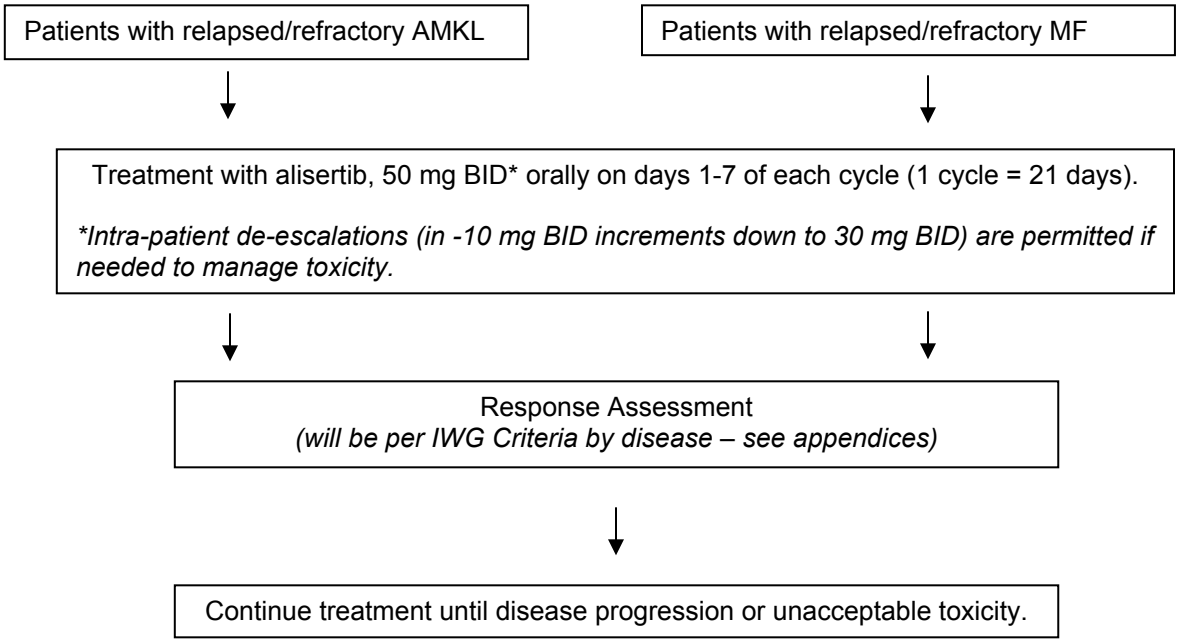
AE	Adverse Event
AMKL	Acute megakaryoblastic leukemia
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AURKA	Aurora A kinase
BID	Twice per daily
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
ECOG	Eastern Cooperative Oncology Group
H&PE	History & Physical Exam
IV (or iv)	Intravenously
MF	Myelofibrosis
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate or Objective Response Rate
OS	Overall Survival
PD	Progressive Disease
PFS	Progression Free Survival
PO (or p.o.)	Per os/by mouth/orally
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease

STUDY SUMMARY

Title	A Multicenter, Open-Label, Pilot Study of Alisertib (MLN8237), A Novel Inhibitor of Aurora Kinases, in Adult Patients with Relapsed/Refractory Acute Megakaryoblastic Leukemia or Myelofibrosis (Including Primary and Post-Essential/Post-Polycythemic Myelofibrosis)
Short Title	Alisertib in Relapsed/Refractory AMKL or MF
Version	March 17, 2017
Study Design	Pilot, two-arm, open-label, multicenter
Study Center(s)	• Northwestern University – Robert H. Lurie Comprehensive Cancer Center (Lead Site) • Miami University – Sylvester Comprehensive Cancer Center • Mayo Clinic
Objectives	Primary The primary objective will be to determine the safety profile of alisertib in patients with AMKL and in patients with MF. Secondary The secondary objective will be to determine preliminary efficacy of alisertib in each population. Exploratory Exploratory objectives will include the following: 1. Describe pharmacodynamics (PD) effects of alisertib in peripheral blood and/or bone marrow samples. 2. Evaluate the relationship between biomarker expression levels and response to alisertib. 3. Evaluate reduction in splenomegaly by palpation (MF arm only) 4. Evaluate improvement in MF symptoms (MF arm only), as assessed by the Myeloproliferative Neoplasm Symptom Assessment form (MPN-SAF)
Sample Size	24 total (Maximum 24 subjects, regardless of the disease breakdown of MF or AMKL. If all 24 were MF, that would be acceptable).
Diagnosis & Key Eligibility Criteria	1. Confirmed diagnosis of one of the following (as defined by the World Health Organization criteria): a. Relapsed/refractory acute megakaryoblastic leukemia (AMKL) b. Myelofibrosis (MF), including primary and post-essential/post-polycythemic myelofibrosis 2. Age 18 years or older with ECOG status of 0-2. 3. Estimated life expectancy of at least 6 months. **Please see Section 3.0 for complete list, including disease-specific criteria for each arm.*
Treatment Plan	Alisertib will be administered orally BID on days 1-7 of each cycle (1 cycle = 21 days). The starting total daily dose (level 1) for all patients will be 50 mg BID. Intra-patient dose de-escalations (in -10 mg BID increments down to 30 mg BID) may be used in the event that 50 mg BID is too toxic. Patients may continue to receive alisertib treatment until progression of disease or unacceptable toxicity.

Statistical Methodology	All adverse events (AEs) will be summarized using frequencies and percentages. Data on type, timing, frequency and attribution of AEs will be included. To determine preliminary efficacy, response (defined as having at least PR in evaluable patients) will be reported as a proportion and 95% confidence interval using exact binomial methods. Proportions for clinical response (defined as having at least PR or stable disease) will also be calculated. Please refer to Section 10 for more information regarding analysis plan for exploratory objectives. Approximate standard errors for the estimated response rate when the true response rate is 10% are +/- 6% with a sample of 24 patients total, +/- 18% with a sample of 18 patients, +/- 9% with 12 patients and +/- 10% to 12% with 6 patients.

STUDY SCHEMA



1.0 INTRODUCTION – BACKGROUND & RATIONALE

1.1 Disease Background

1.1.1 Acute Megakaryoblastic Leukemia (AMKL)

Acute Myeloid Leukemia (AML) is a heterogeneous disorder with respect to morphology, membrane phenotype, cytogenetics, gene expression profiling and natural history. Standard therapy has remained unchanged for the past 4 decades. According to MRC data, there has been little improvement in survival patients for patients with AML during a 30-year period, and improvements noted have been largely due to the use of more intensive regimens and better supportive care. Within AML, Acute megakaryoblastic leukemia (AMKL) occurs in both adults and children but is an uncommon disease comprising approximately 3–5% of cases of AML. Patients with AMKL present with pancytopenia, especially thrombocytopenia, although some may have thrombocytosis. Dysplastic features in neutrophils and platelets may be present. Organomegaly, e.g. hepatosplenomegaly, is usually infrequent. Morphologically, although AMKL may be associated with fibrosis, the histopathology of the biopsy varies from cases with a uniform population of poorly differentiated blasts to a mixture of poorly differentiated blasts and maturing dysplastic megakaryocytes. A variable degree of reticulin fibrosis may be present. Clinically, outcomes for patients with AMKL are dismal. Even for those considered eligible for stem cell transplantation, the majority will succumb to their disease. Newer more effective therapies are urgently needed.

1.1.2 Myelofibrosis (MF)

Myelofibrosis (MF) is a clonal disease of hematopoietic stem cells. It is characterized by cytopenias, a leukoerythroblastic blood picture, marrow fibrosis, and extramedullary hematopoiesis. Allogeneic hematopoietic cell transplantation (HCT) is the only curative treatment for patients with MF at present. Due to significant morbidity and mortality associated with HCT, divergent opinions have emerged about the application of HCT in MF. Significant regimen-related toxicities, graft failure and graft-versus-host disease are major barriers to the success of HCT in MF. Since the majority of patients that present with MF are older and not transplant candidates, the majority of MF patients will not be cured of their disease. Outside of SCT, the goals of therapy in MF are entirely palliative and relate to symptoms control. One non-transplant therapy is approved for this disease and clinical trials of novel agents are critical.

1.2 Pre-clinical Data: Megakaryocytes, AMKL, and PMF

Megakaryocytes undergo a modified form of the cell cycle termed endomitosis, in which cells skip the late stages of mitosis to become polyploid. Murine and human megakaryocytes commonly reach modal ploidy states of 32N and 16N, respectively, and can sometimes achieve DNA contents as high as 128N. Polyploidization is associated with upregulation of megakaryocyte lineage specific genes, proplatelet formation and expression of genes related to apoptosis. Importantly, the choice of a megakaryocyte to undergo polyploidization and differentiation is inextricably linked to exit from the proliferative cell cycle. Thus, hyperproliferation of megakaryocyte precursors is associated within impaired polyploidization and differentiation.

There are two major hematologic disorders that are characterized by aberrant expansion of immature megakaryocytes. The first is AMKL, a rare (3-5%) subtype of AML in which megakaryoblasts continue to proliferate and fail to commit to polyploidization or differentiation. Outcomes for adult patients with AMKL are dismal. A second malignancy with abnormal megakaryocytes is MF, a clonal myeloproliferative neoplasm (MPN) that is characterized by fibrotic destruction of the bone marrow accompanied by immature megakaryocytes. MF megakaryocytes display defective maturation and an immature

gene expression program, and they likely directly contribute to the fibrotic process by secreting increased levels of cytokines, including TGF-β.[1] As a consequence of the failure of the bone marrow to support normal hematopoiesis, blood cell development occurs within the spleen and liver, where it is less effective. Patients with PMF suffer from anemia and are at risk of transformation to AML. There are approximately 12,000 PMF patients in the US. Recent molecular studies have shown that JAK2 and MPL mutations are associated with approximately 50% and 10% of PMF cases respectively[2], while most of the remaining patients harbor mutations in calreticulin.[3, 4] Although the JAK inhibitor ruxolitinib was approved for MF[5], it primarily provides symptomatic relief. Moreover, many patients are intolerant or progress in spite of therapy.[6] Additional treatments to be used alone or in combination with ruxolitinib are clearly needed. Another disorder with immature megakaryocytes that fail to mature properly is myelodysplastic syndrome (MDS). MDS is distinct from the AMKL and MF in that megakaryocytes do not appear to be drivers of the disease.

Given that AMKL blasts are hyperproliferative and fail to undergo differentiation or polyploidization, we hypothesized that small molecule inducers of polyploidization would drive these cells to exit the proliferative cell cycle and undergo terminal differentiation**.** In collaboration with the Broad Institute, we performed a high throughput cell-based screen and identified small molecules that induce polyploidization and proliferative arrest of malignant megakaryocytes, including those that have *MPL* or *JAK2* mutations. We have shown that these compounds, including dimethylfasudil (diMF), selectively increase polyploidization, expression of megakaryocyte cell surface markers, and apoptosis of megakaryocytes and that diMF blocked the growth of primary human AMKL. Given that diMF is known to inhibit multiple kinases, we next performed a multi-pronged target identification approach to uncover the specific target of diMF in malignant megakaryocytes. These approaches included a biochemical assay, two shRNA screens of the kinome, and a SILAC-based proteomic study. Integration of these data revealed that aurora A kinase (AURKA) is a major target of diMF[7], and we found that the selective AURKA inhibitor alisertib (MLN8237) is a potent anti-AMKL agent. We hypothesize that MLN8237 will provide therapeutic benefit to AMKL as well as MF patients. In contrast, since these compounds induce polyploidization, but not platelet production, they are not an appropriate therapy for MDS.

1.3 Aurora Kinase A as a Target for Cancer Therapy

The Aurora kinases are highly conserved serine/threonine kinases that regulate chromosomal alignment and segregation during mitosis and meiosis.[8] Aurora A and Aurora B are expressed in all actively dividing cells, while Aurora C expression is largely restricted to dividing germ cells.[9] The major function of Aurora A is to coordinate centrosome maturation, bipolar spindle assembly and chromosome separation. The AURKA gene is located within a region of chromosome 20q13 that is amplified in many malignancies. The evidence supporting AURKA as a therapeutic target for the treatment of malignancies comes from several sources. First, the AURKA gene is amplified, overexpressed, or both in many tumors, including colon, breast, pancreatic, and bladder cancers, as well as certain lymphomas, leukemias, and myeloma.[10-14] Aurora A overexpression in human cancers has been correlated with increased aneuploidy and centrosome amplification.[15] Second, forced overexpression of Aurora A kinase in experimental models results in the transformation of normal cells, suggesting that Aurora A overexpression may be oncogenic.[10] Lastly, in a number of different experimental systems, Aurora A inhibition leads to mitotic delays and severe chromosome alignment and segregation defects, followed by cell death.[16-19] Overall, the essential role of Aurora A in mitotic progression and its dysregulation in certain cancers makes it an attractive therapeutic target.

A variety of AURKA inhibitors have been tested in patients with varied results. The first generation drugs showed anti-tumor activity in patients, but also showed unacceptable

toxicity. Development of VX-680 was terminated due to its severe toxicities. Given the obligatory role of mitosis in tumor proliferation, an AURKA inhibitor would be expected to have potential applications across a broad range of human tumors. Indeed, alisertib has demonstrated activity against a variety of nonclinical solid tumor and hematological malignancy models grown in vitro and in vivo, as described below. Alisertib is also expected to be toxic to proliferating normal tissues, such as the bone marrow, gastrointestinal epithelium, and hair follicles because any cell that is in mitosis, where Aurora A is expressed and active, should be susceptible to the effects of an Aurora A kinase inhibitor.

1.4 Preclinical Experience with Alisertib

1.4.1 In Vitro Studies

MLN8237 is a second generation, ATP-competitive, and reversible inhibitor of AURKA in vitro with an inhibition constant (Ki) of 0.43 nM.[20] The data from both enzymatic and cell-based assays demonstrated that alisertib is a selective and potent inhibitor of AURKA. Alisertib inhibited proliferation of a wide variety of tumor cell lines grown in culture. Moreover, treatment of tumor cell lines with alisertib induced phenotypes consistent with AURKA inhibition, including mitotic spindle defects, mitotic delay, and apoptosis.[10-14] Treatment of cells with alisertib causes defects in bipolar spindle assembly resulting in chromosomal segregation abnormalities. In addition, cells treated with Aurora A inhibitors arrest in mitosis due to activation of the mitotic checkpoint.

1.4.2 In Vivo Studies

Alisertib demonstrated antitumor activity when administered orally on a daily basis for approximately 21 days (maximal tumor growth inhibition [TGI] > 90%) in several experimental human solid and hematologic tumor models grown as xenografts in immunocompromised mice. The maximally efficacious dose (ED) for each model varied: between 10 and 30 mg/kg if given once daily (QD) and 20 mg/kg if given twice daily (BID). Studies in the HCT-116 colon tumor model showed that less frequent dosing (eg, 5 days on followed by 5 days off) was also efficacious, demonstrating that continuous dosing is not necessary for antitumor activity. A single oral dose of alisertib given to nude mice bearing subcutaneous HCT-116 human colon tumors resulted in inhibition of activated AURKA and an increase in mitotic cells. Therefore, mitotic index (MI) can be used as a pharmacodynamic marker of alisertib in some in vivo settings. The relationship between pharmacokinetics (PK), pharmacodynamics, and efficacy was further studied in HCT-116 xenografts using oral dosing and subcutaneous osmotic mini-pumps. Both a pharmacodynamic response and efficacy (antitumor activity) were achieved using either route of administration. The data from these studies suggest that the maximum pharmacodynamic effect (mitotic accumulation) and efficacy are achieved at steady state plasma concentrations of 1-μM. Moreover, the maximally efficacious oral doses of alisertib in the HCT-116 model (30 mg/kg QD) resulted in plasma concentrations of 1 μM for 8 to 12 hours postdose. Plasma concentrations of alisertib associated with saturating levels of pharmacodynamic and antitumor activity (1 μM) were exceeded at the recommended phase 2 dose (RP2D) of alisertib in patients (50 mg BID).To determine whether alisertib would enhance the antitumor effects of standard of care agents in solid and hematologic malignancies, nonclinical combination studies were performed. Combination therapy with alisertib and docetaxel resulted in additive or synergistic effects during the dosing period, with prolonged tumor growth delay in multiple solid tumor xenograft models after terminating treatment. These effects were also observed in alternative intermittent dosing schedules. In DLBCL xenograft models, combination therapy with alisertib and rituximab resulted in synergistic, additive, or subadditive effects depending on the dose and model; however, prolonged tumor growth delays were observed in

every case after terminating treatment, and in some cases complete cures were maintained.

1.4.3 Safety Pharmacology, Toxicology, and Drug Metabolism

Safety pharmacology studies with alisertib did not identify significant adverse effects in nonclinical studies, including in the central nervous system (CNS) and cardiovascular systems. No alisertib-related effects on clinical signs or physical examination findings indicative of impaired respiratory function (ie, labored or shallow breathing), or microscopic changes in the lungs of animals that survived until scheduled termination, were noted at tolerated doses in Good Laboratory Practice (GLP)-compliant, repeat-dose, toxicology studies. Alisertib exhibited minimal activity against the rapidly activating component of IKr, which is encoded by hERG (IC50 and Ki > 100 μM). Alisertib had in vitro activity against the GABAAα1 benzodiazepine binding site (Ki = 290 nM).

The dose-limiting toxicities (DLTs) for alisertib in both rats and dogs after repeat daily oral dosing for 2 cycles (each cycle consisted of 7 consecutive days separated by a 14-day dose holiday) or for 6 cycles (each cycle consisted of 21 consecutive days of dosing separated by a 7-day dose holiday) were consistent with inhibition of AURKA by alisertib. Principal findings in toxicology studies in rats and dogs included gastrointestinal (GI) signs, panleukopenia, decreased reticulocyte counts, and increased mitotic figures and apoptosis (single-cell necrosis) in tissues with a high basal cellular replication rate. These findings are indicative of toxicity to rapidly replicating cell populations and are consistent with the outcomes associated with Aurora A kinase inhibition. No off-target effects were seen in the GLP-compliant toxicology studies. Alisertib was negative in the bacterial reverse mutation assay (Ames assay) both in the absence and presence of Aroclor™ 1254-induced rat liver S9 fractions. In a rat bone marrow micronucleus assay, alisertib was considered to be equivocal for clastogenicity.

Alisertib is metabolized by multiple phase I (cytochrome P450 [CYP] 3A4, CYP2C9, CYP2C19, and CYP1A2) and phase II (uridine diphosphate glucuronosyltransferase [UGT] 1A1, 1A3, and 1A8) enzymes. Using human liver microsomes with the appropriate cofactors, the percent contribution of CYP and UGT was calculated to be 13.1% and 86.9%, respectively, showing that CYP isozymes play a minor role in the metabolism of MLN8237. MLN8237 is unlikely to inhibit the 5 major CYP enzymes, 1A2, 2C9, 2C19, 2D6, and 3A4/5 (IC50 > 100 □M) when administered at the projected human efficacious dose. MLN8237 is not a mechanism-based inhibitor of CYP3A4/5. Alisertib inhibited the P glycoprotein (Pgp)-mediated efflux of paclitaxel (Taxol®) in Caco 2 cells with an IC50 of 4.0 μM.

Detailed information regarding the nonclinical pharmacology and toxicology of alisertib may be found in the IB.

1.5 Clinical Experience with Alisertib

Alisertib for clinical studies is being developed in 2 dosage formulations: enteric-coated-tablet (ECT) and oral solution (OS). The dose-escalation, phase 1 study, C14007, evaluated multiple dose levels from 10 to 60 mg BID for 7 days in repeat, 21-day cycles and 50 mg BID has been determined to be the MTD.

Alisertib is structurally related to the benzodiazepines (BZD) (eg, diazepam, lorazepam) and also has activity against the GABAAα1 BZD receptor. BZD-like effects (eg, somnolence, confusion, memory loss) have been observed to be associated with the onset of maximal plasma concentration (eg, Tmax [time to maximum plasma concentration]). CNS effects associated with peak plasma levels have been generally

managed by administration of divided doses (eg, BID administration), although dose reductions have sometimes been required. While CNS effects attributed to alisertib were also generally reversible and manageable by dose delay or reduction, the causal relationship, and thus optimal approach to management, were sometimes confounded by diverse causes including, but not limited to, concomitant medications (eg, narcotic analgesics, antianxiety medications), comorbidities (eg, infection, anemia, electrolyte abnormalities), or progressive malignancy (eg, brain metastases). The clinical experience with alisertib includes treatment with multiple doses and schedules and is summarized in the IB.

1.5.1 **Pharmacokinetics**

Upon oral administration to patients with advanced nonhematologic malignancies, absorption of alisertib was fast, with peak plasma concentrations generally achieved by 3 hours postdose. Negligible urinary excretion of alisertib was observed in humans. The renal clearance of alisertib in humans was less than 0.1% of apparent oral clearance. Steady-state plasma exposures of alisertib increased in an approximately dose-proportional manner over the range of 5 to 200 mg/day in patients with advanced solid tumors. Overall mean steady-state terminal half-life following multiple-dose administration in patients with nonhematologic malignancies was approximately 22 hours. The overall mean peak/trough ratios were 2.6 and 5.0 for BID and QD dosing, respectively. The overall mean accumulation ratios were 2.8 and 1.9 for BID and QD dosing, respectively. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration. The PK properties of alisertib in patients with hematologic malignancies were generally consistent with those observed in patients with nonhematologic malignancies. Based on PK and genotype data in patients with nonhematologic malignancies, there was a substantial overlap in exposures (dose-normalized steady-state area under the plasma concentration versus time curve [AUC]) of alisertib in patients with 0, 1, or 2 copies of the UGT1A1 *28 allele, indicating the lack of readily apparent effects of UGT1A1 genotype on alisertib systemic exposure.

Clinical pharmacokinetic data available as of 20 April 2012 are summarized in the IB. Upon oral administration to patients with advanced nonhematologic malignancies, absorption of alisertib was fast, with peak plasma concentrations generally achieved by 2 hours post dose. Negligible urinary excretion of alisertib was observed in humans. The renal clearance of alisertib in humans was less than 0.1% of apparent oral clearance. Steady-state plasma exposures of alisertib increased in an approximately dose proportional manner over the range of 5 to 200 mg/day in patients with advanced solid tumors. Overall mean steady-state terminal half-life following multiple-dose administration in patients with nonhematologic malignancies was approximately 22 hours. The overall mean peak/trough ratios were 2.6 and 5.0 for BID and QD dosing, respectively. The overall mean accumulation ratios were 2.8 and 1.9 for BID and QD dosing, respectively. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration.

Based on the results of a population PK analysis in 294 adult cancer patients, the apparent oral clearance of alisertib CL/F was unaffected by age, body weight, BSA, or the UGT1A1 genotype (number of *28 alleles). These results support the use of a common fixed starting dose of alisertib independent of UGT1A1 genotype status, age or body size in the adult patient population, in the ongoing and planned clinical trials. The absolute bioavailability of alisertib in humans has not been determined; however, the single-dose pharmacokinetics of a prototype oral solution formulation of ancomlisertib (25-mg dose) were characterized in a

cross-over relative bioavailability evaluation in Study C14010 in 15 patients with nonhematologic malignancies.

The effect of a standardized high-fat meal on the PK of single dose alisertib administered as a 50-mg strength was evaluated in 14 patients with advanced solid tumors. The lack of an effect of food on alisertib AUC_{inf} observed in this study supports the conclusion of the lack of a clinically meaningful effect of food on the PK of alisertib. The results of this study, therefore, support a recommendation that alisertib may be dosed without regard to the timing of meals in future clinical studies.

1.5.2 Potential Risks

Seven-hundred fourteen patients (excluding 13 patients from a company-sponsored, non-US IND study in Japan) had been treated with alisertib as of March 29, 2012. Clinical safety data includes experience from patients who received multiple cycles followed by treatment-free periods between each cycle, and from patients who reduced or discontinued treatment. Based on the available clinical data, drug abuse, dependency, and drug withdrawal effects were not observed.

To date, the observed risks associated with alisertib treatment, as detailed in the Safety Management Attachment of the IB, include: (1) reversible myelosuppression including leukopenia, neutropenia, febrile neutropenia, lymphopenia, thrombocytopenia, and anemia; (2) GI toxicity including stomatitis/mucositis/oral pain, nausea, vomiting, anorexia, abdominal pain, dyspepsia, diarrhea, and dehydration; (3) sedation, somnolence, confusional state, disorientation (and associated memory loss), and gait disturbances; (4) alopecia; (5) asthenia/fatigue; (6) fever, (7) infection, (8) abnormal liver function tests (including aspartate transaminase [AST], alanine transaminase [ALT], bilirubin, alkaline phosphatase [ALP], and gamma glutamyl transferase [GGT]), and (9) rash, which may include bullous dermatitis, and palmar-plantar erythrodysaesthesia syndrome. While these toxicities are potentially associated with risk or discomfort to the patient, they are anticipated to be reversible.

1.6 Rationale for the Current Study

Alisertib has proven to be effective in preclinical AML studies. Treatment of AML cell lines, primary AML cells and mouse models of AML with alisertib decreased their viability and colony forming ability on soft agar and increased their apoptosis. In addition, alisertib treatment potentiated the anti-leukemic activity of cytarabine in both primary blasts and AML cell lines.[21] In humans, a phase 1/2 clinical trial reported a 13% response rate for alisertib in relapsed and refractory AML patients. Additionally, in this clinical trial 11% of patients had a partial response and 49% of patients achieved stable disease.[22] Based on the rarity of AMKL, however, it is highly unlikely likely that any patients with this subtype were included in this or other clinical studies of alisertib. Furthermore, there have been no studies of alisertib in MF.

For this reason, while the safety profile is well-documented in other disease sub-groups, it has yet to be documented in either of these specific populations. Further, hematopoiesis is likely to be sufficiently disturbed in those with refractory AMKL and MF (often marked fibrosis and contributions toward hematopoiesis from extramedullary sites), increasing the risk for toxicity, necessitating a careful review of safety. More importantly, alisertib is being used as a differentiation agent in these two entities, and there exists a possibility for a unique differentiation syndrome as can be seen with Acute promyelocytic leukemia when using differentiation therapy. Based on these factors, we feel that a pilot study to assess safety of alisertib in the AMKL and MF would be appropriate.

In summary, our pre-clinical studies show that alisertib has potent activity against both AMKL and MF cell lines, primary cells and animal models. Based on these data, we expect that the drug will provide therapeutic benefit to patients with both AMKL and MF. We propose to conduct a pilot, two-arm trial of alisertib in patients with relapsed/refractory AMKL and MF; the overall goals of this study will be to determine the safety profile and preliminary efficacy of alisertib in each population.

2.0 OBJECTIVES & ENDPOINTS

2.1 Primary Objective & Endpoint

The primary objective of this pilot study is to determine the safety profile of alisertib in patients with AMKL and in patients with MF.

Adverse events will be defined according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0. The number, frequency, and severity of adverse events will be recorded every cycle for each population. All patients who receive at least 1 dose of alisertib will be considered evaluable for this endpoint.

2.2 Secondary Objectives & Endpoints

The secondary objective will be to determine preliminary efficacy of alisertib in both populations.

Serial blood and/or bone marrow samples will be collected at specific timepoints for each disease (see Section 5) to determine response to alisertib treatment. Responses will be categorized according to the revised/modified International Working Group (IWG) Response Criteria. Patients who had measureable disease at baseline, receive at least 1 cycle of treatment, and have at least one post-baseline disease assessment will be considered evaluable for this endpoint. Best response will be reported for all evaluable patients.

2.3 Exploratory Objectives & Endpoints

Exploratory objectives will include the following:

2.3.1 Describe pharmacodynamics (PD) effects of alisertib in peripheral blood and/or bone marrow samples.

Serial blood and/or bone marrow samples will be collected at specific timepoints. Flow cytometry, colony forming assays, AURKA autophosphorylation assays, and in vitro cultures of patient specimens to assess the effect of MLN8237 on megakaryocytes and other hematopoietic cells will be measured.

2.3.2 Evaluate the relationship between biomarker expression levels and response to alisertib.

Changes in biomarker expression levels from baseline will be measured (using PD samples) to correlate with response to alisertib therapy. Biomarkers will include a) genes encoding key enzymes in Aurora kinase signaling, b) markers of cellular aneuploidy and apoptosis, and c) markers of megakaryocytic differentiation.

2.3.3 Evaluate reduction in splenomegaly by palpation (MF arm only).

Patients will be examined for splenomegaly by palpation once per cycle and change from baseline will be calculated over time.

2.3.4 Evaluate improvement in MF symptoms (MF arm only), as assessed by the Myeloproliferative Neoplasm Symptom Assessment form (MPN-SAF).

The MPN-SAF will be administered to patients in the MF arm only once per cycle. Changes in symptom scores over time will be calculated.

2.3.5 Assess change in bone marrow fibrosis in patients in the MF arm. Bone marrow will be assessed at screening and after cycle 6 in this population.

3.0 PATIENT ELIGIBILITY

The target population for this study is patients with either AMKL or MF. This will be a multicenter trial conducted at Northwestern University, Miami University, and Mayo Clinic. Northwestern University will serve as the lead site and coordinating center for this study.

MF and AMKL are rare diseases with total prevalence (total number of patients with the disease in the US at any time) under 15,000. NIH considers prevalence under 200,000 a rare disease. A total of 24 subjects (maximum 24 subjects, regardless of the disease breakdown of MF or AMKL. If all 24 were MF, that would be acceptable) will be needed for this pilot trial. Approximately 4 potentially eligible patients are seen per month, and it is anticipated that at least 1 per month will be accrued (once all sites are up and running). Potential patients may be referred to the Principal Investigator (PI) at Northwestern University, Dr. Brady Stein (312-695-6832 or 312-695-0990), to Dr. Ronan Swords at Miami University, or to Dr. Naseema Gangat at Mayo Clinic.

Eligibility will be evaluated according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

3.1 Inclusion Criteria – AMKL Patients

3.1.1 Patients must have a confirmed diagnosis (by blood or bone marrow) of relapsed/refractory acute megakaryoblastic leukemia (AMKL), as defined by WHO criteria (see appendix I).
NOTE: If diagnosis was performed an outside facility, a copy of the report is sufficient for registration purposes; however, local pathology review at one of the main sites should still be obtained.

3.1.2 Patients must be age 18 or older.

3.1.3 Patients must have an ECOG status 0-2.

3.1.4 Patients must exhibit adequate organ function within 1 week (7 days) prior to registration, defined as:

- Total bilirubin ≤ 1.5 x ULN
- ALT/AST ≤ 2.5 x ULN
- Creatinine < 1.5 x ULN or calculated creatinine clearance > 30 ml/min
- PT and PTT ≤ 1.5 x ULN

3.1.5 Patients must have estimated life expectancy of 6 months or greater.

3.1.6 Female patients of child-bearing potential (FOCBP) must have a negative serum beta-HCG pregnancy test within 7 days prior to registration.

NOTE: A FOCBP is *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- *Has not* undergone a hysterectomy or bilateral oophorectomy
- *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.7 Female patients must meet at least one of the following conditions:

- Must be post-menopausal for at least 1 year prior to registration (not of child-bearing potential as described above)
- Must be surgically sterilized (as described above)
- Willing to use an acceptable method of birth control (i.e. hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study.

3.1.8 Male patients, even if surgically sterilized (i.e. status post-vasectomy) agrees to use an acceptable method for contraception during the entire study treatment period through 120 days after the last dose of alisertib. Likewise, female patients should agree to use acceptable method for contraception during the entire study treatment period through 90 days after the last dose of alisertib.

3.1.9 Patients must be able to understand and willing to sign a written informed consent**.**

3.2 Inclusion Criteria – MF Patients

3.2.1 Patients must have a confirmed diagnosis (by blood or bone marrow) of myelofibrosis (MF), as defined by WHO criteria (see appendix II).
NOTE: If diagnosis was performed an outside facility, a copy of the report is sufficient for registration purposes; however, local pathology review at one of the main sites should still be obtained.

3.2.2 Patients must be intermediate I risk or beyond and meet the following:

- in need of treatment;
- intolerant or refractory to ruxolitinib (or other investigational JAK-inhibitors) OR unlikely to benefit from ruxolitinib
- ineligible or refusal to undergo stem cell transplantation.

3.2.3 Patients must be age ≥ 18 years.

3.2.4 Patients must have an ECOG status 0-2.

3.2.5 Patients must have adequate bone marrow and organ function within 2 weeks (14 days prior to registration, defined as:

- Direct bilirubin ≤ 1.5 x ULN≥
- ALT/AST ≤ 2.5 x ULN
- Creatinine < 1.5 x ULN or calculated creatinine clearance > 30 ml/min
- PT and PTT ≤ 1.5 x ULN
- ANC ≥ 1000/mm^3
- Platelets ≥ 50,000/mm^3
  Note: this must be achieved without transfusion.

3.2.6 Patients must have estimated life expectancy of 6 months or greater.

3.2.7 Female patients of child-bearing potential (FOCBP) must have a negative serum beta-HCG pregnancy test within 7 days prior to registration.
NOTE: See section 3.1 for definition of FOCBP.

3.2.8 Female patients must meet at least one of the following conditions:

- Must be post-menopausal for at least 1 year prior to registration (not of child-bearing potential as described above)
- Must be surgically sterilized (as described above)
- Willing to use an acceptable method of birth control (i.e. hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study.

3.2.9 Male patients, even if surgically sterilized (i.e. status post-vasectomy) agrees to use an acceptable method for contraception during the entire study treatment period through 120 days after the last dose of alisertib. Likewise, female patients should agree to use acceptable method for contraception during the entire study treatment period through 90 days after the last dose of alisertib.

3.2.10 Patients must be able to understand and willing to sign a written informed consent.

3.3 Exclusion Criteria – All Patients

3.3.1 Patients who have received treatment with clinically-significant enzyme inducers within 14 days prior to registration are not eligible. Please refer to Appendix III.

3.3.2 Patients who have received any investigational products, antineoplastic therapies, or radiotherapy within 14 days prior to registration are not eligible.
NOTE: Patients actively receiving hydroxyurea are eligible and may continue to receive hydroxyurea through cycle 1 of protocol treatment.. If the platelet count remains above 1 million after cycle 1, hydroxyurea can be used at the treating physician's discretion, if the platelets are 1000 x 10^9/L, or more, or if there are symptoms from thrombocytosis.

3.3.3 Patients who have received prior administration of an Aurora A kinase-targeted agent (including alisertib) are not eligible.

3.3.4 Patients who have received corticosteroids within 7 days prior registration are not eligible, UNLESS the patient has been taking a continuous dose of no more than 15 mg/day of prednisone for at least 1 month prior.
N*OTE: Low dose steroid use for control of nausea and vomiting will be allowed. Topical steroid use and inhaled steroids are also permitted.*

3.3.5 Patients who are candidates (eligible and willing) for standard and/or potentially curative treatments are not eligible.

3.3.6 Patients who have had major surgery within one month (28 days) prior to registration are not eligible.

3.3.7 Patients within 60 days of allogenic bone transplant are not eligible; patients with solid organ transplant are not eligible.

3.3.8 Patients who have had grade 2 or higher diarrhea, despite optimal antidiarrheal supportive care, within 7 days prior to registration are not eligible.

3.3.9 Patients who have had grade 2 or higher peripheral neuropathy within 14 days prior to registration are not eligible (must have resolved to grade 1 or lower to register).

3.3.10 Patients who have had a myocardial infarction within 6 months (24 weeks) prior to registration are not eligible.

3.3.11 Patients who have class III or IV heart failure (as defined by the New York Heart Association), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities are not eligible.

3.3.12 Patients who have known GI disease or GI procedures which could interfere with the oral absorption or tolerance of alisertib are not eligible. Examples include (but are not limited to) partial gastrectomy, history of small intestine surgery, and celiac disease.

3.3.13 Patients who have a known history of uncontrolled sleep apnea syndrome and other conditions that could result in excessive daytime sleepiness (such as severe chronic obstructive pulmonary disease or requirement for supplemental oxygen) are not eligible.

3.3.14 Patients who have a requirement for constant administration of proton pump inhibitor, H2 antagonist, or pancreatic enzymes are not eligible. Intermittent usage of antacids or H2 antagonists are allowed.

3.3.15 Patients with an active, uncontrolled systemic infection are not eligible until deemed controlled by the treating physician.

3.3.16 Patients who are known human immunodeficiency virus (HIV) positive are not ' eligible.

3.3.17 Patients who are known hepatitis B surface antigen-positive are not eligible.

3.3.18 Patients who have known or suspected active hepatitis C infections are not eligible.
NOTE: Patients who are hepatitis C surface antigen-positive are eligible.

3.3.19 Female patients who are pregnant or breast feeding are not eligible.

3.3.20 Patients who have any of the following severe acute or chronic medical or psychiatric conditions that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study are not eligible:
- uncontrolled diabetes
- malabsorption
- resection of the pancreas or upper small bowel
- requirement for pancreatic enzymes
- any condition that would modify small bowel absorption of oral medications,
- other laboratory abnormality.

3.3.21 Patients who have symptomatic CNS involvement are not eligible.

3.3.22 Patients who have been diagnosed or treated for another malignancy within 3 years prior to registration are not eligible *aside from these exceptions*: completely resected basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, or low-risk prostate cancer after curative therapy. If a patient had a prior MPN that evolved to a blast phase, but with treatment, reverted to Myelofibrosis at the time of screening, these pts are considered eligible at the discretion of the PI, if not considered suitable for stem cell transplantation.

3.3.23 Patients who are unable to swallow oral medication or are unwilling to comply with the administration requirements are not eligible.

4.0 TREATMENT PLAN

4.1 Overview

This will be a two arm pilot study of alisertib treatment in patients with AMKL or MF. The starting dose (level 1) for all patients will be 50 mg BID, administered orally on days 1-7 of each cycle (1 cycle = 21 days), based on the MTD from previous studies. Patients may continue to receive cycles of alisertib until progression of disease or unacceptable toxicity. There will be no long-term follow-up beyond end of treatment, aside from a standard end of treatment visit approximately 30 days after the last dose of study drug, followed by a final off-study visit approximately 6 months after the last dose. Patients with on-going adverse events will be followed until resolution of the event(s), defined as a return to baseline or stabilization of the event.

4.2 Treatment Administration

Alisertib will be administered orally BID on days 1-7 of each cycle; one cycle is defined as 21 days (days 8-21 will be rest days). Patients will be instructed to take each oral dose of alisertib with 8 ounces (1 cup, 240 mL) of water, with or without food. Doses on the same day should be taken approximately 12 hours apart (but *at least* 6 hours apart). All tablets are to be ingested whole; patients who have difficulty swallowing tablets will be excluded from the study. Antiemetogenic agents may be administered at the discretion of the investigator. Neutralizing antacids and calcium-containing supplements cannot be taken from 2 hours prior to alisertib dosing until up to 2 hours after dosing.

Patients will be dispensed the appropriate number of tablets required for each cycle. Treatment compliance will be assessed based on return of unused alisertib tablets and completion of a study medication diary; patients will be asked to bring completed diaries and pill bottles to each visit, and compliance will be reviewed by the study team each cycle. For MF patients only, from Cycle1-6, on Day 1 of each cycle, when the patient comes in for clinic visit, they will be given study drug supply for one cycle (1 cycle=21 days). Beyond Cycle 6, if the patient's condition is stable, then they will be required to come in on Day 1 of every odd cycle (every 6 weeks) for study visit. In that case, patients will be given study drug supply for 2 cycles.

If a dose is missed due to patient error/oversight, they will be instructed to take the dose within 6 hours of the normal time. If more than 6 hours have elapsed, that missed dose should be omitted, and the patient should resume treatment at the next scheduled dosing time point. All missed/omitted doses and the reason will be reported on the diary. If a dose is vomited, it should not be re-taken. If a dose is held due to toxicity or investigator's discretion, the patient should be instructed to resume treatment at the investigators discretion, taking into account all of the dose modification/discontinuation guidelines provided below.

From Cycle1-6, in order for each new cycle of therapy to begin, MF patients must meet the following criteria: (if the cytopenias in AMKL patients are considered-disease related/due to AMKL then the following neutrophil and platelet count requirements do not apply. If cytopenias are considered due to the study drug, then the counts must be

- ANC must be ≥ 1000/mm^3
- Platelet count must be ≥ 50,000/mm^3
- In addition, all toxicities considered to be related to therapy with alisertib must have resolved to ≤ Grade 1or the patient's baseline values

For MF patients only, from Cycle1-6 patients will be required to come in for study visit at beginning of every cycle (every 3 weeks) . Beyond Cycle 6, if the patient's condition is stable, then they will be required to come in on Day 1 of every odd cycle (every 6 weeks) for study visit. In that case, for those visits when an MF patient is not coming to clinic for study visit, CBC and CMP will be done in an outside laboratory convenient for the patient, and results will be have to be faxed to the clinic.

Patients can start their treatment at home only after the treating physician has reviewed the laboratory results and determined that they are able to continue treatment. For these cycles, the study coordinator will contact the patient on/prior to Day 1 to inform them if they can or cannot start the new cycle, and also check compliance regarding study drug intake.

4.3 Dose Modifications & Delays

Any patient who receives at least one dose of study therapy will be evaluable for toxicity endpoints. Each patient will be assessed for the development of toxicity according to the timeframe referenced in Section 5). Toxicity will be evaluated according to the NCI's CTCAE v 4. To manage excessive toxicity, reduction of the total alisertib dose can be done by reducing the daily dose administered and/or by interruption of the schedule treatment within a cycle.

In general treatment delays of up to 1 week for any reason are permitted. If a patient fails to meet the above-cited criteria for a new cycle of therapy, then initiation of the next cycle *should be delayed* for up to 1 week. At the end of that week, the patient should be re-evaluated to determine whether the criteria for retreatment have been met. Should treatment need to be delayed for more than 1 week because of incomplete recovery from treatment-related toxicity, the dose of alisertib will be reduced (see table below) to 40 mg BID when therapy resumes. A second dose reduction to 30 mg BID may occur should treatment need to be delayed for more than 1 week because of incomplete recovery from treatment-related toxicity at the 40 mg BID dose. Patients who require further dose reduction will be removed from the study. Should treatment need to be delayed for more than 2 weeks at any dose, therapy with alisertib will be discontinued.

Dose Level	Alisertib[1]
Level 1 (starting dose)	50 mg
Level -1	40 mg
Level -2	30 mg
Level -3	Discontinue

[1] Doses are administered orally, BID on days 1-7 of each cycle.

4.3.1 **Dose Modifications for Hematological Toxicities**

If a patient experiences any of the following hematological toxicities during the dosing period, dosing will be discontinued for the remainder of that cycle and the dose will be decreased by 1 level (-10 mg) for all subsequent cycles of treatment.

- Grade 4 neutropenia (ANC $<$ 500 cells/mm^3) lasting more than 7 consecutive days
- Grade 4 thrombocytopenia (platelet count < 25,000/μL) lasting more than 7 consecutive days
- Platelet count less than 10,000/μL at any time
- Grade 3 neutropenia with fever or infection, or both, where fever is defined as an oral temperature greater than 38.5°C
- Grade 3 thrombocytopenia with clinically significant bleeding

4.3.2 **Dose Modifications for Non-hematological Toxicities**

If a patient experiences any of the following toxicities during the dosing period, dosing will be held for the remainder of that cycle and the dose will be decreased by 1 level (-10 mg) for all subsequent cycles of treatment, and treatment may resume after drug related toxicities have resolved to ≤ Grade 1 or to baseline.

- Any Grade 3 non-hematological toxicity that is considered by the investigator to be related to study drug other than:
    - Grade 3 or greater nausea or emesis, or both, that occurs in the absence of optimal antiemetic therapy (5-hydroxytryptamine 3 [5-HT3] serotonin receptor antagonist);
    - Grade 3 or greater diarrhea that occurs in the absence of optimal supportive therapy with loperamide or a comparable anti-diarrheal;
    - Grade 3 fatigue that lasts less than 1 week
- Grade 2 non-hematological toxicities that are considered by the investigator to be related to study drug and in the opinion of the investigator require dose reduction.
- Any grade 4 non-hematological toxicity that is considered by the investigator to be related to study drug. If, in the opinion of the investigator and the DMC, it is in the patient's interest to continue therapy with alisertib, the patient may resume therapy after recovery from a grade 4 to ≤ Grade 1 or to baseline values; the dose of alisertib should be reduced by at least 1 dose level for subsequent cycles of therapy.

When intra-patient dose reduction of alisertib is required, no re-escalation of dose will be permitted. If a patient requires more than 2 dose reductions, therapy with alisertib will be discontinued.

4.4 Management of Clinical Events

4.4.1 **Nausea and vomiting**

Prophylactic antiemetic therapy will not be used in this study unless it becomes clear that alisertib causes acute nausea and vomiting. Although this study will not initially employ prophylactic antiemetics, there is no prohibition against antiemetic use in the management of a patient who develops nausea or vomiting, or both. If

prophylactic antiemetic therapy is needed, 5 HT3 receptor antagonists (without corticosteroids) should be tried first. Because of the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

4.4.2 **Diarrhea**

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, at the occurrence of the first loose stool and then 2 mg every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

4.4.3 **Central nervous system effects**

If a patient experiences excessive sedation believed to be related to alisertib, treatment with alisertib should be interrupted. Patients whose sedation is not considered immediately life-threatening should be carefully monitored and given appropriate supportive care. Patients and family members/caregivers will be explicitly counseled regarding the possibility for sedation during the informed consent process, prior to cycle 1, and with each study visit/AE assessment. Patients and family members/caregivers will be counseled not to drive, operate heavy or dangerous machinery, or perform any other dangerous activities if they experience any sedation with alisertib.

If the patient's level of consciousness is considered to be life-threatening, necessary measures should be instituted to secure the airway, ventilation, and intravenous access. Flumazenil (Romazicon®) is a selective benzodiazepine receptor antagonist that is intended as an adjunct to, not as a substitute for, the proper management of benzodiazepine overdose. Although there is neither preclinical nor clinical experience with flumazenil and alisertib, the use of flumazenil should be considered if the level of alisertib-associated sedation is considered to be life-threatening. Patients treated with flumazenil should be monitored for resedation, respiratory depression, and other residual benzodiazepine effects for an appropriate period after treatment. Continued monitoring is particularly important in the case of alisertib given its half-life and the comparatively brief half-life of flumazenil in the CNS (20-30 minutes). Flumazenil should be administered according to its label.

4.4.4 Differentiation syndrome

In this study, alisertib is being used as a differentiation agent in the treatment of AMKL and MF, and theoretically, there exists a possibility for a unique differentiation syndrome as can be seen with acute promyelocytic leukemia when using differentiation therapy. Therefore, patients will be monitored for symptoms of a differentiation syndrome. Taking from the experience in APL, patients will be monitored for the following symptoms or signs: fever, peripheral edema, hypoxemia, respiratory distress, hypotension, renal and hepatic dysfunction, and serositis (shortness of breath, pleurisy, abdominal distension) The study team will be counseled about the presenting features of this acute illness. With regard to the monitoring plan, with suspected differentiation syndrome, alisertib will be held. Patients will require hospitalization for careful monitoring and observation. Supportive care maneuvers will include supplemental oxygen for those with hypoxia, In severe cases, ventilatory support will be required. In the presence of volume overload, if hemodynamics permit, diuresis can be attempted. Those with dyspnea or abdominal discomfort will require imaging to evaluate for the presence of effusion, pulmonary opacity, and ascites. Patients will be assessed for the presence of coagulopathy, and if present, supportive care with fresh frozen plasma, platelet transfusions, or

cryoprecipitate will be considered. Intravenous dexamethasone (10mg IV q12 hours x 3 days) will be administered in those with differentiation syndrome.

4.5 Concomitant Medications/Treatments

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient is permitted, except as specifically prohibited (see Sections 4.5.1 and 4.5.2 below),

4.5.1 Prohibited Concomitant Medications/Treatments

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy other than alisertib
- Alternative therapy, including palliative radiotherapy, for treatment of the patient's malignancy
- Any investigational therapy other than alisertib
- Requirement for administration of any proton pump inhibitor. Use of any PPI in either continued or intermittent use will be prohibited during the conduct of the study and patients must discontinue any use of PPI within five days prior to the first dose of alisertib. Patients may be administered alternative agents to manage gastric acidity or reflux (eg, H2 receptor antagonists, antacids) with exceptions described below:
    - Histamine-2 (H2) receptor antagonists are not permitted from the day prior (Day -1) through to the end of alisertib dosing (e.g., Day 7)
- Strong UGT 1A1/2/8 and CYP 3A4, 2C9, 2C19, and 1A2 enzyme inducers or inhibitors, such as the enzyme-inducing antiepileptic drugs phenytoin, carbamazepine or phenobarbital, or rifampin, rifabutin, rifapentine unless medically necessary (see Appendix III)
- Patients should be strongly encouraged to avoid herbal remedies, including:
    - St. John's wort (strong inducer of CYP3A4)
    - Grapefruit juice (strong inhibitor of CYP3A4)

4.5.2 Permitted Concomitant Medications/Treatments

- Myeloid growth factors to treat patients with neutropenia according to the ASCO guidelines.
- Antiemetic agents may be administered at the discretion of the investigator but are not commonly required as a prophylactic agent.
- Antacids are permitted; however, they should be administered more than 2 hours before or 2 hours after administration of alisertib.
- Medications with potential CNS effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with alisertib. Because of alisertib's structural and pharmacological similarity to the benzodiazepines, concomitant therapy with benzodiazepines is discouraged but not prohibited.
- Patients actively receiving hydroxyurea are eligible and may continue to receive hydroxyurea through cycle 1 of protocol treatment.
  *(Note: **I**f the platelet count remains above 1 million after cycle 1, hydroxyurea can be used at the treating physician's discretion, if the platelets are 1000 x 10^9/L, or more, or if there are symptoms from thrombocytosis).*

- Medications that can potentially reduce or increase effectiveness of alisertib, UGT 1A1/2/8 and moderate CYP 3A4, 2C9, 2C19, and 1A2 enzyme inducers or inhibitors, should be used with caution (see appendix III).
- All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

4.6 Duration of Therapy

Patients may continue to receive alisertib treatment until any of the following occur:

- Disease progression
- Development of an inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from either study treatment or the as a whole study
- The treating investigator determines that the patient should be taken off treatment for any reason (i.e. changes in condition, inability to comply with study treatment or procedures)

4.7 Duration of Follow Up

Once off treatment for any reason, patients will have an end-of-treatment visit at approximately 30 days after their last dose of alisertib. Patients who are experiencing on-going toxicities at this time will continue to be followed until resolution of the toxicity (either return to baseline or stabilization). In addition, there will be a final off-study visit approximately 6 months after the last dose of treatment to assess for late-onset toxicity.

4.8 Termination of Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted)
- Patient withdraws consent (no follow-up permitted)
- Patient is unable to comply with protocol requirements
- Patient demonstrates disease progression
- Patient experiences unacceptable toxicity
- Patient experiences treatment delay of > 2 weeks due to toxicity
- Treating physician determines that continuation on the study would not be in the patient's best interest
- Patient becomes pregnant
- Patient develops a second malignancy that requires treatment which would interfere with this study
- Patient develops an intercurrent illness that interferes with study treatment.
- Patient becomes lost to follow-up (LTF)

5.0 STUDY PROCEDURES

5.1 Schedule of Events for MF Patients

	Baseline	On-Treatment[i]							Off-Treatment	
	Screening	Cycle 1[a]				Cycles 2-6[q] +/- 2 days		Cycle 7+[m] +/- 2 days	EOT Visit[o]	EOS Visit[p]
		D1[i]	D4 (+/- 1d)	D8 (+/-2 d)	D15 (+/-2 d)	D1[i]	D15	D1		
Informed consent[v]	X									
Medical history[r]	X									
Physical exam[b]	X	X		X	X	X		X	X	X
ECOG status[s]	X	X		X	X	X		X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X
12-lead ECG[d]	X									
Hematology[e,t]	X	X	X	X	X	X	X	X	X	X
Chemistry[f,t]	X	X	X	X	X	X	X	X	X	X
DIC screen[g,t]	X									
PT(INR)/aPTT[g]		X	X	X	X	X				
Pregnancy test[h,t]	X	X								
Palpation for splenomegaly[l]	X	X				X		X		
MPN-SAF[l]	X	X				X		X		
Bone marrow biopsy & aspirate[k]	X							X[k]	X	
Bone marrow collection for PD studies [i, j]	X									
Blood collection for PD studies [i,j]		X			X	X	X			
Treatment[n]		X								
Adverse events[u]	X	X		X	X	X		X	X	X
Concomitant meds	X	X		X	X	X		X	X	

[a] One cycle = 21 days.

[b] Includes height (baseline only) and weight. Baseline physical exam within 14 days of registration.

[c] Vital signs include diastolic and systolic blood pressure, heart rate, and oral temperature;.C1D1 vital signs should be taken pre-dose(+/-30 mins) as well as about 2 hours(+/-30mins) post dose.

[d] A 12-lead ECG will be performed at baseline and thereafter only as clinically indicated. Baseline ECG within 14 days of registration

[e] Hematology panel to include hematocrit, hemoglobin, white blood cell count with differential, and platelet count. Samples should be collected days 1, 4, 8, and 15 of cycle 1, days 1 and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all timepoints). See footnote t.

[f] Chemistry panel to include the following: BUN, creatinine, sodium, potassium, chloride, bicarbonate, glucose, uric acid, LDH, total bilirubin, direct bilirubin, ALP, AST (SGOT), ALT (SGPT), albumin, magnesium, phosphate, and calcium. Samples should be collected days 1, 4, 8, and 15 of cycle1, days 1 and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all timepoints). See footnote t. Beyond Cycle 6, magnesium and phosphate testing is not required(as part of the chemistry panel).

[g] Disseminated intravascular coagulation (DIC) screen should include: PT/INR, aPTTt, fibrinogen, and D-dimer. Cycle 1 day 1 PT/INR/aPTT does not need to be performed if the screening DIC was within 3 days of this visit. If the initial DIC is positive follow-up coagulation studies should include the full DIC panel (rather than the PT/INR/aPTT alone). See footnote t.
Note: Beyond Cycle 6, PT/INR,aPTT testing is not required.

[h] Serum pregnancy test required for women of child-bearing potential during screening AND pre-dose on Cycle 1 day 1. Results must be available and negative prior to dosing. See footnote t.

[i] It is strongly preferred that correlative samples be collected and shipped only on Monday – Thursday (with same day shipment preferred). Friday collections/shipments should be avoided to ensure sample stability. Since these samples are collected on Day 1 of each cycle, it is preferred that Day 1 visits be conducted on Monday through Thursday when correlative samples are being collected. Sites should use the available windows to achieve this. Please contact the QAM if there are any questions

.[j] Peripheral blood (25 ml) and/or bone marrow (bone marrow only if done clinically – will collect about 10-20 ml) samples will be collected for determination of alisertib pharmacodynamics (PD studies). Blood samples will be collected at the start of each cycle(D1). Sample collection at D15 will be optional.(Samples will be collected for a maximum of 6 cycles). Correlative bone marrow samples should be obtained any time bone marrows are collected as clinically indicated during study participation. . At baseline, correlative bone marrow collection is not mandatory for study entry. However, study team should request 3-4 unstained slides from any prior bone marrow testing performed within the last year before study entry. Please refer to laboratory manual for details.

[k] Bone marrow aspirate and biopsy is required within 1 year prior to registration(provided the PI confirms the diagnosis and there is no suspicion for acceleration to blast phase),on C7D1 (+/-14 days) and thereafter only as clinically indicated.

[l] Palpation for splenomegaly and MPN-SAF will be done only on MF patients. Baseline MPN-SAF and palpation for Splenomegaly within 14 days of registration

[m] Beyond Cycle 6, if the patient's condition is stable, then they will be required to come in only on Day 1 of every even cycle (every 6 weeks) for study visit. In that case, for those visits when patient is not coming to clinic for study visit, CBC and CMP only will be done in an outside laboratory convenient for the patient, and results will be have to be faxed to the clinic. Patients can start their treatment at home only after the treating physician has reviewed the laboratory results and determined that they are able to continue treatment. For these cycles, the study coordinator will contact the patient on/prior to Day 1 to inform them if they can or cannot start the new cycle, and also check compliance regarding study drug intake.

[n] Patients will be administered orally on days 1-7 of each cycle (1 cycle = 21 days); patients may remain on treatment until development of unacceptable toxicity or progression of disease.

[o] Patients will have an end of treatment (EOT) visit approximately 30 days (+10 days) after the last dose of treatment; if a bone marrow was carried out at disease progression, this will be considered the EOT marrow and does not need to be repeated at the EOT visit.

[p] Patients will have a final end of study (EOS) visit approximately 6 months (+/- 30 days) after the last dose of treatment to assess for late-onset toxicity.

[q] Labs required only on days 1 and 15 of cycles 2-6, but in the event of cycle 1 toxicity or at treating investigator's discretion, additional interim labs may be performed per standard of care**.**

[r] Baseline Medical History within 14 days of registration

[s] Baseline ECOG Status within 14 days of registration.

[t] Baseline Hematology, Chemistry, DIC screen, Pregnancy Test, within 14 days of registration

[u] Baseline Adverse Events and Concomitant Medications within 14 days of registration.

[v]Informed consent should be obtained within 28 days of registration.

5.2 Schedule of Events for AMKL Patients

	Baseline	On-Treatment[i]							Off-Treatment	
	Screening	Cycle 1[a]				Cycles 2-6[p] +/- 2 days		Cycle 7+[l] +/- 2 days	EOT Visit[n]	EOS Visit[o]
		D1[i]	D4 (+/-1d)	D8 (+/-2 d)	D15 (+/-2 d)	D1[i]	D15			
Informed consent[v]	X									
Medical history[q]	X									
Physical exam[b]	X	X		X	X	X		X	X	X
ECOG status[r]	X	X		X	X	X		X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X
12-lead ECG[d]	X									
Hematology[e,s]	X	X	X	X	X	X	X	X	X	X
Chemistry[f,s]	X	X	X	X	X	X	X	X	X	X
DIC screen[g,s]	X									
PT(INR)/aPTT[g]		X	X	X	X	X				
Pregnancy test[h,s]	X	X								
Bone marrow biopsy & aspirate[k]	X					X		X	X	
Bone marrow collection for PD studies [i, j]	X									
Blood collection for PD studies [i,j]		X			X	X	X			
Buccal swab (Optional)[u]	X									
Treatment[m]		X								
Adverse events[t]	X	X		X	X	X		X	X	X
Concomitant meds	X	X		X	X	X		X	X	

[a] One cycle = 21 days

[b] Includes height (baseline only) and weight. Baseline physical Exam within 14 days of registration.

[c] Vital signs include diastolic and systolic blood pressure, heart rate, and oral temperature; C1D1 vital signs should be taken pre-dose(+/-30 mins) as well as about 2 hours(+/-30mins) post dose.

[d] A 12-lead ECG will be performed at baseline and thereafter only as clinically indicated. Baseline ECG within 14 days of registration

[e] Hematology panel to include hematocrit, hemoglobin, white blood cell count with differential, and platelet count. Samples should be collected days 1, 4, 8, and 15 of cycle 1, days 1 and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all timepoints). See footnote s.

[f] Chemistry panel to include the following: BUN, creatinine, sodium, potassium, chloride, bicarbonate, glucose, uric acid, LDH, total bilirubin, direct bilirubin, ALP, AST (SGOT), ALT (SGPT), albumin, magnesium, phosphate, and calcium. Samples should be collected days 1, 4, 8, and 15 of cycle1, days 1 and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all timepoints). See footnote s.

[g] Disseminated intravascular coagulation (DIC) screen should include: PT/INR, aPTT, fibrinogen, and D-dimer. Cycle 1 day 1 PT/INR/aPTT does not need to be performed if the screening DIC was within 3 days of this visit. If the initial DIC is positive follow-up coagulation studies should include the full DIC panel (rather than the PT/INR/aPTT alone). See footnote s.

[h] Serum pregnancy test required for women of child-bearing potential during screening AND pre-dose on Cycle 1 day 1. Results must be available and negative prior to dosing. See footnote s.

[i] It is strongly preferred that correlative samples be collected and shipped only on Monday – Thursday (with same day shipment preferred). Friday collections/shipments should be avoided to ensure sample stability. Since these samples are collected on Day 1 of each cycle, it is preferred that Day 1 visits be conducted on Monday through Thursday when correlative samples are being collected. Sites should use the available windows to achieve this. Please contact the QAM if there are any questions.

[j] Peripheral blood (25 ml) and/or bone marrow (if done will collect about 10-20 ml) samples will be collected for determination of alisertib pharmacodynamics (PD studies). Samples will be collected at the start of each cycle(D1). Sample collection at D15 will be optional Samples will be collected for a maximum of 6 cycles . Please refer to laboratory manual for details.

[k] Bone marrow aspirate and biopsy will be performed at screening (within 21 days prior to registration), cycle 2 day 1, and after cycle 4 (between days 21 and 35). For patients not yet in CR after cycle 4, bone marrow aspirate and biopsy should be repeated after cycle 6 (between days 21 and 35). Thereafter bone marrows should be repeated as clinically indicated.

[l] Labs and exam only required on day 1 of cycles 7 and beyond (although additional may be performed as clinically indicated).

[m] Patients will be administered orally on days 1-7 of each cycle (1 cycle = 21 days); patients may remain on treatment until development of unacceptable toxicity or progression of disease.

[n] Patients will have an end of treatment (EOT) visit approximately 30 days (+10 days) after the last dose of treatment; if a bone marrow was carried out at disease progression, this will be considered the EOT marrow and does not need to be repeated at the EOT visit.

[o] Patients will have a final end of study (EOS) visit approximately 6 months (+/- 30 days) after the last dose of treatment to assess for late-onset toxicity.

[p] Labs *required* only on days 1 and 15 of cycles 2-6, but in the event of cycle 1 toxicity or at treating investigator's discretion, additional interim labs may be performed per standard of care.

[q] Baseline Medical History within 14 days of registration

[r] Baseline ECOG Status within 14 days of registration.

[s] Baseline Hematology, Chemistry, DIC screen, Pregnancy Test, within 7 days of registration

[t] Baseline Adverse Events and Concomitant Medications within 14 days of registration.

[u] For subjects signing optional consent.

[v]Informed consent should be obtained within 28 days of registration.

6.0 ENDPOINT ASSESSMENT

6.1 Primary Endpoint

The primary objective of this pilot study is to determine the safety profile of alisertib in patients with AMKL and in patients with MF.

Adverse events will be defined according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0. The number, frequency, and severity of adverse events will be recorded every cycle for each population. All patients who receive at least 1 dose of alisertib will be considered evaluable for this endpoint.

6.2 Secondary Endpoints

The secondary objective will be to determine preliminary efficacy of alisertib in both populations.

Serial blood and/or bone marrow samples will be collected at specific timepoints for each disease (see Section 5) to determine response to alisertib treatment. Responses will be categorized according to the revised/modified International Working Group (IWG) Response Criteria. Please refer to criteria located in the appendices. Patients who had measureable disease at baseline, receive at least 1 cycle of treatment, and have at least one post-baseline disease assessment will be considered evaluable for this endpoint. Response after cycle 6 (upon completion of C7D1 marrow) will be reported for all evaluable patients.

7.0 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to http://cancer.northwestern.edu/cro/data/DataandSafetyMonitoringPlanMay2014.pdf). The level of risk attributed to this study requires high intensity monitoring, as outlined in the DSMP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations and as required by the NCI.

7.1 Adverse Event Monitoring

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 (see Section 5 for timepoints). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed 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.

7.2 Definitions & Descriptions

7.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, “hepatitis” and not “elevated liver function tests” should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

7.2.2 Severity of AEs

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

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.

7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 30 days after the last administration of study drug, must be reported upon discovery or occurrence.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **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.**
  Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".
  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.2.4 Unanticipated Problems Involving Risks to Subject or Others

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is *unanticipated* in terms of nature, severity, or frequency
- Places the research subject or others at a different or *greater risk of harm*
- Is deemed to be *at least possibly related* to participation in the study.

7.3 Adverse Event Reporting

7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF according to the time intervals noted in the appendices. Routine AEs will be reviewed by the Data Monitoring Committee (DMC) according to the study's phase and risk level, as outlined in the DSMP.

7.3.2 Determining if Expedited Reporting is Required

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.

1) Identify the type of adverse event using the NCI CTCAE v 4.0.
2) Grade the adverse event using the NCI CTCAE v 4.0.
3) Determine whether the adverse event is related to the protocol therapy. Attribution categories are as follows:
   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.
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 protocol
   - the current Investigator's Brochure

7.3.3 Expedited Reporting of SAEs/Other Events

7.3.3.1 Reporting to the Northwestern University QAM/DMC

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Completion of the NU CRO SAE Form is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the DMC at their next meeting.

7.3.3.2 Reporting to the Northwestern University IRB

The following information pertains to the responsibilities of the lead site (Northwestern University). Additional participating sites should follow their local IRB guidelines for reporting to their local IRBs.

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

7.3.3.3 Reporting to the FDA

All notifications to the FDA will be handled centrally the QA team at Northwestern.

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

7.3.3.4 Reporting to Takeda

Regardless of expectedness or causality, all SAEs must also be reported to Takeda or designee:

- **Fatal and Life Threatening SAEs** within 24 hours but no later than 4 calendar days from observation or awareness of the event
- **All other serious (non-fatal/non-life threatening) events** within 4 calendar days of observation or awareness of the event

The NU CRO SAE report form will be completed and submitted for all SAEs requiring reporting to Takeda. Reports will include event term(s), serious criteria, intensity of the event(s), and causality of the event(s). Follow-up information on the SAE may be requested by Takeda.

The NU PI is responsible to ensure that the SAE reports are sent to Takeda (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the NU CRO QAM (as outlined above). NU must also provide Takeda with a copy of all communications with applicable regulatory authorities related to the study product(s) as soon as possible but no later than 4 calendar days of such communication.

The contact for reporting is:
Takeda or Designee
SAE and Pregnancy Reporting Contact Information
FAX Number 1-800-963-6290
Email: TakedaOncoCases@cognizant.com
If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator and NU CRO QAM immediately and permanently discontinue study drug. The QAM must fax a completed Pregnancy Form to Takeda or designee. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the QAM must also immediately fax a completed Pregnancy Form Takeda or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Takeda Quality representative.

For Product Complaints, call MedComm Solutions at:
877-674-3784 (877 MPI DRUG)

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Takeda.

8.0 DRUG INFORMATION

8.1 Alisertib

8.1.1 Other names

MLN8237

8.1.2 Classification - type of agent

Alisertib is an adenosine triphosphate (ATP)-competitive and reversible inhibitor of Aurora A kinase.

8.1.3 **Mode of action**

Consistent with the mechanism of action for an Aurora A kinase inhibitor, alisertib treatment results in formation of abnormal mitotic spindles, an accumulation of mitotic cells, and a decrease in the proliferation of a broad range of tumor cell lines grown in culture.

8.1.4 **Storage and stability**

Tablets should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30□C; 59-86□F) and used before the retest expiry date provided by Takeda. Containers should be kept closed during storage. Patients should be instructed on proper storage, accountability, and administration of alisertib, including that alisertib is to be taken as intact tablets.

8.1.5 **Protocol dose specifics**

The starting dose for patients on both arms will be 50 mg orally BID; each day the doses must be taken at least 6 hours apart, with or without food.

8.1.6 **Preparation**

No preparation is necessary for drug used in this study. Alisertib is an investigational agent and therefore should be handled with due care. In case of contact with broken tablets, raising dust should be avoided during the cleanup operation. The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during preparation and the cleanup operation. The area should be ventilated and the spill site washed after material pick-up is complete. The spilled material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations. In case of contact with the powder (eg, from a broken tablet), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes. In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified.

8.1.7 **Route of administration for this study**

Oral. Patients will be instructed to take each oral dose of alisertib with 8 ounces (1 cup, 240 mL) of water. All tablets are to be ingested whole; patients who have difficulty swallowing tablets will be excluded from the study.

8.1.8 **Incompatibilities**

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Patients should be instructed to limit the use of alcohol while enrolled in this study. Patients should consume no more than 1 standard unit of alcohol per day during the study and for 30 days from the last dose of alisertib. A standard unit of alcohol is defined as a 12 oz beer (350 mL), 1.5 oz (45 mL) of 80-proof alcohol, or one 6-oz (175 mL) glass of wine.

8.1.9 **Availability & Supply**

Alisertib will be provided by Takeda and will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid). Alisertib ECT are packaged in a 60-cc high density polyethylene (HDPE) bottle with a rayon

coil, induction seal, desiccant packs, and a polypropylene child resistant cap. Patients will be instructed on the home use of alisertib, including the requirement that alisertib be administered as intact tablets.

8.1.10 **Side effects**

Common

In prior clinical trials, the following have been considered as emerging as a consequence of the treatment in ≥ 10% of patients:

- Gastrointestinal Disorders
  - Diarrhea
  - Nausea
  - Stomatitis
  - Vomiting
  - Constipation
  - Abdominal discomfort
- Hematological abnormalities
  - Neutropenia
  - Anemia
  - Thrombocytopenia
  - Febrile neutropenia
- General abnormalities
  - Fatigue
  - Fever
  - Edema
  - Asthenia
  - Infection, even in absence of neutropenia (including thrush)
- Dermatological abnormalities
  - Alopecia
  - Rash
- Nervous system disorders
  - Somnolence
  - Headache
  - Dizziness
  - Difficulty walking
  - Psychiatric illness
  - Confusional state/disorientation
- Metabolic/Nutritional consequences
  - Dehydration
  - Decreased appetite
  - Low potassium and blood magnesium levels
- Respiratory disorders
  - Cough
  - Difficulty breathing

In prior clinical trials, there have been also been reports of eye disorders (not further specified), muscle/joint disorders (back and extremity pain), and vascular disorders (low blood pressure, sometimes severe (“shock”)

Less common (occurring between 1% and 10% of the time):

- Liver function test abnormalities

Infrequent (occurring less than 1% of the time):

- Cardiac abnormalities have been reported in 2 patients, including reduced cardiac function, and abnormal heart rhythm (QTc prolongation)

8.1.11 **Nursing implications**

Antiemetogenic agents may be administered at the discretion of the investigator. Neutralizing antacids and calcium-containing supplements cannot be taken from 2 hours prior to alisertib dosing until up to 2 hours after dosing.

9.0 CORRELATIVE STUDIES

9.1 Sample Collection

Blood and/or bone marrow samples for PD studies will be collected at multiple timepoints for cycles 1-6. Samples will be collected at the time of routine blood and/or bone marrow collection on Day 1 of each cycle. Sample collection on Day 15 will be optional. For MF patients, only peripheral blood samples will be collected unless bone marrow is deemed medically necessary; AMKL patients may include both blood and bone marrow procurement. Approximately 25 ml of peripheral blood and 10-20 ml of bone marrow will be collected in lavender top EDTA tubes and dark green top tubes respectively, and labeled with the patient initials, study number, and date of collection. Samples may sit at room temperature until they are picked up or shipped.,.

As mentioned above, correlative bone marrow samples should be obtained any time bone marrows are collected as clinically indicated during study participation. At baseline, bone marrow collection is not required for study entry, but study team should request 3-4 unstained slides from prior bone marrow testing performed within the last year. Unstained slides should be shipped at room temperature to Dr. Crispino

It is strongly preferred that correlative samples be collected and shipped only on Monday – Thursday (with same day shipment preferred). Friday collections/shipments should be avoided to ensure sample stability. Since these samples are collected on Day 1 of each cycle, it is preferred that Day 1 visits be conducted on Monday through Thursday when correlative samples are being collected. Sites should use the available windows to achieve this. Please contact the QAM if there are any questions.

9.2 Transport/Shipment

Samples will be picked up by Dr. Crispino's lab (for NU samples) or shipped at room temperature by overnight courier to Dr. Crispino's laboratory (for University of Miami and Mayo Clinic specimens). Samples will be shipped the day of collection(preferred), or within the week after collection in the event that additional samples will be collected from other patients in the same week. Samples will only be sent Monday through Thursday. Any samples collected between Thursday late afternoon and Sunday night will be shipped on Monday morning.

ATTN: Crispino Laboratory
Northwestern University
303 East Superior Street
Lurie Building Room 5-250
Chicago, IL 60611

The phone number to contact Dr. Crispino's lab for questions or to confirm receipt of samples is 312-503-1433. Alternatively, Dr. Crispino may be reached via email at j-crispino@northwestern.edu.

9.3 Processing and Analysis

Upon receipt, monocuclear cells will be collected from the samples by Ficoll preparation. Aliquots of the cells will be used in the following ways:

1. To extract RNA for gene expression studies. We will determine the pathways that are up or down-regulated upon Alisertib treatment. We anticipate changes in cell cycle regulatory and megakaryocyte differentiation pathways.

2. To isolate proteins for analysis of levels of key factors, such as transcription factors, ribosome assembly proteins, and signaling molecules. We will use western blot assays to determine the changes that occur with Alisertib treatment.
3. To perform flow cytometry will be performed to analyze the state of maturation of the cells, including CD41, CD42 expression and DNA content. We will use FlowJo software to analyze the flow cytometry data to determine the levels of these differentiation parameter.
4. To perform flow cytometry to assess the state of signaling cascades, such as the AKT, ERK, and STAT pathways. We will use FlowJo software to analyze the flow cytometry data to determine the levels of activation of these pathways.
5. To perform flow cytometry to assess the hematopoietic stem cell compartment. We will use FlowJo software to determine the different populations of hematopoietic stem and progenitor populations.
6. To culture in vitro and in vivo to assess the state and differentiation potential of hematopoietic progenitor cells.
7. We may elect to use samples at a later time point to directly compare differences in gene expression and signaling pathways from sequential patient specimens. Thus, we will freeze down aliquots of cells, and/or or RNA, and/or protein for future cell culture, flow cytometry, DNA, RNA and protein extractions. Samples will be kept for up to two years.
8. An optional correlative study (separate patient consent) will involve exome or whole genome sequencing on normal and tumor DNA for patients with AMKL. DNA from tumor samples will be available as in line 7 above; to obtain germline DNA, buccal swabs will be obtained.

Samples will be labeled with a number and not the patient names. Identifying information will be retained by the PI and co-investigators and not shared with the laboratory staff. Identities of the subjects will not be reported in presentations or publications.

Samples will be retained indefinitely in Dr. Crispino's laboratory located at Northwestern University.

Please refer to laboratory manual for more details.

10.0 STATISTICAL CONSIDERATIONS

10.1 Statistical Analysis

10.1.1 Primary Endpoint

The primary objective of this pilot study is to determine the safety profile. All adverse events (AEs) will be summarized using frequencies and percentages. These percentages will be subclassified by type of AE, timing of AE in the treatment cycle, frequency and attribution of AEs (treatment related or not).

10.1.2 Secondary Endpoint

To determine preliminary efficacy, response (defined as having at least PR in evaluable patients) will be reported as a proportion and 95% confidence interval using exact binomial methods. Given that this is a purely descriptive protocol, there is no set hypothesis regarding the response rate. Proportions for clinical response (defined as having at least PR or stable disease) will also be calculated. Please refer to appendices for detailed explanations of the clinical response criteria for each population.

10.1.3 Exploratory Endpoints

To describe pharmacodynamic effects, serial measures of megakaryocytes and other hematopoietic cells will be summarized using descriptive statistics such as means, medians and ranges for each time point. These measures will be plotted

for each patient, and these patient specific plots will be plotted for all patients together. Analyses for change over time will be done using repeated measures general linear model analysis.

To evaluate the relationship between biomarker expression levels and response, changes in biomarker expression levels will be related to response using a Wilcoxon rank sum test. To evaluate reduction in splenomegaly by palpation in the MF arm, a categorical variable measuring the extent of splenomegaly will be tracked over time descriptively, since the small sample size will preclude longitudinal analysis. To evaluate improvement in MF symptoms in the MF arm, the Myeloproliferative Neoplasm Symptom Assessment (MPN-SAF) score will be summarized descriptively over time using means, medians, standard errors and ranges.

10.2 Sample Size and Accrual

MF and AMKL are rare diseases with total prevalence (total number of patients with the disease in the US at any time) under 15,000. NIH considers prevalence under 200,000 a rare disease. A total of 24 subjects (maximum 24 subjects, regardless of the disease breakdown of MF or AMKL. If all 24 were MF, that would be acceptable) will be accrued to this trial. Approximately 4 potentially eligible patients are seen per month at all sites, and it is anticipated that at least 1 per month will be accrued, leading to a 2 year accrual period.

. Approximate standard errors for the estimated response rate when the true response rate is 10% are +/- 6% with a sample of 24 patients total, +/- 18% with a sample of 18 patients, +/- 9% with 12 patients and +/- 10% to 12% with 6 patients. In the event that a patient consents and is registered to the study but never begins treatment, that patient may be replaced. The QAM must be notified and, if necessary, DMC approval must be obtained.

11.0 STUDY MANAGEMENT

11.1 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 should 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.

11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by

Janssen Scientific Affairs. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

11.2 Registration Procedures

Patients may not begin protocol treatment prior to registration. All patient registrations will be registered centrally through the Clinical Research Office at Northwestern University before enrollment to study. Please contact the assigned Quality Assurance Monitor (QAM) or email the QA Department (croqualityassurance@northwestern.edu) for questions regarding patient registration. For all potential patients, study teams are asked to inform the QAM of the date and time that the patient will need to be registered.

Prior to registration, eligibility criteria must be confirmed by the assigned QAM. The study coordinator will screen all subjects for potential registration via the web-based application NOTIS (Northwestern Oncology Trial Information System), which is available at: https://notis.nubic.northwestern.edu. Please note that a username and password is required to use this program, and will be provided during site activation prior to training on the NOTIS system.

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.3 Instructions for Participating Sites

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Research Office at Northwestern University:

- Signed and completed Letter of Invitation to participate in the study.
- Signed copy of Northwestern University's Data Monitoring Committee policy pertaining to data submission.
- Draft informed consent form should for review/approval prior to submission to the local IRB
- A copy of the official IRB approval letter for the protocol and informed consent.
- CVs and medical licensure for the local PI and any sub-investigators who will be involved in the study at the site.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation.

Additional activities may be required prior to site activation (i.e. contract execution, study-specific training). Full requirements will be outlined in a memo upon receipt of the signed Letter of Invitation.

11.4 Data Submission and Monitoring/Auditing

Once a subject is confirmed and registered to the study, eCRFs should be submitted through NOTIS according to the detailed data submission guidelines (will provided in a

separate document). Generally, all data during the first cycle must be submitted on a weekly basis. Thereafter data will be due at the end of each cycle.

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to Appendices for additional information). The level of risk attributed to this study requires high intensity monitoring, as outlined in the DSMP. The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level.

11.5 Adherence to the Protocol

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

11.5.1 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.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

11.5.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.
- Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation may be considered an instance of RNI(Reportable New Information) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.6 Investigator Obligations

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 PI is responsible for personally overseeing the treatment of all study patients. The PI 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, entered onto the appropriate eCRFs, and submitted

within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

11.7 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. The assigned QAM will prepare a preliminary data summary (to be approved by the DMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data collection for the primary endpoint). If the investigator's wish to obtain DMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DMC at their next available meeting, and a final, DMC-approved dataset will be released along with any DMC decisions regarding publication. The investigators are expected to use only DMC-approved data in future publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers.

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Appendix I – International Working Group Response Criteria for AML

Definitions of response criteria are based primary on those given by Cheson et al.

Category	Definition
Complete remission (CR)[1]	Bone marrow blasts <5 percent; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 109/L (1000/μL); platelet count >100 x 10^9/L (100,000/μL); independence of red cell transfusions
CR with incomplete recovery (CRi)[2]	All CR criteria except for residual neutropenia (<1.0 x 1^{09}/L (1000/μL)) or thrombocytopenia (<100 x 109/L (100,000/μL))
Morphologic leukemia-free state[3]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required.
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%.
Cytogenic CR (CRc)[4]	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow.
Molecular CR (CRm)[5]	No standard definition; depends on molecular target.
Treatment failure	
Resistant disease (RD)	Failure to achieve CR, CRi or PR; only includes patients surviving ≥7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination.
Death in aplasia	Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia.
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available.
Relapse[6]	Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease.

[1] All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5 to 7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[2] The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

[3] This category may be useful in the clinical development of novel agents within phase I clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

[4] Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.

[5] As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 104 copies of ABL1 in accordance with standardized criteria, transcript levels below 10 to 12 copies appear to be predictive for long-term remission.

[6] In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.

Appendix II – Revised IWG-MRT & ELN Response Criteria for MF

The criteria below are a revision of the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria for treatment response in myelofibrosis (MF) and represents a collaborative effort by the IWG-MRT and the European LeukemiaNet to objectively assess the value of new drugs in inducing morphologic remission or improvement in MF-associated symptomatic burden (MF-SB).

Response Categories	Required Criteria[1]
CR (complete remission)	• Bone marrow[2]: age-adjusted normocellularity; < 5% blasts; ≤ grade 1 MF[3] • Peripheral blood: hemoglobin ≥ 100 g/L and < ULN; neutrophil count ≥ 1 x 10^9 and < ULN • Platelet count ≥ 100 x 10^9 and < ULN; < 2% immature myeloid cells[4] • Clinical: resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH.
PR (partial remission)	• 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[4] and • Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH or • Bone marrow[2]: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF[3], and peripheral blood: Hemoglobin ≥85 but <100 g/L and <UNL; neutrophil count ≥1 × 109/L and <UNL; platelet count ≥50, but <100 × 109/L and <UNL; <2% immature myeloid cells[4] 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[5]
Anemia response	• Transfusion-independent patients: a ≥ 20 g/L increase in hemoglobin level[6] • Transfusion-dependent patients: becoming transfusion independent[7]
Spleen response[8]	• A baseline splenomegaly that is palpable at 5-10 CM, below the LCM, becomes not palpable[9] OR • A baseline splenomegaly that is palpable at > 10 cm, below the LCM, decreases by ≥ 50% A baseline splenomegaly that is palpable at <5 cm, below the LCM, is not eligible for spleen response
Symptoms response	A ≥ 50% reduction in the MPN-SAF TSS[10]
Progressive disease[11]	• 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.

[1] For all response categories, benefit must last for ≥12 wk to qualify as a response.

[2] 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.

[3] Grading of MF is according to the European classification. 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.

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

[5] 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 CTCAE v 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.

[6] 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.

[7] 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.

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

[9] For the purpose of this study, spleen or liver responses must be confirmed based on palpation/physical examination, or ultrasound if the body habitus is prohibitive, in keeping with standard care.

(*Note: The original criteria mandates the use of 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.)*

[10] 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.

[11] 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.

Appendix III – CYP 1A2, 2C9, 2C19, 3A4 and UGT 1A1/2/8 Inhibitors and Inducers

CYP 1A2, 2C9, 2C19, 3A4 and UGT1A1/2/8 INHIBITORS				
1A2	**2C9**	**2C19**	**3A4**	**UGT1A1/2/8**
Strong • fluvoxamine • ciprofloxacin **Weak** • cimetidine **Other** • amiodarone • efavirenz • fluoroquinolones • fluvoxamine • furafylline • interferon • methoxsalen • mibefradil	**Strong** • fluconazole **Moderate** • amiodarone • miconazole **Other** • efavirenz • fenofibrate • fluconazole • fluvastatin • fluvoxamine • isoniazid • lovastatin • metronidazole • paroxetine • phenylbutazone • probenicid • sertraline • sulfamethoxazole • sulfaphenazole • teniposide • voriconazole • zafirlukast	**Strong** • fluvoxamine • ticlopidine • fluconazole **Moderate** • esomeprazole • fluoxetine • lansoprazole • omeprazole • voriconazole **Other** • pantoprazole • chloramphenicol • cimetidine • felbamate • indomethacin • isoniazid • ketoconazole • modafinil • oxcarbazepine • probenicid • topiramate	**Strong** • indinavir • nelfinavir • ritonavir • clarithromycin • itraconazole • ketoconazole • nefazodone • saquinavir • telithromycin • grapefruit juice **Moderate** • amiodarone • aprepitant • erythromycin • fluconazole • verapamil • diltiazem **Weak** • cimetidine • chloramphenicol • boceprevir • ciprofloxacin • fluvoxamine • imatinib • mibefradil • mifepristone • norfloxacin • norfluoxetine • starfruit • telaprevir • voriconazole	• Atazanavir • Indinavir
CYP 1A2, 2C9, 2C19, 3A4 and UGT1A1/2/8 INDUCERS				
1A2	**2C9**	**2C19**	**3A4**	**UGT1A1/2/8**
Moderate • tobacco • Phenytoin **Other** • broccoli • brussel sprouts • carbanazepine • char-grilled meat • insulin • modafinil • omeprazole • rifampin	**Strong** • carbamazepine • rifampin **Other** • nevirapine • phenobarbital • St. John's Wort	**Strong** • carbamazepine • rifampin **Moderate** • norethindrone • prednisone • St. John's Wort	**Strong** • carbamazepine • phenytoin • rifampin • St. John's Wort **Moderate** • efavirenz • modafinil • barbiturates • prednisone • oxcarbazepine • phenobarbital • pioglitazone • rifabutin	• Carbamazepine • Nicotine

Note: for full list refer to: http://medicine.iupui.edu/clinpharm/ddis/main-table/ and http://www.pharmacologyweekly.com/content/pages/ugt-enzymes-medications-herbs-substrate-inhibitor-inducer

Reference: *Flockhart DA. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007). "http://medicine.iupui.edu/clinpharm/ddis/clinical-table/" Accessed 3/25/15.*

Appendix IV – Protocol History of Changes

Original Protocol – June 02, 2015			
Amendment 1 – Aug 14, 2015			
Section(s) Affected	***Prior Version***	***Amendment 1 Changes***	***Rationale***
Section 4.4 (Management of Clinical Events)	1) Did not have explicit description of the possibility of sedation due to the study drug. 2) N/A	1) Included a statement in section 4.4.3 (Central nervous system effects) that potential participants will be explicitly counseled during the consenting process on the possible effects of sedation due to the study drug and advise them against driving or operating heavy machinery after taking the drug. 2) Included a section (section 4.4.4) on clinical management of differentiation syndrome	Based on Clinical deficiencies pointed out by FDA reviewers
Amendment 1 – Aug 14, 2015			
Amendment 2 – Oct 22, 2015			
Section(s) Affected	***Prior Version***	***Amendment 2 Changes***	***Rationale***
Sec 3.1 (Inclusion Criteria for AMKL patients)	3.14 - Prior version included the following organ function tests as an inclusion criteria: adequate bone marrow function; ANC ≥ 1500/mm^3 condition; Platelets ≥ 100,000/mm3; Hemoglobin > 9 g/dL	3.1.4 - criteria for organ function tests modified to delete the following: adequate bone marrow function ANC ≥ 1500/mm^3 condition Platelets ≥ 100,000/mm3 Hemoglobin > 9 g/dL	To broaden the inclusion criteria and make the study available as an option for relapsed/refractory AMKL patients, who otherwise, have extremely limited treatment options.

Sec 3.3.7 (Exclusion Criteria)	3.3.7 - Previous version did not: specify an upper threshold for excluding patients who have had prior allogenic bone marrow transplant and did not set prior solid organ transplant as an exclusion criteria 3.3.24 – Previous version excluded patients who required administration of myeloid growth factors/platelet	3.3.7 – changed exclusion criteria: 1) to set 60 days as an upper threshold for exclusion of patients with prior allogenic bone marrow transplantation. 2) to exclude patients with prior solid organ transplants. 3.3.24 – deleted criteria to exclude patients with a requirement for administration of myeloid growth factors/platelet transfusions.	As in the case of the inclusion criteria (above), changing this exclusion criteria also can help make the study available to this patient population.
Sec 4.2 (Treatment Administration)	For the statement "In order for each …."„ the previous version did not have specific criteria with respect to cytopenias for MF and AMKL patients	For the statement "In order for each ….", the criteria was clarified to be applicable to MF patients. Also clarified the condition for cytopenias in AMKL patients.	For clarity and to ensure that the amended neutrophil and platelet count criteria can help make the study available to this patient population.
Section 5.0 (Study Procedures – Schdule of events 5.1 and 5.2)	N/A	Study table footnotes updated to include the information on windows for baseline testing, which was clarified via Amnd 1 clarification memo dt 09.21.15 that was IRB approved on 10.23.15 Optional buccal swab added to study table for AMKL (sec 5.2)	To integrate clarification memo information into protocol amendment 2. To incorporate optional correlative study described in sec
Sec 9.3 (Processing and Analysis)	N/A	Description of optional correlative study added.	To gain a better understanding of genetic changes underlying the disease.
Amendment 3 – Feb 1, 2016			
Section(s) Affected	***Prior Version***	***Amendment 3Changes***	***Rationale***
Cover page, Study Summary, Sec 3.0 (Patient Eligibility), Sec 9.2 (Transport/Shipment)	N/A	Adds Mayo Clinic as affiliate site with Dr. Naseema Gangat as the PI and Dr. Ayalew Tefferi as co-investigator	Mayo Clinic has been approved as an affiliate site

	Amendment 4	**April 21, 2016**	
Section(s) Affected	***Prior Version***	***Amendment 4 Changes***	
Exclusion Criteria 3.3.15	Patients who have a systemic infection requiring IV antibiotic therapy within 14 days prior to registration (or other severe infection) are not eligible.	Patients with an active, uncontrolled systemic infection are not eligible until deemed controlled by the treating physician.	To increase flexibility for inclusion of such patients
Section 5.1 & 5.2 Study procedures Footnote i	Urinalysis with microscopic analysis ***(+/-2 PD analysis***) will be performed days 1, 4, 8, and 15 of cycle 1, days 1 and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all time points).	Removed: (+/-2 PD analysis) **Current language:** Urinalysis with microscopic analysis will be performed days 1, 4, 8, and 15 of cycle 1,days and 15 of cycles 2-6, and then day 1 of all cycles thereafter (a window of +/- 2 days is permitted for all time points).	Correction of error. PD analysis is not done with urine
Section 5.1 and 5.2	Windows of +/-2 for each cycle was mentioned in the footnotes	Windows of +/-2 is added to each cycle in both table headings itself to increase clarity : e.g Cycle 1(+/-2 days)	For clarity

	Amendment 5	**July 12, 2016**	
Section(s) Affected	**Prior Version**	**Amendment 5 Changes**	**Rationale**
Inclusion criteria 3.1.8 and 3.2.9	Male patients, even if surgically sterilized (i.e. status post-vasectomy) agrees to use an acceptable method for contraception during the entire study treatment period through 4 months after the last dose of alisertib	Male patients, even if surgically sterilized (i.e. status post-vasectomy) agrees to use an acceptable method for contraception during the entire study treatment period through 120 days after the last dose of alisertib. Likewise, female patients should agree to use acceptable method for contraception during the entire study treatment period through 90 days after the last dose of alisertib.	Per Takeda safety board and current IB
Exclusion Criteria 3.3.22	Patients who have been diagnosed or treated for another malignancy within 3 years prior to registration are not eligible *aside from these exceptions*: completely resected basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, or low-risk prostate cancer after curative therapy.	If a patient had a prior MPN that evolved to a blast phase, but with treatment, reverted to Myelofibrosis at the time of screening, these pts are considered eligible at the discretion of the PI, if not considered suitable for stem cell transplantation.	In order to accommodate patients that fall within this disease spectrum

Section 5.1 Study procedures table	Footnote J Peripheral blood (25 ml) and/or bone marrow (bone marrow only if done clinically – will collect about 10-20 ml) samples will be collected for determination of alisertib pharmacodynamics (PD studies). Samples will be collected in dark green top tubes at the start of each cycle and at the time of the Day 15 labs, for a maximum of 6 cycles (or 12 samples total)	Removed: dark green top tubes. Added: Please refer to laboratory manual for details.	To maintain consistency with the lab manual.
Section 5.2	Footnote Peripheral blood (25 ml) and/or bone marrow (if done will collect about 10-20 ml) samples will be collected for determination of alisertib pharmacodynamics (PD studies). Samples will be collected in dark green top tubes	Removed: dark green top tubes. Added: Please refer to laboratory manual for details	To maintain consistency with the lab manual
	at the start of each cycle and at the time of the Day 15 labs, for a maximum of 6 cycles (or 12 samples total).		
Section 5.1 and 5.2	Windows of+/-2 days for each visit starting rom C1D1	Windows of +/-1 day for D1D4 and +/- 2 days for all subsequent visits. NO WINDOW FOR C1D1	For logistical convenience and to minimize protocol deviation. No window for C1 D1 as it sets up the dates for subsequent visits.

Section 9.0 Correlatives	Approximately 25 ml of peripheral blood and 10-20 ml of bone marrow will be collected in dark green top tubes, and labeled with the patient initials, study number, and date of collection. Samples may sit at room temperature until they are picked up or shipped.	Approximately 25 ml of peripheral blood and 10-20 ml of bone marrow will be collected in lavender top EDTA tubes and dark green top tubes respectively, and labeled with the patient initials, study number, and date of collection. Samples may sit at room temperature until they are picked up or shipped.	To maintain consistency with the lab manual
Appendix II	Footnote 9 Spleen or liver responses must be confirmed using 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. In the spleen response section of the table: "A spleen response requires confirmation by MRI or computed tomography showing ≥ 35% spleen volume reduction." In the spleen response section of the table: "A spleen response requires confirmation by MRI or computed tomography showing ≥ 35% spleen volume reduction."	Footnote 9 modified. For the purpose of this study, spleen or liver responses must be confirmed based on palpation/physical examination, or ultrasound if the body habitus is prohibitive, in keeping with standard care. (*Note: The original criteria mandates the use of 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.)* *Deleted this criteria*	Since this study is not performing CT or MRI for for assessment of spleen response , due to limited resources.

Previous amendment Summary of changes (dated April 21,2016	Rationale for each change was not inserted in the Summary of changes.	Rationale inserted for each change in the Summary of changes	Correction of error and to maintain protocol/amendment format and consistency.
Amendment 6 October 12, 2016			
Section(s) Affected	Prior Version	Amendment 6 Changes	Rationale
Section 3.2.5 Eligibility criteria	Adequate bone marrow and organ function for eligibility included ANC ≥1500/mm3 Platelet≥100,000/ mm3	Adequate bone marrow and organ function for eligibility included ANC ≥1000/mm3 Platelet ≥50,000/ mm3	Given observations in NU patients, in keeping with consistency of active MF clinical trials in , and to include patients who may have limited treatment options in such situations
Section 4.2 Treatment administration	In order for each new cycle of therapy to begin, criteria that MF patients must meet included: • ANC must be ≥ 1500/mm3 • Platelet count must be ≥ 75,000/mm3	These requirements have been modified to: • ANC must be ≥ 1000/mm3 • Platelet count must be ≥ 50,000/mm3 Other language in this section modified to align with this change.	In order to the make protocol more specific/explicit, and consistent across the two arms. This is in keeping with the relaxing of the inclusion criteria, and also to be consistent for both MK and AMKL patients

Section 5.1 and 5.2 Urinalysis	Urinalysis was scheduled throughout the study	Urinalysis to be done only at screening. Related footnotes modified accordingly	Beyond screening, there is no use for the urinalysis. There is no correlative study that requires urine.
Section 3.3.2(Eligibility criteria) & Section 4.5.2(Permitted Concomitant medications/treatments)	Patients actively receiving hydroxyurea are eligible and may continue to receive hydroxyurea through cycle 1 of protocol treatment.	Added Note: If the platelet count remains above 1 million after cycle 1, hydroxyurea can be used at the treating physician's discretion, if the platelets are 1000 x 109/L, or more, or if there are symptoms from thrombocytosis	For clarity
Section 9.1	Details about blood and bone marrow sample collection.	Added language: In addition, following any bone marrow procedure, two unstained slides should be shipped at room temperature to Dr. Crispino Laboratory manual has been updated accordingly.	For added correlative analysis.

Amendment 7 December 14, 2016			
Section(s) Affected	Prior Version	Amendment 7 Changes	Rationale
Study summary and Section 3.0(eligibility) and 10.2 statistics section	Sample size : 24 total (12 with MF and 12 with AMKL).	24 total (Maximum 24 subjects, regardless of the disease breakdown of MF or AMKL. If all 24 were MF, that would be acceptable)	To accommodate more patients on the MF arm, since there are more patients available for MF than for AMKL.
Section 3.2.2 Inclusion criteria –MF patients	Patients must be intermediate I risk or beyond and meet criteria such as : ineligible for stem cell transplantation.	Modified to state that patients can be either "ineligible or refusal to undergo stem cell transplantation"	For flexibility, so that various scenarios are covered.
Section 5.1, 5.2 and Section 9.1 Schedule of Events tables and Correlative studies section.	Footnote j: Peripheral blood samples to determine pharmacodynamics of alisertib(correlative studies) were to be collected at D1 and D15 of each cycle for a maximum of 6 cycles.	Footnotej:The D15 sample has been made optional. Also, Footnote i reworded in tables in 5.1 and 5.2: " It is strongly preferred that correlative samples be collected and shipped only on Monday – Thursday (with same day shipment preferred). Friday collections/shipments should be avoided to ensure sample stability. Since these samples are collected on Day 1 of each cycle, it is preferred that Day 1 visits be conducted on Monday through Thursday when correlative samples are being collected. Sites should use the available windows to achieve this. Please contact the QAM if there are any questions." Same language inserted in Section 9.1	For convenience of patients commuting long distances. To ensure stability of correlative samples

Section 5.1 and 5.2 Schedule of events tables for MF and AMKL arms	C1D1 vital signs should be taken before and after dosing. Urinalysis to be done at screening	C1D1 vitals to be done pre-dose(+/-30 mins) as well as about 2 hours (+/- 30mins) post-dose. Urinalysis removed from screening tests. Related footnote removed/modified.	For clarity and convenience. Urinalysis not required for this study at any point-per PI
Study summary and Section 10. 2 (Statistics)	Approximate standard errors for the estimated response rate when the true response rate is 10% are +/- 6% with a sample of 24 patients total, and +/- 9% with a sample of 12 patients in each disease group. Approximately 4 potentially eligible patients are seen per month at both sites DLT language within the statistics section	Approximate standard errors for the estimated response rate when the true response rate is 10% are +/- 6% with a sample of 24 patients total, +/- 18% with a sample of 18 patients, +/- 9% with 12 patients and +/- 10% to 12% with 6 patients. Both sites corrected to read'all sites' Removed DLT language	Updated statistics to align with modification to disease allocation of patients. Correction of typographical error(since there are more than 2 sites). Correction of error, since there are no DLTs in this study.

Amendment 8 March17, 2017			
Section(s) Affected	**Prior Version**	**Amendment 8 Changes**	**Rationale**
Section 4.2 Treatment administration Section 5.1 Schedule of events for MF patients	Description of treatment administration Footnote m: statement that lab and exams will be done only on day 1 of Cycle 7 and beyond.	Language modified: to state that beyond cycle 6, for MF patients who are stable, they will be asked to come to clinic on D1 of every even cycle and they will be given drug supply for 2 cycles to facilitate this. During these visits,labs and exams will be done as indicated in the schedule.During the odd numbered cycles, outside lab tests(only CBC and CMP) will be ordered. Patients can start their treatment at home only after the treating physician has reviewed the laboratory results and determined that they are able to continue treatment. For these cycles, the study coordinator will contact the patient on/prior to Day 1 to inform them if they can or cannot start the new cycle, and also check compliance regarding study drug intake. A ‘D1’ added under the Cycle 7+ column	To facilitate patient treatment convenience and flexibility.

Section 5.1 Schedule of events for MF patients	Footnote f: description of chemistry panel	Added note to state that: Beyond Cycle 6, magnesium and phosphate testing is not required(as part of the chemistry panel).	Per PI: These tests are not required after cycle 6. Hence, they have been removed for convenience.
	Footnote g: description of DIC screen and coagulation studies.	Added note to state that: Beyond Cycle 6, PT/INR,aPTT testing is not required.	
	Footnote j: Description of blood and bone marrow samples for PD studies.	Added language to clarify that : "Correlative bone marrow samples should be obtained any time bone marrows are collected as clinically indicated during study participation. At baseline, correlative bone marrow collection is not mandatory for study entry. However, study team should request 3-4 unstained slides from any prior bone marrow testing performed within the last year before study entry". Number of slides have been increased from 2 to 3-4 slides(described clearly in section 9.1)	For clarity and convenience
	Footnote k: Bone marrow aspirate and biopsy is to be done within 1 year prior to registration(provided the PI confirms the diagnosis and there is no suspicion for acceleration to blast phase), and thereafter only as clinically indicated.	Footnote k updated to add timepoint C7D1(+/-14 days) to bone marrow aspirate and biopsy.	

Section 5.1 Schedule of events for MF patients and section 5.2 Schedule of events for AMKL patients	Footnote c: Vital signs included supine and standing blood pressure measurement. Footnote g: 3 pt listed as part of DIC screen at baseline. In both tables, row demonstrating 'samples for PD studies' which included both blood and bone marrow samples	Footnote c: Removed the detail about 'supine and standing' from the blood pressure measurement. Footnote g: 3 pt replaced by aPTT. In both tables, this row has been removed and replaced by by 2 different rows demonstrating 'Bone marrow collection for PD studies and 'Blood collection for PD studies'.	Such detail is not required for this study. Hence, removed in order to minimize protocol deviations. For clarity For increased clarity
Section 5.1 Schedule of events for MF patients and section 5.2 Schedule of events for AMKL patients (contd)	In both tables, window for signing informed consent not defined.	Added footnote v in both tables stating that informed consent should be obtained within 28 days of registration	For clarity and convenience
Section 6.2 Secondary endpoints	Response at 6 months will be reported for all evaluable patients.	Modified to state: "Response after cycle 6 (upon completion of C7D1 marrow) will be reported for all evaluable patients."	For clarity

Section 9.1	Details about Correlative sample collection. 10-20ml of bone marrow at multiple timepoints for cycles 1-6.Two unstained bone marrow slides were required following any bone marrow procedure.	Language added to clarify : 1. Collection of 10-20ml of bone marrow at multiple timepoints for cycles 1-6. Collection of 3-4 unstained slides whenever a bone marrow collection is done at baseline or within I year prior to study entry. 2. Language stating that bone marrow collection for correlative testing is not mandatory for study entry. However, study team should encourage collection of 3-4 unstained slides from bone marrow collected within the last year prior to enrollment.	For clarity, convenience and flexibility.
Section 11.5.2 Study management: Other protocol deviations	Promptly reportable Non Compliance (PRNC) definitions listed	The term Promptly reportable Non Compliance (PRNC) is replaced by Reportable New Information (RNI)	Per NU IRB directive