Mayo Clinic Cancer Center

MC200805, Phase II study to determine the efficacy and safety of luspatercept (ACE-536) in patients with myelodysplastic/myeloproliferative neoplasms with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) and myelodysplastic/myeloproliferative neoplasms, unclassifiable with ring sideroblasts (MDS/MPN-U with RS)

Sponsor/Principal Investigator: [redacted]

Co-Principal Investigator: [redacted]

Co-Investigators: [redacted]

Statistician: [redacted]

√Study contributor(s) not responsible for patient care

FDA IND #: IND EXEMPT

Trial Supported by:
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Commercial Agents: Hydroxyurea, aspirin
Drug Company Supplied: Luspatercept-aamt

BMS Protocol ID: LU-CL-MDS-PI-14022

Site Investigators & Participating sites:

[redacted]

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Protocol Resources

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Forms completion and submission	[redacted]
Protocol document, consent form, regulatory issues	[redacted]
Non-paraffin biospecimens	[redacted]
Serious Adverse Event Reporting	[redacted]

*No waivers of eligibility allowed

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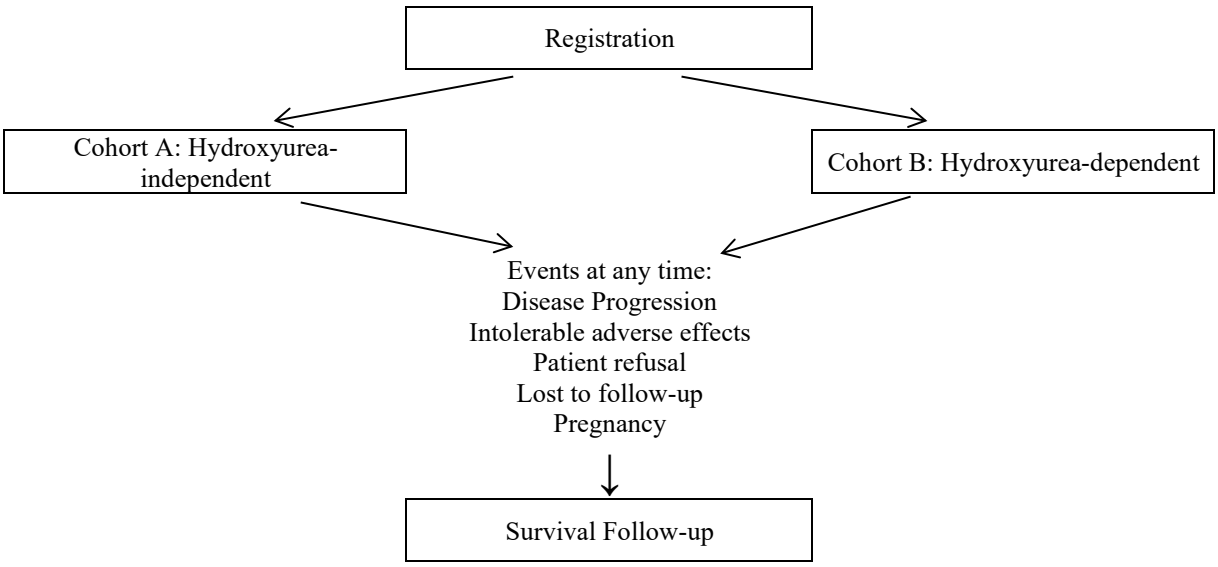
Schema



Cycle = 21 days

Generic name: Luspatercept-aamt Brand name(s): Reblozyl Mayo Abbreviation: LUSPATERCEPT Availability: BMS	Generic name: Hydroxyurea Brand name(s): Hydrea Mayo Abbreviation: HYDREA Availability: Commercial

1.0 Background

Myelodysplastic syndrome/myeloproliferative (MDS/MPN) overlap syndromes are classified by the World Health Organization (WHO) as a distinct entity under myeloid neoplasms due to their unique clinical and biological characteristics. The categories include chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), juvenile myelomonocytic leukemia, MDS/MPN with ring sideroblasts (RS) and thrombocytosis (T) and MDS/MPN, unclassifiable (U) (1). MDS/MPN-RS-T has been defined by the presence of erythroid lineage dysplasia with the presence or absence of multi lineage dysplasia and persistent thrombocytosis (platelet count $\geq$ 450 x 10^9/L), without other disease defining genetic abnormalities. MDS/MPN-U is a separate category under MDS/MPN overlap syndrome under which patients meet the criteria for MDS/MPN overlap syndrome but do not met criteria for CMML, aCML, JMML, or MDS/MPN-RS-T. MDS/MPN-U patients can also have $\geq$ 15% ring sideroblasts but not classified under MDS/MPN-RS-T due to $\geq$ 1% peripheral blood blasts or $\geq$ 5% bone marrow blasts (2, 3).

MDS/MPN-RS-T and MDS/MPN-U with RS are characterized by distinct clinical features such as anemia, increased risk for thrombosis and differential response rates to conventional agents, when compared to similar myeloid neoplasm such as myelodysplastic syndrome with ring sideroblasts (MDS-RS). Anemia is a frequent cause of morbidity due to ineffective erythropoiesis as a consequence of intracellular iron locking in mitochondria and accelerated erythroid precursor apoptosis (4). Lower hemoglobin level and red blood cell (RBC) transfusion-dependence have been associated with adverse cardiovascular outcomes. Erythropoiesis stimulating agents (ESA) are often used as first-line agents for the treatment of anemia in myeloid neoplasm with ring sideroblasts, including MDS/MPN/RS-T and MDS/MPN-U with RS. However, erythroid response rates are seen in approximately 45% patients and often not long-lasting (5). Additional therapeutic options such as lenalidomide and hypomethylating agents are effective only in a handful of patients with short-lived responses (2, 5). Therefore, restoration of arrested erythropoiesis and reducing morbidity associated with anemia is a significant area of need in patients with MDS/MPN overlap syndromes.

Luspatercept-aamt is a first-in-class erythroid maturation agent that specifically binds to transforming growth factor-beta superfamily ligands to reduce *Smad* 2/3 signaling and increase late-stage erythropoiesis. The phase 3 MEDALIST trial formally evaluated this drug in lower risk MDS-RS and found to achieve a decrease in red blood cell transfusion independence for 8 weeks or longer in 38% patients during the first 24 weeks (6). The most frequent adverse events included asthenia, fatigue, bone pain, diarrhea, nausea, dizziness and back pain. Based on this study, regulatory approval was granted for MDS-RS and MDS/MPN overlap syndrome patients. However, the MEDALIST trial did not prospectively test the safety and efficacy of luspatercept-aamt in a separate large cohort of patients with MDS/MPN overlap syndromes (only a limited cohort of 23 MDS/MPN-RS-T patients were included). Further, erythroid responses were assessed per the IWG MDS response criteria, whereas contemporary response adjudication in MDS/MPN overlap syndromes is done as per the MDS/MPN IWG criteria(7), which is proposed in the current trial design.

Due to the unique clinical characteristics among patients with MDS/MPN-RS-T and MDS/MPN-U with RS such as the presence of thrombocytosis (in the former), increased risk of thrombosis (in both) (5), it is important to establish safety of luspatercept-aamt prior to its use in these patients. Our proposed clinical trial is aimed to specifically test the safety and efficacy of luspatercept-aamt in myelodysplastic/myeloproliferative neoplasms with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) and myelodysplastic/myeloproliferative neoplasms, unclassifiable with≥15% ring sideroblasts (MDS/MPN-U with RS), both orphan cancers with no

effective disease-modifying drugs. Although hypomethylating agents, lenalidomide, and erythropoiesis stimulating agents (ESA) are commonly used for these patients, no adequately powered clinical trials have confirmed disease biology modification or survival benefit. Transfusion-dependent anemia is a frequent clinical manifestation for these patients. In a Mayo Clinic study of 40 patients with MDS/MPN-RS-T, erythroid responses to ESA therapy were found in 45% patients, and predicted by an EPO level of $\leq$ 50 IU/L (5). However, a majority of patients either lose this response or developed dose-limiting adverse effects such as hypertension, thrombocytosis, and venous or arterial thromboembolism. Our group has also shown that patients with MDS/MPN-U with RS ($\geq$15% RS in diagnostic bone marrow biopsies) have comparable clinical features and outcomes to MDS/MPN-RS-T (2).

Due to the clinical equipoise of whether luspatercept-aamt is specifically effective and safe in MDS/MPN overlap syndromes, we have designed this phase II clinical study with the aim to study the effects of the drug prospectively in a cohort of patients with MDS/MPN-RS-T and MDS/MPN-U with RS. As opposed to MDS-RS, hydroxyurea use is an important part of treatment strategy in MDS/MPN overlap syndromes, such as MDS/MPN-RS-T due to the concomitant thrombocytosis. Therefore, it is also not known whether hydroxyurea can be safely combined with luspatercept-aamt and whether it will have impact on erythroid responses (15). Concomitant hydroxyurea and aspirin use as per the Essential Thrombocythemia (ET) risk stratification system (12) will be assessed along with luspatercept-aamt will also be formally assessed, since this a common clinical decision-making process for clinicians caring for patients with these disorders.

1.1 Treatment

The disease studied is MDS/MPN-RS-T and MDS/MPN-U with RS, defined as per the latest World Health Organization 2016 (WHO) criteria. As iterated above, these diseases do not have any effective disease modifying agents. The aim of this study is to test the safety and efficacy of luspatercept-aamt in these patients.

1.2 Correlative Research

The goal of the correlative studies would be to identify a biomarker of response to luspatercept-aamt. At enrollment, plasma, DNA, and RNA will be collected and stored for patients. We propose measuring the following parameters at enrollment in all patients:

1.21 Plasma: Ineffective erythropoiesis due to a perturbed TGF-β superfamily signaling and increased Smad-2/3 phosphorylation is a hallmark of myeloid neoplasms with RS. The recently discovered hormone, erythroferrone (ERFE) has been shown to act as a ligand trap for Smad proteins (8) and implicated in iron homeostasis in both Thalassemia Major (9) and Sickle Cell Disease (10). Variant ERFE secondary to SF3B1 mutant-induced aberrant splicing has been shown to disrupt iron homeostasis in MDS-RS (11).

Due to the known mechanism of luspatercept-aamt in reducing Smad pathway signaling through TGF-β modulation, we propose measuring hepcidin, and its known inhibitors related to TGF- β signaling, such as ERFE, growth-differential factor-15 (GDF-15) to identify potential biomarkers of erythroid response. Iron status will be documented through clinical assays prior to enrollment. In addition, we will perform cytokine profiling through a luminex assay. All parameters will be measured through ELISA as per previously published methods.

1.22 DNA – Stored peripheral blood mononuclear cell (PBMC) DNA at enrollment will be used to perform next generation sequencing for myeloid-specific genes (if

not done clinically) in order to identify potential genomic biomarkers of response in MDS/MPN-RS-T and MDS/MPN-U with RS.

1.23 RNA – Bulk RNA-seq will be performed on RNA extracted from PBMCs at enrollment and pathway analysis will be carried out to identify predictive biomarkers of response.

All correlative studies will be performed at Mayo Clinic.

2.0 Goals

2.1 Primary Goal

To document the erythroid response rate assessed as per the 2015 International Working Group (IWG) MDS/MPN response criteria (7)

2.2 Secondary Goals

2.21 To document response duration, time to acute myeloid leukemia (AML) transformation, AML-free survival (LFS) and overall survival (OS) in patients with MDS/MPN-RS-T and MDS/MPN-U with RS.

2.22 To document safety of luspatercept-aamt in patients with MDS/MPN-RS-T and MDS/MPN-U with RS.

2.3 Exploratory goals

2.31 To assess overall health-related quality of life as measured by Hematological Malignancy Specific Patient-Reported Outcome Measure (HM-PRO)

2.4 Correlative Research

The goal of correlative research as enumerated above is to find an effective biomarker of response to luspatercept-aamt.

3.0 Registration Patient Eligibility

3.1 Registration – Inclusion Criteria

3.11 Age ≥18 years

3.12 Patients with a WHO-defined diagnosis of MDS/MPN-RS-T or MDS/MPN-U with ≥15% RS (1).

3.13 Prior treatment with lenalidomide, hypomethylating agents, immunosuppressive therapy, ESA, or investigational agent is allowed as long as patients have not received luspatercept-aamt or sotatercept. If there is prior history of investigational agent, there should be an interval equivalent to at least four elimination half-lives of the agent prior to enrollment.

Note: For patients who have received prior lenalidomide, hypomethylating agents, or immunosuppressive therapy, there must be ≥6 weeks since the last dose before luspatercept-aamt treatment is started.

3.14 ECOG Performance Score of 0, 1 or 2.

3.15 Requirement of red blood cell transfusions (≥2 units ≤ 8-weeks prior to registration) OR symptomatic anemia with hemoglobin <9.5 g/dL OR hematocrit <30% (as long as there is documentation of adequate iron stores (ferritin > 50 mg/L) ≤ 5 weeks prior to registration). Symptomatic anemia is defined as fatigue with or without exertion, shortness of breath with or without exertion, or decrease in exercise tolerance.

3.16 The following laboratory values obtained ≤14 days prior to registration:

- Hemoglobin ≤9.5 g/dL
- Total bilirubin ≤1.5 x ULN
- Alanine aminotransferase (ALT) and aspartate transaminase (AST) ≤3 x ULN (≤5 x ULN for patients with liver involvement)
- Calculated creatinine clearance ≥30 ml/min using the Cockcroft-Gault formula below:

Cockcroft-Gault Equation:

Creatinine clearance for males = $\frac{\textbf{(140 - age)(weight in kg)}}{\text{(72)(serum creatinine in mg/dL)}}$

Creatinine clearance for females = $\frac{\textbf{(140 - age)(weight in kg)(0.85)}}{\text{(72)(serum creatinine in mg/dL)}}$

3.17 Female of childbearing potential (FCBP) defined as a sexually mature woman who:

1) has achieved menarche at some point,

2) has not undergone a hysterectomy or bilateral oophorectomy, or

3) has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (ie, has had menses at any time in the preceding 24 consecutive months) and must:

   a. Must have two negative urine or serum pregnancy tests as verified by the investigator prior to starting study therapy. A negative pregnancy test

must be done ≤7 days prior to registration. Patient must agree to ongoing pregnancy testing during the course of the study, and after end of study therapy. This applies even if the subject practices true abstinence* from heterosexual contact.

b. Either commit to true abstinence*from heterosexual contact (which must be reviewed on a monthly basis and source documented) or agree to use, and be able to comply with highly effective, contraception without interruption during the study therapy (including dose interruptions), and for 84 days after discontinuation of study therapy.

3.18 Male participants must:

Practice true abstinence* (which must be reviewed on a monthly basis) or agree to use a condom during sexual contact with a pregnant female or a female of childbearing potential while participating in the study, during dose interruptions and for at least 84 days following investigational product discontinuation even if he has undergone a successful vasectomy. * True abstinence is acceptable when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception).

3.19a Provide written informed consent.

3.19b Willing to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.19c Willingness to provide mandatory blood specimens for correlative research

3.2 Registration - Exclusion criteria

3.21 Any of the following because this study involves an agent that has known genotoxic, mutagenic and teratogenic effects:

- Pregnant persons
- Nursing persons
- Persons of childbearing potential who are unwilling to employ highly effective contraception

3.22 Any of the following prior therapies:

- Surgery ≤3 weeks prior to registration
- Chemotherapy or other agents ≤2 weeks prior to registration.

3.23 Uncontrolled intercurrent non-cardiac illness including, but not limited to:

- Ongoing or active infection
- Uncontrolled hypertension (defined as systolic blood pressure ≥140 mmHg or diagnostic blood pressure ≥90 mmHg despite use of ≥ 3 anti-hypertensive drugs at optimal doses).
- Psychiatric illness/social situations
- Dyspnea at rest due to complications of advanced malignancy or other disease that requires continuous oxygen therapy
- Clinically significant (symptomatic) anemia either due to nutritional deficiencies or iron, vitamin B12, folate or GI bleeding.
- Any other conditions that would limit compliance with study requirements.

3.24 Immunocompromised patients and patients known to be HIV positive and currently receiving antiretroviral therapy.
NOTE: Patients known to be HIV positive, but without clinical evidence of an immunocompromised state (CD4 ≤200 x 10^6/L), are eligible for this trial.

3.25 Receiving any other drug (except hydroxyurea) or investigational agent which would be considered as a treatment for the primary disease, that is, MDS/MPN-RS-T or MDS/MPN-U with RS ≤2 weeks prior to registration.

3.26 Other active malignancy ≤3 years prior to registration. Patients on hormonal therapy for treated breast or prostate cancer are permitted if they meet other eligibility criteria.

EXCEPTIONS: Non-melanotic skin cancer or carcinoma-in-situ of the cervix.
NOTE: If there is a history of prior malignancy, they must not be receiving other specific treatment (luteinizing hormone-releasing hormone (LHRH) agonists for prostate cancer, and treatment with bisphosphonates and receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitors) for their cancer.

3.27 History of myocardial infarction, stroke, embolism, deep vein or arterial thrombosis ≤6 months prior to registration, or congestive heart failure requiring use of ongoing maintenance therapy for life-threatening ventricular arrhythmias.

4.0 Test Schedule

4.1 Test schedule for MDS/MPN-RS-T and MDS/MPN-U with RS

Tests and procedures		Active Monitoring Phase		
	Screening (≤30 days prior to registration	**Every Cycle** (ie,1,2,3+ up to max 8 cycles) **Day 1**	**Cycle1 Days 8 and 15**	**Week 25[1] Response Assessment Visit/EOT** *24 calendar* weeks *after first dose regardless of dose delays.*
Window		±3 days	±3 days	±14 days
Medical History	X			
Height	X			
Physical Examination, weight, ECOG PS	X	X		X
Prior and concomitant medications/procedures	X	Continuous		
Adverse Event Assessment	X	X	X	X
Serum or Urine Pregnancy Test and Counseling[2,R]	X	X		X
Vital Signs to include BP	X	X	X	X
Serum Chemistry[3]	X[10]	X		X
Hematology[4]	X[10]	X	X	X
Von-Willebrand factor (vWF) activity	X			
Serum erythropoietin (EPO)	X			
Serum Ferritin[5]	X			X
HIV-1/2 Antigen or Antibody screen [R,11]	X			
MDS-MPN overlap syndrome disease Assessment[6]	X			X
Bone Marrow Biopsy and Aspirate (BMA) and Peripheral Blood for cytomorphology and cytogenetic testing[6,7]	X			
Hydroxyurea Medication Diary[8] (Cohort B only)		X	X	
Optional Hematological Malignancy Patient-Reported Outcome Measure (HM-PRO)[13]		X		X

Tests and procedures		Active Monitoring Phase		
	Screening (≤30 days prior to registration	**Every Cycle** (ie,1,2,3+ up to max 8 cycles) **Day 1**	**Cycle1 Days 8 and 15**	**Week 25[1] Response Assessment Visit/EOT** *24 calendar* weeks *after first dose regardless of dose delays.*
Mandatory Research Blood (plasma, DNA, RNA) for Exploratory/ Biomarkers (eg, ERFE, cytokines, genomic and transcriptomic) Assays[R] (See Section 14.0)	X[12]			X
Optional Research Bone Marrow Biopsy and Aspirate for Exploratory/Biomarkers (eg, TGF-ß superfamily, MDS-associated molecular mutations) Assays[9,R] (See Section 14.0)	X[12]			

Cycle = 21 days

[1] Week 25 Visit and End of Treatment (EOT) Visit may be the same visit. If a subject is discontinued during a regular scheduled visit, all EOT procedures should be completed at that visit.

[2] Pregnancy test is required for all female participants of childbearing potential (FCBP) ≤ 7 days prior to registration. Serum beta human chorionic gonadotropin (β-hCG) will be performed at screening. A urine (or serum) pregnancy test will be repeated prior to the first administration of IP on C1D1, unless the screening pregnancy test was done within 72 hours of C1D1. During the Treatment Period, urine or serum pregnancy test is allowed. For males and FCBP, counseling about pregnancy precautions and the potential risks of fetal exposure must be conducted prior to each IP administration or on a monthly basis (eg, in the event of dose delays).

[3] For serum chemistry, draw a Comprehensive Metabolic Panel (CMP) and uric acid only. CMP includes albumin, total bilirubin, total calcium, bicarbonate (CO2), chloride, creatinine, glucose, alk phosphatase, potassium, total protein, sodium, AST, ALT, urea nitrogen

[4] Hematology assessment includes a CBC with differential and absolute reticulocyte count

[5] Ferritin samples should be collected within 5 weeks prior to registration.

[6] During the Treatment Period, MDS-MPN overlap syndrome Disease Assessment should be completed by the investigator in conjunction with clinical bone marrow collections for cytomorphology and cytogenetics, and/or peripheral blood assessment.

[7] During the Screening Period, bone marrow biopsy AND bone marrow aspirate are not required at enrollment, if done clinically ≤30 days prior to enrollment. After enrollment in the primary treatment phase, a bone marrow biopsy is collected only when adequate aspirate is not attainable. No additional bone marrow biopsies are necessary and will be performed only as per clinical indications. Clinical benefit for the drug will be assessed per MDS/MPN response assessment criteria [Section 11, (7)]. Marrow response will only be assessed if additional bone marrow biopsy evaluations are available.

[8] Patients will use the pill diary to confirm their daily dose of hydroxyurea, note dose holds, and dose changes.

[9] Remaining BMA, (after quantity sufficient is allocated towards cytomorphology and cytogenetics analysis), will be used for exploratory biomarker studies (eg, ERFE, cytokines, genomic and transcriptomic). An additional bone marrow procedure should not be performed for these samples. Refer to Section 14.0 for additional information related to biomarker/exploratory samples.

[10] Hemoglobin, total bilirubin, ALT, and AST must be done ≤14 days prior to registration

[11] If HIV positive, a CD4 count must be drawn (this will be research funded)

[12].Research blood and bone marrow samples must be collected after registration, but prior to Cycle 1 Day 1 treatment

[13] HM-PRO will be done every cycle, if patient consents, it does not need to be completed prior to drug infusion. It will be given either during in person visits or sent to patient electronically (±7 days of D1 and EOT).

[R] Research funded

4.2 Survival Follow-up

Window of ±30 days

	Survival Follow-up[1]		
	Every 3 months[1]	Death	New Primary
Survival Follow-up[1]	X	X	At each occurrence

1 If a patient is still alive 1.5 years after registration, no further follow-up is required.

5.0 Grouping Factor:

5.1 Cohort A (hydroxyurea-independent patients) vs. Cohort B (hydroxyurea-dependent patients)

Definition of groups:

Hydroxyurea-independent group: Patients who meet the inclusion criteria and either have a platelet count of $< 450 \times 10^9$/L or they do not meet the criteria for hydroxyurea use per ET risk stratification criteria. Patients with prior use of hydroxyurea will be allowed in this group as long as use is more than 2 weeks before enrollment (Appendix II).

Hydroxyurea-dependent group: This group includes patients who meet the inclusion criteria and have a platelet count $\geq 450 \times 10^9$/L AND meet criteria for hydroxyurea use as per the ET risk stratification criteria (Appendix II).

5.2 There will be no stratification factors.

6.0 Registration/Randomization Procedures

6.1 Registration:

6.11 Registering a patient

To register a patient, access the Research Registration Application at [redacted] The Research Registration Application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call Research Site Management at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

Access and training instructions for the Research Registration Application are available on the Office of Clinical Trials web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the Research Registration Application can be confirmed in any of the following ways:

- Contact Research Site Management [redacted] If the patient was fully registered, the Research Site Management staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.2 Verification of materials

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility

- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.3 Documentation of IRB approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office (fax: [redacted]). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Correlative Research

6.41 Mandatory

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.*0,* 14.0 and/or 17.0).

6.42 Optional

An optional correlative research component is part of this study, there will be an option to select if the patient is to be registered onto this component (see Section 14.0).

Patient has/has not given permission to give his/her bone marrow sample for research testing.

6.5 Banking

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research of MDS/MPN overlap syndromes at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.6 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic Rochester, Mayo Clinic Arizona, or Mayo Clinic Florida under the supervision of a hematologist.

6.7 Treatment start

Treatment cannot begin prior to registration and must begin ≤ 30 days after registration.

6.8 Pretreatment

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.9a Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

6.9b Study drug

Study drug is available on site.

6.9c Blood draw kits

For MCF and MCA, blood draw kit is available on site. MCR will not use kits.

6.9d Patient Questionnaire Booklets, if patient consents

Patient questionnaire booklets can be found in the Protocol Catalog. They may be printed or distributed electronically for each patient.

6.9e Study Conduct

The clinical trial will be conducted in compliance with regulations (21 CFR 312, 50, and 56), guidelines for Good Clinical Practice (ICH Guidance E6), and in accordance with general ethical principles outlined in the Declaration of Helsinki; informed consent will be obtained from all participating patients; the protocol and any amendments will be subject to approval by the designated IRB prior to implementation, in accordance with 21 CFR 56.103(a); and subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

7.0 Protocol Treatment

7.1 Treatment Groups

This parallel cohort phase II protocol will stratify eligible patients based on hydroxyurea use at enrollment.

- Cohort A (hydroxyurea-independent)
- Cohort B (hydroxyurea-dependent)

7.11 Treatment

Use actual weight or estimated dry weight if fluid retention. Both Cohorts will receive the same schedule of luspatercept-aamt.

Cohort A – Hydroxyurea independent:

Agent	Starting Dose Level	Route	Day	ReRx
Luspatercept-aamt[1]	1 mg/kg	Subcutaneous	1	Every 21 Days

Cohort B – Hydroxyurea dependent:

Agent	Starting Dose Level	Route	Day	ReRx
Luspatercept-aamt[1]	1 mg/kg	Subcutaneous	1	Every 21 Days
Hydroxyurea[§] (optional)	500 mg (or stable dose for 2 weeks prior to enrollment)	Oral	Daily*	Ongoing

[§]Hydroxyurea use is optional. The starting dose and ongoing schedule are at the primary provider's discretion based on the recommended Essential Thrombocythemia (ET) risk stratification system (12) (See Appendix II). Patient will be provided a prescription. Doses and adjustments will be collected via a patient medication diary
*Hydroxyurea may be taken before or after luspatercept-aamt injection.

1. Starting as soon as Cycle 3, Day 1 and assessed by the investigator prior to every subsequent treatment cycle, participants may have the luspatercept-aamt dose level increased in a stepwise manner beyond the starting dose of 1.0 mg/kg to 1.33 mg/kg, and up to a maximum of 1.75 mg/kg if all the following criteria are met:
   - Subject has ≥ 1 RBC transfusion event (for pre-transfusion Hgb of < 9 g/dL) during the 2 most recent prior treatment cycles (~6-weeks).
   - The <u>two</u> most recent prior treatment cycles assessed must be at the same dose level.
   - Subject must not have met protocol dose delay and/or reduction criteria in the two most recent treatment cycles (exception of dose delay required due to influence of RBC transfusions).

   If all criteria above are met, the dose may be increased by 1 dose level.

The dose level should be titrated individually for each subject and must not exceed 1.75 mg/kg. Starting dose with dose increases and reductions are presented in Table 8.1.

7.2 Self-administration

Patients may not self-administer luspatercept-aamt. Preparation and administration by a healthcare provider is required.

Patient may self-administer hydroxyurea.

7.3 Return to consenting institution

For this protocol, the patient must return to the consenting institution for evaluation at least every 21 days during treatment (Active Monitoring Phase).

7.4 Treatment by a local medical doctor (LMD) is not allowed.

8.0 Dosage Modification Based on Adverse Events

Strictly follow the modifications in this table for the first **two** cycles, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose.

→ ***ALERT:*** ***ADR reporting may be required for some adverse events (See Section 10.0)*** ←

8.1 Dose Levels (Based on Adverse Events in Tables 8.*2* and 8.3)

Dose Level	Luspatercept-aamt
+2	1.75 mg/kg
+1	1.33 mg/kg
1*	1 mg/kg
-1	0.8 mg/kg
-2	0.6 mg/kg
-3	Discontinue

*Dose level 1 refers to the starting dose. Hydroxyurea dose changes will be per provider discretion.

NOTE: If hydroxyurea is discontinued, luspatercept-aamt may be continued. If luspatercept-aamt is discontinued, the patient will go to survival follow up (section 4.2.).

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) current version 5.0* unless otherwise specified*** ← ←

* Located at [redacted]

8.2 Dose Modifications at the start of new cycle of therapy

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

- The ANC is ≥ 500/μL
- The platelet count is ≥ 50 X 10^9/L.
- Any other non-hematologic treatment –related (possibly, probably, or definitely related) adverse event that may have occurred has resolved to baseline severity.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of therapy will be held until the toxicity has resolved as described above.

If the study drug (luspatercept-aamt) dosing was halted during the previous cycle and was restarted with a one-level dose reduction without requiring an interruption for the remainder of the cycle, then that reduced dose level will be initiated on Day 1 of the new cycle.

If any drug dosing was omitted for the remainder of the previous cycle or if the new cycle is held due to known hematologic toxicity newly encountered on the scheduled Day 1, then the new cycle will be started with a one-level dose reduction when toxicity resolves to the required level. If a new cycle of therapy cannot be restarted within 4 weeks of the scheduled Day 1 due to non-resolution of drug related toxicities, the patient will be removed from protocol therapy and will go to survival follow-up.

8.3 Dose Modifications Based on **Interval** Adverse Events (occurring within a cycle of treatment)

8.31 COHORT A: HYDROXYUREA-INDEPENDENT COHORT:

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Blood and lymphatic system disorders	Any suspected related AE ≥ grade 3	Luspatercept-aamt	Dose delay until resolved to < grade 1 or baseline, and then reduce dose by one level as above.
Investigations	≥2 dose reductions for suspected related AE	Luspatercept-aamt	Discontinue treatment
	Hemoglobin increase by ≥ 2 g/dL compared to pre-dose Hb of previous cycle	Luspatercept-aamt	Reduce dose by one level (if change in Hb is not influenced by RBC transfusions).
	Predose Hb ≥ 11.5 g/dL	Luspatercept-aamt	Dose delay until Hb is ≤ 11 gm/dL
	White blood cell increased by >50% compared pre-dose WBC of previous treatment	Luspatercept-aamt	Dose delay; recheck CBC, including WBC weekly during dose delay. Treatment may be resumed if WBC values below upper limit of normal within 2 weeks. If WBC remains above upper limit of normal for >2 consecutive weeks in absence of associated condition (such as infection or steroid use), continue dose delay and collect bone marrow sample to assess disease status. Treatment may be resumed if: There is absence of disease progression per MDS/MPN response criteria (Savona M et al. Blood 2015, Appendix III) AND WBC values return below upper limit of normal Discontinue treatment if there is evidence of disease progression or WBC count remains above upper limit of normal.

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
	Platelet count increase by >25% compared to pre-dose platelet count of previous treatment	Luspatercept-aamt	Reduce luspatercept-aamt dose by 1 dose level. Hydroxyurea addition is not allowed. If hydroxyurea needs to be added, patients will be taken off study. Addition of aspirin (along with dosing determinations) will be per provider discretion
	Grade 3 or 4 hypersensitivity reactions	Luspatercept-aamt	Discontinue treatment
	Other grade 3 or 4 adverse reactions	Luspatercept-aamt	• Interrupt treatment. • When the adverse reaction resolves to no more than grade 1, restart treatment at the next lower dose level as mentioned in section 8.1

** Use the following to describe actions in the Action column:

- **Omit** = The current dose(s) for the specified drug(s) during a cycle is skipped. The patient does not make up the omitted dose(s) at a later time.
- **Hold/Delay** = The current dose(s) of all drugs during a cycle is delayed. The patient does make up the delayed dose(s) when the patient meets the protocol criteria to restart drugs.
- **Discontinue** = The specified drug(s) are totally stopped.

8.32 COHORT B: HYDROXYUREA-DEPENDENT COHORT

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Blood and lymphatic system disorders	Any suspected related AE ≥ grade 3	Luspatercept-aamt	Dose delay until resolved to < grade 1 or baseline, and then reduce dose by one level as above.
Investigations	≥2 dose reductions for suspected related AE	Luspatercept-aamt	Discontinue treatment
	Hemoglobin increase by ≥ 2 g/dL compared to pre-dose Hb of previous cycle	Luspatercept-aamt	Reduce dose by one level (if change in Hb is not influenced by RBC transfusions).
	Predose Hb ≥ 11.5 g/dL	Luspatercept-aamt	Dose delay until Hb is ≤ 11 gm/dL
	White blood cell increased by >50% compared pre-dose WBC of previous treatment	Luspatercept-aamt	Dose delay; recheck CBC, including WBC weekly during dose delay. Treatment may be resumed if WBC values below upper limit of normal within 2 weeks. If WBC remains above upper limit of normal for >2 consecutive weeks in absence of associated condition (such as infection or steroid use), continue dose delay and collect bone marrow sample to assess disease status. Treatment may be resumed if: There is absence of disease progression per MDS/MPN response criteria (Savona M et al. Blood 2015, Appendix III) AND WBC values return below upper limit of normal Discontinue treatment if there is evidence of disease progression or WBC count remains above upper limit of normal.

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Investigations	Platelet count increase by >25% compared to pre-dose platelet count of previous treatment	Luspatercept-aamt	Reduce luspatercept-aamt dose by 1 dose level. Hydroxyurea dose changes will be per provider discretion
	Grade 3 or 4 hypersensitivity reactions	Luspatercept-aamt	Discontinue treatment
	Other grade 3 or 4 adverse reactions	Luspatercept-aamt	• Interrupt treatment. • When the adverse reaction resolves to no more than grade 1, restart treatment at the next lower dose level as mentioned in section 8.1 • Hydroxyurea dose changes will be per provider discretion.

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Blood products

Concurrent treatment for anemia with blood transfusions is recommended when Hgb is ≤ 9.0 g/dL or at the discretion of the investigator if Hgb is ≥ 9.0 g/dL and associated with symptom(s) of anemia (eg, hemodynamic or pulmonary compromise requiring treatment) or comorbidity justifying a threshold ≥ 9.0 g/dL Hgb. Dose adjustment and discontinuation of hydroxyurea, per provider discretion, should be considered for Cohort B if cytopenias occur.

For any RBC transfusion received during the study, collect hemoglobin value just prior to transfusion.

9.3 Corticosteroids

Concurrent corticosteroids used for medical conditions other than MDS/MPN overlap disorders is allowed provided subject is on a stable or decreasing dose for ≥ 1 week prior to randomization.

9.4 Iron Chelation Therapy

Participants who are using iron-chelating therapies at time of randomization should be on a stable dose for at least 8 weeks.

Concurrent treatment with iron chelation therapies during the Treatment Period is allowed at the discretion of the investigator and is recommended to be used per product label.

9.5 Antiemetics

Antiemetics may be used at the discretion of the attending physician.

9.6 Diarrhea

Diarrhea could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of Grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (Grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

9.7 Prohibited concomitant medications and procedures

Best supportive care for this study specifically excludes cancer surgery, immunotherapy,

biologic therapy, radiotherapy, and systemic chemotherapy where the goal is to eradicate or slow the progression of the disease.

The following concomitant medications are specifically excluded during the course of the study:

- Chemotherapeutic, targeted or investigational agents/therapies
- Azacitidine, decitabine or other hypomethylating agents including oral hypomethylating agents and combinations
- Lenalidomide, thalidomide and other immunomodulating drugs (IMiDs)
- Erythropoietin stimulating agents (ESAs) and other RBC hematopoietic growth factors (eg, Interleukin-3).
- Granulocyte colony stimulating factors (ie, G-CSF, GM-CSF), except in cases of neutropenic fever or as clinically indicated per product label.
- Androgens, unless to treat hypogonadism
- Oral retinoids (topical retinoids are permitted)
- Arsenic trioxide
- Interferon

10.0 Adverse Event (AE) Monitoring and Reporting

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human participants, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send[1]:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form [redacted] Will automatically be sent to [redacted]
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	Will automatically be sent to [redacted]

1.Also send SAE report to CELGENE at [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0 A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site:
[redacted]

a. Identify the grade and severity of the event using the CTCAE version 5.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.
An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.
NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 **AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME (Combination) Arm**

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the **entire combination (arm) is then considered an investigational intervention for reporting.**

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for **investigational** agents/interventions.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report, unless hospitalization is required. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

- An increased incidence of an expected adverse event (AE) is based on the patients treated for this study at their site. A list of known/expected AEs is reported in the package insert or the literature, including AEs resulting from a drug overdose.

10.32 **EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting**

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for IND Agents

10.41 **Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND within 30 Days of the Last Administration of the Investigational Agent/Intervention**[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.32 for exceptions to Expedited Reporting

10.42 General reporting instructions

The Mayo IND and/or MCCC Compliance will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form [redacted] for investigational agents or commercial/investigational agents on the same arm.

Also submit the SAE Report to CELGENE at [redacted]

10.43 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Participants or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Participants or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place participants or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that participants should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form

[redacted] The Mayo Clinic Compliance Unit will review and process the submission to the Mayo Clinic IRB and work with the IND Coordinator for submission to FDA.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease that cannot be attributed to a CTCAE term associated with Grade 5 should be reported as **Grade 5 "Disease Progression"** under the system organ class (SOC) of General disorders and administration site conditions. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm
- All secondary malignancies that occur following treatment with an agent under an IND will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

For any occurrence of a possible exposure of a pregnant woman to luspatercept-aamt (this could involve a pregnant patient or female partner of a male subject, or a pregnant female who came in contact with the medication while dispensing) or exposure (to infant) during lactation. , the subject should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these participants if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation including all reports of elevated/questionable or indeterminate beta human chorionic gonadotropins (b hCGs) or positive urine pregnancy test after administration of luspatercept-aamt. Include this form:

[redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 **Baseline and Adverse Events Evaluations**

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v5.0**unless** alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Anemia	X	X
	White blood cell decreased	X	X
	Investigations – Other, specify: Platelet count increased	X	X
	Platelet count decreased	X	X
	Neutrophil count decreased	X	X
	Alanine aminotransferase increased	X	X
	Aspartate aminotransferase increased	X	X
	Blood bilirubin increased	X	X
	Creatinine increased	X	X
Blood and lymphatic system disorder	Leukocytosis	X	X
Musculoskeletal and Connective Tissue Disorders	Bone pain	X	X
	Arthralgia	X	X
Infections and Infestations	Upper Respiratory Infection	X	X
	Infections and infestations Other, specify: Other infections	X	X
Nervous System disorders	Headache	X	X
	Dizziness	X	X
	Vertigo	X	X
	Presyncope	X	X
	Syncope	X	X

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
General disorders and administration site conditions	Fatigue	X	X
	Flu like symptoms	X	X
Gastrointestinal disorders	Baseline number of stools	X	
	Abdominal pain	X	X
	Diarrhea		X
	Nausea	X	X
Vascular disorders	Hypertension	X	X
Metabolism and nutritional disorders	Hyperuricemia	X	X
Respiratory, thoracic and mediastinal disorders	Cough	X	X
	Dyspnea	X	X
Immune system disorder	Allergic reaction	X	X
	Anaphylaxis	X	X
Cardiac disorders	Sinus tachycardia	X	X

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

10.8 BMS Additional Event Reporting Instructions

10.81 Additional Safety Information:
All adverse events and Other Reportable Events associated with luspatercept-aamt (as defined at [redacted] in accordance with the protocol and applicable law must be reported to CELGENE using the SAE report

and timelines as per Sections 10.0 and 10.4. INSTITUTION shall comply with CELGENE's reasonable follow-up requests.

10.82 Product Quality Complaints
All Product Quality Complaints (as defined at [redacted]) must be reported promptly to CELGENE at [redacted], but not to exceed the lessor of one (1) business day or three (3) calendar days of becoming aware of the event. INSTITUTION shall comply with CELGENE's reasonable follow-up requests.

10.83 Yearly summary safety reports will be submitted for 2 years and will provide summaries related to data collected on malignancies including secondary primary malignancies. These summary safety data will include incidence of secondary hematological malignancies (including de-novo AML and transformation to AML), solid tumors, and non-melanoma skin cancer.

11.0 Treatment Evaluation/Measurement of Effect

Primary end-point, that is, erythroid response is defined as hemoglobin increase by $\geq$ 2 g/dL from baseline or transfusion independence for $\geq$ 8 weeks for patients requiring at least 4 packed red blood cell transfusions in the previous 8 weeks.

Secondary end-point is to assess safety of luspatercept-aamt in patients with MDS/MPN-RS-T and MDS/MPN-U with RS.

Secondary endpoints such as response will be documented per the standard MDS-MPN response criteria per Savona et al. Blood 2015 (5).

Complete remission is defined by presence of all the following improvements.

a) Bone marrow: $\leq$ 5% myeloblasts with normal maturation of all cell lines and return to normal cellularity (presence of dysplastic changes, which may be interpreted within the scope of normal range of dysplastic changes may still exist in the presence of CR as allowed in the MDS IWG criteria. Marrow should exhibit age-adjusted normocellularity in CR).
b) Osteomyelofibrosis absent or equal to "mild reticulin fibrosis ($\leq$grade 1 fibrosis)
c) Peripheral blood: 1. WBC $\leq$ 10 x 10^9 cells/L, 2. Hgb $\geq$11 g/dL, 3. Neutrophils $\geq$ 1 x 10^9/L, 4. No blasts (peripheral blood), 5. Neutrophil precursors reduced to $\leq$ 2%, 6. Monocytes $\leq$ 1 x 10^9/L.
d) Extramedullary disease: Complete resolution of extramedullary disease if present before therapy, including palpable hepatosplenomegaly.

Complete cytogenetic remission: Resolution of previously present chromosomal abnormality as seen on classic karyotyping with minimal of 20 metaphases or FISH.

Partial remission: Normalization of peripheral counts and hepatosplenomegaly with bone marrow blasts reduced by 50%, but remaining more than 5% cellularity except in cases of MDS MPN with $\leq$ 5% bone marrow blasts at baseline.

Marrow response:

Optimal marrow response: Presence of all marrow criteria necessary for CR without normalization of peripheral blood in disease is presented above.
Partial marrow response: Bone marrow blasts reduced by 50% but remaining more than 5% cellularity or reduction and grading of reticulin fibrosis from baseline on at least 2 bone marrow evaluations paced at least 2 months apart.

Clinical benefit:
Requires 1 of the following in the absence of progression or CR/PR:
Erythroid response: as defined above

Platelet response: Transfusion independence been previously requiring platelet transfusions of at least 4 platelet transfusions the previous 8 weeks.

Neutrophil response
Pre-treatment $\leq$ 0.5 x 10^9/L: At least 100% increase and an absolute increase $\geq$ 0.5 x 10^9/L
Pre-treatment > 0.5 x 10^9/L and $\leq$ 1 x 10^9/L: At least 50% increase and an absolute increase of $\geq$ 0.5 x 10^9/L.

Spleen response: Either a minimum 50% reduction in palpable splenomegaly of a spleen that is at least 10 cm at baseline or a spleen that is palpable at more than 5 cm at baseline becomes not palpable.

For details, please refer to standard MDS-MPN response criteria per Savona et al. Blood 2015 (7).

Stable disease: Failure to achieve at least a partial remission (PR), but no evidence of progression for > 8 weeks

Disease Progression criteria as per Appendix III

12.0 Descriptive Factors

12.1 Prior treatment: 1 vs 2 vs 3+

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who achieve a response per criteria mentioned in section 11.0 will continue treatment per protocol.

13.2 Progressive disease (PD)

Patients who develop PD (Appendix III) while receiving therapy will go to the survival follow-up phase.

13.3 Off protocol treatment

Patients who go off protocol treatment for reasons other than PD (ie. Intolerable adverse events, subject refusal/withdrawal of consent, lost to follow-up, pregnancy) will go to the Survival Follow up phase per Section 4.0.

13.4 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will remain on treatment, patient will be followed , as per section 4.0 of the protocol.

- If the patient never received treatment, on-study material must be submitted. Patient will go off study and no follow up is required.

13.5 Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will continue in the Active Monitoring/Treatment phase of the study, as per section 4.0 of the protocol.

13.6 Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel

Notification Form must be submitted. The patient will go off study. No further follow up is required.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

Research (Section for more information)	Specimen Purpose (check all that apply)	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Baseline[1]	Week 25 Response	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping
DNA/RNA	Correlative	Mandatory	Peripheral blood,	EDTA	50 ml (five 10 ml EDTA tubes)	X	X	Yes (MCR); No (MCA/ MCF)	See Section 14.3
Exploratory/Biomarkers (eg, TGF-ß superfamily, MDS-associated molecular mutations) Assay	Correlative	Optional	Bone Marrow aspirate	EDTA	20 ml (five 4ml EDTA tubes)	X		Yes (MCR); No (MCA/ MCF)	See Section 14.3
Future Research	Banking	Optional	Bone marrow biopsy	Formalin Fixed, Paraffin Embedded	One block or 5 slides 5µ thickness	X		Yes (MCR); No (MCA/ MCF)	See Section 14.3

1. Baseline research blood and bone marrow samples must be collected after registration but prior to Cycle 1 Day 1 treatment

14.2 Collection and Processing

14.21 Research blood collections as per table in Section 14.1 will be done at each treating site. All processing will be done at the lead site, Mayo Clinic in Rochester, MN.

14.22 All samples must be collected Monday-Friday ONLY for specimens collected at Mayo Clinic Rochester and Monday-Thursday for all other locations.

14.23 Label specimen tube(s) with MCCC protocol number, MCCC Registration number, and time and date of blood drawn.

14.24 If questions, contact [redacted] at Predolin Foundation Biobank at [redacted]

14.3 Shipping and Handling

14.31 MCA and MCF will use kits for this study. MCR will not use kits for this study

14.311 For MCA, kits will be supplied by the Predolin Foundation Biobank on Stabile 6.

14.311a The kit contains supplies and instructions for collecting and shipping specimens.

14.311b Participating institutions may obtain kits by emailing [redacted] Because we are charged for all outgoing kits, a small, but sufficient, supply of the specimen collection kits should be ordered prior to patient entry.

14.311c Kits will be sent via Fed Ex® Ground at no additional cost to the participating institutions. **Allow at least two weeks to receive the kits.**

14.311d Kits will not be sent via rush delivery service unless the participating institution provides their own Fed Ex® account number or alternate billing number for express mail. **Cost for rush delivery of kits will not be covered by the study.**

14.311e **All specimens must be collected and shipped Monday – Thursday ONLY.**

14.312 For MCF, kits will be supplied by FL BAP

14.312a Please contact BAP Lab FL to order kit(s)

14.312b **All specimens must be collected and shipped Monday – Thursday ONLY.**

14.32 Shipping Specimens

For MCA and MCF, baseline blood and bone marrow samples will be shipped with a Cool Pak the same day they are collected (Monday-Thursday). Note: Week 25 blood samples may be shipped ambient. They should be shipped priority overnight.

If unavoidable, Friday shipping with Saturday delivery can be arranged by contacting the laboratory **in advance**.

Please notify Mayo Clinic by email to [redacted] when specimens are being shipped. If questions, please contact [redacted]

[redacted]

14.33 Handling Specimens

MNC Collection for Bone marrow or Peripheral blood done at the Predolin Foundation Biobank:
Follow the AML SOPs for processing both the bone marrow (plasma and MNCs) and peripheral blood (plasma and MNCs). **NOTE: No purity slides.**
Store the bone marrow and peripheral blood plasma in the current AML plasma box.
The bone marrow and peripheral blood MNCs will be divided as follows:
<u>If less than 5 x 10^6:</u>

- Store in RNA*Later* (priority)

<u>5 x 10^6 to 10 x 10^6 recovered:</u>

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: Remaining cells x 10^6 stored in RNA*Later*

<u>>10 x 10^6 to 15 x 10^6 MNCs recovered divide in **3 ways**:</u>

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: 5 x 10^6 stored in RNA*Later*
- Third vial: Remaining cells x 10^6 stored as a pellet

<u>>15 x 10^6 to 25 x 10^6 MNCs recovered divide in **4 ways**:</u>

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: 5 x 10^6 stored in RNA*Later*
- Third vial: 5 x 10^6 stored as a pellet
- Fourth vial: Remaining cells up to 15 x 10^6 stored as a 2^{nd} pellet

<u>>25 x 10^6 to 30 x 10^6 MNCs recovered divide in **5 ways**:</u>

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: 5 x 10^6 stored in RNA*Later*
- Third vial: 5 x 10^6 stored as a pellet
- Fourth vial: 5 x 10^6 stored as a 2^{nd} pellet
- Fifth vial: 5-10 x 10^6 stored in DMSO and <u>banked</u>

<u>35 x 10^6 MNCs recovered divide in **6 ways**:</u>

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: 5 x 10^6 stored in RNA*Later*
- Third vial: 5 x 10^6 stored as a pellet
- Fourth vial: 5 x 10^6 stored as a 2^{nd} pellet
- Fifth vial: 5-10 x 10^6 stored in DMSO and <u>banked</u>
- Remaining **<u>bone marrow cells</u>**: Cells go to [redacted] laboratory [redacted] add some **S10** and leave at room temperature.

- Remaining **peripheral blood cells**: Freeze ten million cells in DMSO (place with our aliquots in the CoolCell). The next day transfer the vial to the CMD group's storage box located on our working shelf in the -80 freezer. Transport to Terra Lasho.

Greater than 35 x 10^6 MNCs recovered divide **as follows in this order of priority**:

- First vial: 5 x 10^6 stored in RNA*Later* (priority)
- Second vial: 5 x 10^6 stored in RNA*Later*
- Third vial: 5-15 x 10^6 stored as a pellet
- Fourth vial: 5-15 x 10^6 stored as a 2nd pellet
- Fifth vial: 10-30 x 10^6 stored in DMSO and banked
- **Peripheral blood cells**: Freeze ten million cells in DMSO (place with our aliquots in the CoolCell). The next day transfer the vial to the CMD group's storage box located on our working shelf in the -80 freezer. Transport to Terra Lasho.
- Sixth thru Tenth vials: 10.0 x 10^6 stored in DMSO
- Remaining **bone marrow or peripheral blood cells**: Cells go to [redacted] laboratory [redacted] add some **S10** and leave at room temperature.

Freeze the RNA*Later* and pellet vial at -80º for a minimum of 10 minutes. Freeze ALL the DMSO vials per SOP (CoolCell).
Transport the samples (RNA Later, pellets, DMSO vials 6 thru 10 and any fresh cells) in the AML tracking system to [redacted] using **IRB 15-003786**.
Place the processing worksheet on the 7am technologist's desk.
The next day the 1st DMSO aliquot is stored in our active AML/CMML/CAR-T Liquid Nitrogen box.

14.4 Background and Methodology

14.41 *Plasma*: Ineffective erythropoiesis due to a perturbed TGF-β signaling and increased Smad-2/3 phosphorylation is a hallmark of myeloid neoplasms with RS. The recently discovered hormone, erythroferrone (ERFE) has been shown to act as a ligand trap for *Smad* proteins (Arezes J et al. *Blood* 2018) and implicated in iron homeostasis in both Thalassemia Major (Kautz L et al. *Blood* 2015) and Sickle Cell Disease (Mangaonkar A et al. *Br J Haematol* 2020). Variant ERFE secondary to *SF3B1* mutant-induced aberrant splicing has been shown to disrupt iron homeostasis in MDS-RS (Bondu S et al. *Sci Trans Med* 2019).

14.42 Due to the known mechanism of luspatercept-aamt in reducing *Smad* pathway signaling through TGF-β modulation, we propose measuring hepcidin, and its known inhibitors related to TGF- β signaling, namely ERFE, and growth-differential factor-15 (GDF-15) to identify potential biomarkers of erythroid response. Iron status will be documented through clinical assays prior to enrollment. All parameters will be measured through ELISA as per previously published methods.

14.43 *DNA* – Stored peripheral blood mononuclear cell (PBMC) DNA at enrollment will be used to perform next generation sequencing for myeloid-specific genes

(if not done clinically) in order to identify potential genomic biomarkers of response in MDS/MPN-RS-T and MDS/MPN-U with RS.

14.44 *RNA* – Bulk RNA-seq will be performed on RNA extracted from PBMCs at enrollment and pathway analysis will be carried out to identify predictive biomarkers of response.

All correlative studies will be performed at Mayo Clinic (Rochester, MN)

15.0 Drug Information

IND exempt

- Investigator brochure

  BMS contact information:

  [redacted]

15.1 Luspatercept-aamt (Reblozyl®, ACE-536, BMS-986346)

15.11 **Background:** Luspatercept-aamt is a recombinant fusion protein that binds several endogenous TGF-β superfamily ligands, thereby diminishing Smad2/3 signaling. Luspatercept-aamt promoted erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts), decreased abnormally elevated Smad2/3 signaling and improved hematology parameters associated with ineffective erythropoiesis.

15.12 **Formulation**: Luspatercept-aamt for injection is a sterile, preservative-free, lyophilized cake/powder. Upon reconstitution it consists of 50 mg/mL luspatercept-aamt in a 10 mM citrate buffer-based solution (pH 6.5, 9% sucrose, 0.02% polysorbate 80). The drug product is packaged in a 3-mL glass vial in the following strengths: 25 mg/vial and 75 mg/vial.

15.13 **Preparation and storage:** Store luspatercept-aamt vials at 2°C to 8°C. Luspatercept-aamt is reconstituted with sterile water for injection (SWFI). Luspatercept-aamt should not be reconstituted with bacteriostatic water or any other solution.

A. Reconstitute 25 mg vial with 0.68 mL of SWFI to a final concentration of 25 mg/0.5 mL. Reconstitute 75 mg vial with 1.68 mL of SWFI to a final concentration of 75 mg/1.5 mL (50 mg/mL). Direct the stream onto the lyophilized powder. Let stand for one minute.
B. Discard the needle and syringe used for reconstitution. The needle and syringe used for reconstitution should not be used for subcutaneous injections.
C. Gently swirl the vial in a circular motion for 30 seconds. Stop swirling and let the vial sit in an upright position for 30 seconds.
D. Inspect the vial for undissolved particles in the solution. If undissolved powder is observed, repeat Step C until the powder is completely dissolved.
E. Invert the vial and gently swirl in an inverted position for 30 seconds. Bring the vial back to the upright position, and let it sit for 30 seconds.
F. Repeat Step E seven more times to ensure complete reconstitution of material on the sides of the vial.

If the reconstituted solution is not used immediately, store at room temperature at 20°C to 25°C (68°F to 77°F) in the original vial for up to 8 hours. Discard if not used within 8 hours of reconstitution. Alternatively, store refrigerated at 2°C to 8°C (36°F to 46°F) for up to 24 hours in the original vial. Remove from refrigerated condition 15-30 minutes prior to injection to allow solution to reach room temperature for a more comfortable injection. Discard if not used within 24 hours of reconstitution. Do not freeze the reconstituted solution. Discard any

unused portion. Do not pool unused portions from the vials. Do not administer more than 1 dose from a vial. Do not mix with other medications.

15.14 **Administration**: Divide doses requiring larger reconstituted volumes (i.e., greater than 1.2 mL) into separate similar volume injections and inject into separate sites. If multiple injections are required, use a new syringe and needle for each subcutaneous injection. Administer the injection subcutaneously into the upper arm, thigh, and/or abdomen.

15.15 **Pharmacokinetic information:**

a) Absorption – Median time to Cmax is 7 days (range, 6-10 days) in beta thalassemia or 7 days (range, 5-21 days) in MDS. The absorption of luspatercept was not significantly affected by the SC injection sites (upper arm, thigh, or abdomen)
b) Distribution – The mean volume of distribution of luspatercept-aamt was 7 L for patients with beta thalassemia and 9.68L for patients with MDS
c) Metabolism – Luspatercept-aamt is expected to be catabolized into small peptides and amino acids by general protein degradation processes in multiple tissues
d) Excretion – The mean t½ of luspatercept-aamt was approximately 11 days in beta thalassemia and 14 days in MDS patients. Luspatercept is not expected to be significantly excreted into urine due to the fact that its large molecular mass is above the glomerular filtration size exclusion threshold

15.16 **Potential drug interactions:** No significant drug-drug interactions are known. No clinically significant differences in luspatercept-aamt pharmacokinetics were noted with concomitant iron-chelation therapy.

15.17 Known potential adverse events:

Common (> 10%):
Cardiovascular: Hypertension
Gastrointestinal: Abdominal pain, diarrhea, nausea
Hepatic: Increased serum alanine aminotransferase, increased serum aspartate aminotransferase, increased serum bilirubin
Nervous system: Dizziness, fatigue, headache, vertigo
Neuromuscular & skeletal: Arthralgia, musculoskeletal pain, ostealgia
Renal: Decreased creatinine clearance
Respiratory: Cough, dyspnea

Less Common (1-10%):
Cardiovascular: Presyncope, syncope, tachycardia, thromboembolism including deep vein thrombosis, ischemic stroke, portal vein thrombosis, pulmonary embolism,
Endocrine & metabolic: Hyperuricemia
Genitourinary: Urinary tract infection
Hepatic: Increased direct serum bilirubin, increased serum alkaline phosphatase
Hypersensitivity: Hypersensitivity reaction
Immunologic: Antibody development
Infection: Influenza
Local: Injection site reaction

Nervous system: Cerebrovascular accident, spinal cord compression
Renal: Renal insufficiency
Respiratory: Bronchitis, upper respiratory tract infection, viral upper respiratory tract infection
Miscellaneous: Mass

Frequency not defined: Neuromuscular and skeletal: Back pain
Splenectomized β-thalassemia subjects are at increased risk of developing thromboembolic complications. Caution is advised when treating splenectomized subjects who had at least 1 other risk factor for developing thromboembolic events such as a history of thrombocytosis or concomitant use of hormone-replacement therapy. Thromboprophylaxis should be considered in subjects with β-thalassemia at higher risk. Patients with β-thalassemia are at increased risk of developing extramedullary hematopoietic masses; some in the paraspinal region having the potential of causing spinal compression.

15.18 Drug procurement:

The Mayo Clinic pharmacist will obtain drug from BMS using the drug ordering system, IDOS:

Outdated or remaining drug is to be destroyed on-site per procedures in place at each institution.

15.19 Nursing guidelines

15.191 GI side effects have been seen. Most commonly abdominal pain, diarrhea, and nausea. Manage symptomatically and monitor for effectiveness.

15.192 Monitor LFT's

15.193 Patients may experience dizziness and vertigo. Warn patients of this possibility and to use caution when standing or doing activities that require concentration.

15.194 Arthralgia and musculoskeletal pain have been seen. Treat symptomatically and monitor for effectiveness.

15.195 Monitor renal function and encourage patients to stay well hydrated.

15.196 Rarely thrombotic and cardiac events can be seen. Instruct patients to report any edema, shortness of breath, chest pain or palpitations to the study team immediately or seek out urgent evaluation. Thrombotic events are more common in patients with β-thalassemia who have undergone splenectomy or those with splenectomy who have at least 1 other risk factor for developing thromboembolic events.

15.197 Hypertension is common. Instruct patients on the importance of taking any medications for blood pressure as prescribed. Monitor BP and report elevated readings to the local investigator.

15.2 Hydroxyurea for Oral Administration (Hydrea®)

15.21 **Background**: Hydroxyurea is an antimetabolite which selectively inhibits ribonucleoside diphosphate reductase, preventing the conversion of

ribonucleotides to deoxyribonucleotides, halting the cell cycle at the G1/S phase and therefore has radiation sensitizing activity by maintaining cells in the G_1 phase and interfering with DNA repair. In sickle cell anemia, Hydroxyurea increases red blood cell (RBC) hemoglobin F levels, RBC water content, deformability of sickled cells, and alters adhesion of RBCs to endothelium.

15.22 **Formulation**: Commercially available for oral administration as:

Capsules: 500 mg

15.23 **Preparation, storage, and stability**: Store at room temperature preferably below 25ºC (77ºF); excursions permitted between 15°C and 30°C (59°F and 86°F). The manufacturer does not recommend opening the capsules; observe proper handling procedures (eg, wear gloves).

15.24 **Administration:** Refer to the treatment section for specific administration instructions. The manufacturer does not recommend opening the capsules.

15.25 Pharmacokinetic information:

Distribution: Readily crosses blood-brain barrier; distributes into intestine, brain, lung, kidney tissues, effusions and ascites
Protein binding: 10% to 60%
Time to peak: 1-4 hours
Metabolism: 60% via hepatic and GI tract
Half-life elimination: 3-4 hours
Excretion: Urine (sickle cell anemia: 40% of administered dose)

15.26 Potential Drug Interactions:

Avoid Concomitant Use
Avoid concomitant use of Hydroxyurea with any of the following: BCG; Didanosine; Natalizumab; Pimecrolimus; Stavudine; Tacrolimus (Topical); Vaccines (Live)
Increased Effect/Toxicity: Hydroxyurea may increase the levels/effects of: Didanosine; Leflunomide; Natalizumab; Stavudine; Vaccines (Live). The levels/effects of Hydroxyurea may be increased by: Denosumab; Didanosine; Pimecrolimus; Stavudine; Tacrolimus (Topical); Trastuzumab
Decreased Effect: Hydroxyurea may decrease the levels/effects of: BCG; Sipuleucel-T; Vaccines (Inactivated); Vaccines (Live). The levels/effects of Hydroxyurea may be decreased by: Echinacea

15.27 **Known potential adverse events**: Consult the package insert for the most current and complete information. U.S. Boxed Warning: Hydroxyurea is mutagenic and clastogenic. Treatment of myeloproliferative disorders with long-term Hydroxyurea is associated with secondary leukemia.

Known potential toxicities, frequency not defined:
Cardiovascular: Edema
Central nervous system: Chills, disorientation, dizziness, drowsiness (dose-related), fever, hallucinations, headache, malaise, seizure
Dermatologic: Alopecia, cutaneous vasculitic toxicities, dermatomyositis-like skin changes, facial erythema, gangrene, hyperpigmentation, maculopapular rash, nail atrophy, nail discoloration, peripheral erythema, scaling skin atrophy, skin cancer, skin ulcer, vasculitis ulcerations, violet papules

Endocrine & metabolic: Hyperuricemia
Gastrointestinal: Anorexia, constipation, diarrhea, gastrointestinal irritation and mucositis, (potentiated with radiation therapy), nausea, pancreatitis, stomatitis, vomiting
Genitourinary: Dysuria
Hematologic: Myelosuppression (anemia, leukopenia [common; reversal of WBC count occurs rapidly], thrombocytopenia); macrocytosis, megaloblastic erythropoiesis, secondary leukemias (long-term use)
Hepatic: Hepatic enzymes increased, hepatotoxicity
Neuromuscular & skeletal: Peripheral neuropathy, weakness
Renal: BUN increased, creatinine increased

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Respiratory: Acute diffuse pulmonary infiltrates (rare), dyspnea, pulmonary fibrosis (rare)

15.28 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.29 Nursing Guidelines:

15.291 Hydroxurea is mutagenic and clastogenic, and therefore has the potential to cause chromosomal abnormalities in the pateint that could lead to other conditions. Warn patient of this possibility.

15.292 Myelosuppression is common. Monitor CBC with differential. Instruct patient to report any signs or symptoms of infection, or any unusal bruising or bleeding to the study team.

15.293 Patients who are on this agent for long term have an increased risk of developing secondary leukemias. Warn patient of this possibility.

15.294 Nurmerous dermatologic conditions can be seen with hyrdoxurea. Refer to section 15.17 for these and instruct patient to report any dermatologic signs and symptoms to the study team.

15.295 Gastrointestinal side effects can be seen (diarrhea, constipation, nausea, vomitin, anorexia, etc.). Treat symptomatically and monitor for effectiveness.

15.296 While rare, pulmonary infiltrates, dyspnea and pulmonary fibrosis can occur. Instruct patients to report any respiratory symptoms to the study team.

16.0 Statistical Considerations and Methodology

16.1 Study Design Overview: This is a phase 2 trial with two parallel cohorts with independent statistical designs and decision rules. The two cohorts will be based on dependence on concurrent hydroxyurea in these patients. The following designs and decision criteria will be used for each of the cohorts:

- Hydroxyurea-independent cohort: This phase 2 evaluation within this cohort will utilize a Simon minimax two-stage design. Based on published data and our experiences with this patient population, we would expect that if this regimen was not effective that we would observe at most 10% of patients with an erythroid-based response. To be considered promising in these hydroxyurea-independent patients, we hypothesize a true response rate of at least 40%. Constraining Type I error to 0.05 and Type II errors to 0.20 each (i.e. with 80% power, α=0.05), this design requires a minimum of 8 patients and a maximum of 13 patients in this cohort.
  - Decision Rule: Overall, if we observe 4 or more patients with an erythroid response in the 13 evaluable patients, we will consider this to be sufficient evidence of promising activity in this cohort.
  - Interim analysis: This two-stage design also includes an interim analysis after the first 8 evaluable patients have been accrued. If in these 8 patients we see at least 2 patients with an erythroid response, we will continue accrual to the total of 13 evaluable patients. If at most 1 of these 8 patients have an erythroid response, then we will consider this sufficient evidence that this agent is not promising in this cohort of HU-independent MDS/MPN-RS-T patients. Note that while the first 8 patients are being evaluated for response, we will allow accrual to continue given the relatively rare nature of this patient population.
- Hydroxyurea-dependent cohort: For this cohort, we will utilize a Simon optimal two-stage phase 2 trial design. Given that these patients will be dependent on concurrent hydroxyurea, we assume that their likelihood of an erythroid response in the absence of other treatment is lower (5%). For this more compromised group, we will consider a true response rate of 25% or greater to be promising in this cohort of patients. Constraining our Type I to 0.05 and the Type II error rate to 0.2 (i.e. with 80% power, α=0.05), this design requires a minimum of 9 patients and a maximum of 17 patients in this cohort.
  - Decision Rule: Overall, if we observe 3 or more patients with an erythroid response in 17 evaluable patients, we will consider this to be sufficient evidence of promising activity in this HU-dependent cohort.
  - Interim analysis: This two-stage design also includes an interim analysis after the first 9 evaluable patients have been accrued. If in these 9 patients we see at least 1 patient with an erythroid response, we will continue accrual to the total of 17 evaluable patients. If none of these 9 patients has an erythroid response, then we will consider this sufficient evidence that this agent is not promising in this cohort of HU-dependent MDS/MPN-RS-T patients. Note

that while the first 9 patients are being evaluated for response, we will allow accrual to continue given the relatively rare nature of this patient population.

16.2 Sample Size

16.2.1 Sample Size: The maximum sample size required in this study is 30 evaluable patients (13 HU-dependent, 17 HU-independent). We will accrue up to an additional 10% (3 patients) in order to account for potential ineligible patients or patients who withdraw prior to treatment. Thus, the total number of patients enrolled is expected to be up to a maximum of 33 patients.

16.3 Study Endpoints and Analysis Plans

Since the two HU dependence-based cohorts will be evaluated in parallel, their endpoints will also be evaluated separately. However, for safety endpoints as well as some clinical outcomes, we will also evaluate these across all patients while adjusting for HU dependence status (i.e. concurrent HU vs. not).

- Primary Endpoint: erythroid response rate will be defined as the proportion of patients who achieve an erythroid response out of the total number of evaluable patients (i.e. eligible patients who received at least one dose of treatment on study). Assuming that the number of patients who achieve a response is binomially distributed, we will calculate the proportion of erythroid responses in each cohort along with the corresponding binomial confidence intervals. For evaluation of erythroid response across all participants, we will also in an ancillary manner evaluate the incidence of erythroid response using a multivariable logistic regression model, where we will assess the influence of HU dependence as well as other factors of interest.
- Secondary Endpoints:
  - Safety and tolerability: The incidence of adverse events (AEs) and the ability of patients to stay on treatment will be used to assess safety and tolerability of treatment with luspatercept-aamt in each of the cohorts. Incidence of severe (grade 3+) AEs as well as toxicities (AEs felt to be at least possibly related to study treatment) will be tabulated and evaluated in each cohort along with tabulation of specific types of AEs. Time to end of active treatment will be evaluated as the time from treatment start date to the time patients go off treatment for any reason. This will be summarized within and across cohorts using the methods of Kaplan and Meier, and the reasons patients discontinue treatment will be summarized.
  - Time-to-event outcomes: Several time-to-event outcomes will be evaluated in the context of this study. These endpoints will be used to help characterize the patients within and across cohorts, and factors that affect the distributions of these endpoints in these cohorts may be explored. Each of these will be evaluated and characterized within each cohort using the methods of Kaplan and Meier. Patients who have not had the event of interest by the time of their last follow-up for that event will be censored at that time point. Any factors influencing these time-to-event outcomes will also be explored using

Cox proportional hazards models. The specific time-to-event outcomes to be evaluated are as follows:

- Duration of response: will be defined as the time from first documented erythroid response to the time of progression and/or relapse.
- Time to leukemic transformation: will be defined as the time from study entry to the time of transformation to AML.
- Leukemia-free survival: will be evaluated in all patients who from time of treatment to progression to acute myeloid leukemia or death from any cause.
- Overall survival: will be defined as the time from study entry to the time of death due to any cause.

- Exploratory outcomes: An optional study will assess health-related quality of life using the HM-PRO questionnaire. These assessments will be completed at the beginning of each cycle of treatment and at end of treatment. Given that this is an optional and exploratory endpoint, change from baseline HM-PRO scores will be analyzed in a descriptive manner.
- Correlative outcomes: Several correlative markers of interest as outlined above will be evaluated within and across cohorts. These markers will be assessed and summarized alone as well as in relation to erythroid response and/or other outcomes of interest. Analyses will also be explored across all patients, where cohort (HU dependence) will be adjusted for in the model and/or evaluated in the context of effect modification on the outcomes of interest and other factors of interest.

16.4 Data & Safety Monitoring

16.41 Safety review

The principal investigator(s) and the study statistician will review the study monthly to identify accrual, adverse event, and any endpoint problems that might be developing. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least biannually, based on reports provided by the MCCC Statistical Office.

16.42 Adverse Event Stopping Rules

The safety stopping rules to be used in this trial will be across both cohorts using a Bayesian toxicity monitoring rule. This rule constrains the maximum probability of unacceptable or excessive toxicity at 0.20. For the purposes of stopping rules for this patient population, where it is not uncommon for patients to experience severe adverse events, we will define excessive or unacceptable toxicity as any grade 3 or greater non-hematologic toxicity (an adverse event considered to be at least possibly related to study treatment) that is not responsive to supportive care. Excluded from this would be grade 3 fatigue as we would not consider this treatment-limiting. Assuming a prior distribution of this probability of *Beta*(1,1) and a maximum of N=33 patients, we will utilize these rules starting when n=6 patients have been accrued overall and to be evaluated after each patient (i.e. cohort size =1) and with a posterior probability >80% (i.e. probability of 0.80 that we will stop for excessive toxicity if the true toxicity rate

is >0.20). If the stopping rule thresholds are met, accrual will be temporarily suspended for full review by the study team.

Table of rules for toxicity stopping boundaries

# patients	# patients with unacceptable/excessive toxicity to suspend accrual for full review
6	2 or more
7 to 10	3 or more
11 to 14	4 or more
15 to 19	5 or more
20 to 23	6 or more
24 to 27	7 or more
28 to 31	8 or more
32 to 33	9 or more

16.5 Subset Analyses for Minorities

16.51 Study availability

This study will be available to all eligible patients, regardless of gender, race or ethnic origin.

16.52 Statistical analysis by subset

There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analyses will look for differences in treatment effect based on racial groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.53 Regional population

The geographical region served by MCCC has a population which includes approximately 3% minorities. With accrual open at all three Mayo Clinic sites, we will target improved accrual of minority patient participation. Expected sizes of racial by gender subsets are shown in the following table, which also includes the planned potential over accrual of 10% for a total of N=33 patients:

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	13	18	31
Ethnic Category: Total of all participants	**14**	**19**	**33**
Racial Category			
American Indian or Alaskan Native	0	1	1
Asian	0	0	0
Black or African American	0	1	1
Native Hawaiian or other Pacific Islander	0	0	0
White	14	18	31
Racial Category: Total of all participants	**14**	**19**	**33**

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa.

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

NONE

18.0 Records and Data Collection Procedures .

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Survival Follow-up

See Section 4.

18.3 CRF completion

This study will use Medidata Rave® for remote data capture (rdc) of all study data. Data collection for this study will be done exclusively through the Medidata Rave® clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active account and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on the organization roster at the enrolling site.

18.4 Site responsibilities

Each site will be responsible for ensuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for diagnosis and progression prior to study entry as well as for evidence of response to study therapy and progression after study therapy. These documents should be submitted within 14 days of registration (for prior to study entry materials) or within 14 days after the visit at which response or progression is determined.

ESA therapies ≤ 4 weeks prior to registration and RBC and Platelet transfusions ≤8 weeks prior to registration will be collected

18.6 Labeling of materials

Each site will be responsible for ensuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Overdue lists

A list of overdue forms and outstanding queries will be available in Rave through the Rave Task Summary. In addition to this, the Overdue Materials report is available on the Cancer Center Systems homepage.

19.0 Budget

19.1 Costs charged to patient: routine clinical care and hydroxyurea

19.2 Tests to be research funded:

HIV screening, CD4, if needed, and pregnancy tests.

Research blood and bone marrow specimen processing and analyzing

Luspatercept-aamt SQ injection administration

19.3 Other budget concerns:

BMS will provide Mayo Clinic with funding to support the costs of running this study.

BMS will provide study drug, luspatercept-aamt, for use in this study

20.0 References

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

[redacted]

APPENDIX II: Hydroxyurea and/or aspirin use guidelines.

Since there are no established guidelines to provide cytoreductive and/or anti-platelet therapy to manage patients with MDS/MPN overlap syndromes with thrombocytosis (platelet count $\geq$ 450, 000 X 10^9/L), we recommend the use of risk stratification system of essential thrombocythemia in estimating thrombotic risk, also known as IPSET-Thrombosis (12). This risk stratification will only be applied in patients with thrombocytosis (platelet count $\geq$ 450, 000 X 10^9/L). In this, patients are stratified into low, intermediate and high risk, based on the points assigned based on the following variables:

1. Age > 60 years: 1 point
2. Presence of thrombosis history (arterial or venous): 2 points
3. Cardiovascular risk factors (i.e hypertension, diabetes mellitus, and active tobacco use): 1 point
4. Presence of JAK2V617F mutation: 2 points.

Low risk is defined as <2 points; intermediate risk as 2 points, and high risk as > 2 points.

If patient is low risk, then once daily aspirin only is recommended. However, low risk in the presence of cardiovascular risk factors, twice-daily aspirin can be considered.

If patient is in the intermediate-risk category, then once-daily aspirin plus hydroxyurea therapy is recommended. If no cardiovascular risk factors are present, then twice-daily aspirin only can be considered.

In patients in the high-risk category, then hydroxyurea plus twice-daily aspirin should be considered. If there is a history of venous thrombosis, then systemic anticoagulation can be substituted in place of twice-daily aspirin. The treatment algorithm was derived and modified from Tefferi A et al. Blood Cancer J 2018 (13).

*Avoid aspirin therapy in the presence of extreme thrombocytosis and acquired Von Willebrand Syndrome.

APPENDIX III: Disease Progression Criteria

This appendix contains disease progression criteria derived from Savona MR et al. Blood 2015.

Combination of 2 major criteria, 1 major and 2 minor criteria or 3 minor criteria is defined as disease progression.

Major Criteria:

- Increase in blast count*
  - <5% blasts: ≥50% increase and to >5% blasts.
  - 5-10% blasts: ≥50% increase and to >10% blasts.
  - 10-20% blasts: ≥50% increase and to >20% blasts.

- Evidence of cytogenetic evolution**:
  - Appearance of a previously present or new cytogenetic abnormality in complete cytogenetic remission via FISH or classic karyotyping.
  - Increase in cytogenetic burden of disease by >50% in partial cytogenetic remission via FISH or classic karyotyping.

- New extramedullary disease

- Worsening splenomegaly:
  - Progressive splenomegaly that is defined by IWG-MRT: the appearance of a previously absent splenomegaly that is palpable at .5 cm below the left costal margin or a minimum 100% increase in palpable distance for baseline splenomegaly of 5-10 cm or a minimum 50% increase in palpable distance for baseline splenomegaly of 10 cm

- Extramedullary disease outside the spleen: To include new/worsening hepatomegaly, granulocytic sarcoma, skin lesions, etc.

Minor criteria:
- Transfusion dependence***
- Significant loss of maximal response or cytopenias ≥50% decrement from maximum remission/response in granulocytes
- Reduction in Hgb by 1.5g/dL from best response or from baseline as noted on complete blood count
- Increasing symptoms as noted by increase in ≥ 50% as per the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) (14)
- Evidence of clonal evolution****

*Blast count measured from bone marrow.
**Increase in cytogenetic burden of disease by ≥ 50% (via FISH or classic karyotyping). Given variability of fluorescent probes used in FISH, cytogenetic normalization via FISH will depend on specific probes used.
***Transfusion dependency is defined by a history of at least 2 units of red blood cell transfusions in the past month for a hemoglobin level, 8.5 g/dL that was not associated with clinically overt bleeding. Cytopenias resulting from therapy should not be considered in assessment of progression.
****The identification of new abnormalities using single nucleotide polymorphism arrays or sequencing or a clearly significant increase in mutational burden of a previously detected abnormality. Precise criteria for defining new abnormalities and what exactly constitutes a significant increase in mutational burden are open to interpretation; we suggest that this criterion should be used conservatively based on current evidence.