CLINICAL RESEARCH PROJECT

Protocol #09-H-0199
Drug Name: eltrombopag (Promacta®)
IND number: 105,207
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Title: A Pilot Study of a Thrombopoietin-receptor Agonist (TPO-R agonist), Eltrombopag, in Patients with Low to Int-2 Risk Myelodysplastic Syndrome (MDS)

Other Identifying Words: Hematopoiesis, autoimmunity, thrombocytopenia, megakaryocyte, acute myeloid leukemia, bone marrow fibrosis, stem cells, cytokine, Promacta® (eltrombopag), revolade

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Subjects of Study:	Number	Sex	Age-range
	30	Either	≥ 18 years

Project Involves Ionizing Radiation?	No (only when medically indicated)
Off-Site Project?	No
Multi-center trial?	No
DSMB Involvement?	Yes

PRECIS

The myelodysplastic syndromes (MDS) are bone marrow disorders characterized by anemia, neutropenia, and thrombocytopenia. Patients with MDS are at risk for symptomatic anemia, infection, and bleeding, as well as a variable risk of progression to acute leukemia. With the exception of stem cell transplant, the standard treatments for MDS are rarely curative, and relapse rates are significant. MDS patients with cytopenias who fail standard therapies require regular blood or platelet transfusions which are expensive and inconvenient, and are at risk for serious bleeding complications.

Thrombopoietin (TPO) is the principal regulator of platelet production by megakaryocytes in the bone marrow. A 2nd generation TPO-agonist, eltrombopag (Promacta®) has been shown to increase platelets in thrombocytopenic patients with chronic immune thrombocytopenic purpura (ITP). Eltrombopag is administered orally, is well-tolerated, and is FDA approved for the treatment of thrombocytopenia in patients with chronic ITP who failed to respond to standard treatment.

Because the management of MDS patients with persistent cytopenias remains unsatisfactory and novel therapeutic approaches are needed, we propose a non-randomized, pilot, phase II study of eltrombopag in low to Int-2 risk MDS subjects with thrombocytopenia and anemia cytopenias who are either untreated or cytopenias that persist despite treatment with standard therapies to assess its utility in these settings.

Subjects will initiate study medication at an oral dose of 50 mg/day (25 mg/day for East Asians), which will be adjusted as clinically indicated to the lowest dose that maintains a stable platelet count $\geq$ 20,000/μL above baseline while maximizing tolerability. Treatment response will be any increase in a cytopenia, in the lineage that fulfilled eligibility criteria for enrollment and will be defined as: **(a)** platelet count increases to 20,000/μL above baseline at 16 or 20 weeks, or stable platelet counts with transfusion independence for a minimum of 8 weeks in subjects who were previously transfusion dependent; **(b)** erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment compared with the pretreatment transfusion number in the previous 8 weeks; **(c)** neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L. Subjects meeting a response may remain on the extended access until they meet an off study criteria or the study is closed.

Subjects with response at 16 or 20 weeks may be consented for entry into the extended access part of the trial. In the event that a subject is transfused platelets for a count >10,000/μL without a medical indication during the study period, the subject may continue on study drug and the response assessment may be extended for an additional 4 weeks, to week 20, at the discretion of the principal investigator. Subjects with evidence for a clinical response in any lineage at 16 weeks but not yet meeting full primary endpoint response criteria, and who are tolerating investigational treatment, may receive an additional 4 weeks of eltrombopag and be reassessed after 20 weeks. At that time, if they meet primary endpoint response criteria, they will be eligible to enter the extended access part of the study. If they do not meet primary endpoint response criteria, eltrombopag will be discontinued.

Primary objective is to assess the efficacy of eltrombopag in patients with low to Int-2 risk MDS. Safety of eltrombopag in this subject population will be assessed concurrently.

Secondary objectives include the toxicity profile of extended treatment with eltrombopag (treatment longer than 4 months), reduction in incidence and severity of bleeding episodes, and response following extended access to study drug (treatment longer than 4 months).

The ***primary endpoint*** will be the portion of drug responders as defined by changes in the platelet count and/or platelet transfusion requirements, or the proportion of subjects who meet erythroid response, or neutrophil response criteria[1]. Platelet response is defined as platelet count increases to 20,000/□L above baseline at 16 or 20 weeks, or stable platelet counts with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment - compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L. Subjects with an erythroid, and/or neutrophil response at 16 weeks may continue study medication (extended access) until they meet an off study criteria. Subjects with erythroid, or neutrophil response at 16 weeks may continue study medication for an additional 4 weeks (to ensure eligibility) prior to being consented for entry into the extended access part of the trial. Patients may remain on the extended access until they met an off study criteria.

The toxicity profile will be measured using the CTCAE Version 4.0 criteria.

Secondary endpoints will include incidence of grade 2 or higher bleeding events as measured by CTCAE v. 4.0; changes in serum thrombopoietin level, measured at 4 months; and progression to higher risk MDS as measured by IWG criteria[1].

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1. OBJECTIVES

Primary objective is to assess the efficacy of eltrombopag in patients with either untreated low to Int-2 risk MDS with cytopenias or those with cytopenias refractory to standard therapy. Safety of eltrombopag in this subject population will be assessed concurrently.

Secondary objectives include the toxicity profile of extended treatment with eltrombopag (treatment longer than 4 months), reduction in incidence and severity of bleeding episodes, erythroid and neutrophil response, and response following extended access to study drug (treatment longer than 4 months).

2. BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 Pathophysiology of cytopenias in MDS patients

Myelodysplasia is a clonal disorder which originates from the hematopoietic stem cell. Ineffective hematopoeisis occurs which results in peripheral cytopenias. Anemia is present in the majority of patients at presentation but transfusion dependency usually occurs later in the disease course. Neutropenia is less common but predisposes patients to sometimes severe infections, predominantly of bacterial and fungal origin. Thrombocytopenia is a major cause of morbidity and mortality in patients with MDS. At presentation, 65% of patients with MDS are thrombocytopenic, and hemorrhage is a significant cause of morbidity and mortality in MDS patients[2]. Thrombocytopenia in MDS patients is caused by decreased hematopoietic stem and progenitor cell function, resulting in dysplastic megakaryocytes that cannot produce mature platelets, and consequently undergo inappropriate programmed cell death[3]. The precise molecular mechanisms underlying this process are not well defined, although perturbed signaling through the thrombopoietin receptor *mpl* may contribute to this process[4,5]. The regulation of endogenous TPO production is also not yet fully understood. The major site of production is the liver, and the primary determinant of circulating TPO levels appears to be platelet and megakaryocyte mass, with low numbers resulting in higher than baseline TPO levels (for review see [6]). In MDS, TPO levels are generally significantly increased, in contrast to TPO levels in ITP, which are generally within the normal range or only moderately increased[7] [8].

2.2 Clinical consequences of cytopenias

All patients with myelodysplastic syndromes will develop cytopenias at some stage in their clinical course. Patients with anemia complain of fatigue and may develop dyspnea, palpitations, headache and chest pain. Neutropenia results in recurrent infections which are usually bacterial in origin but fungal infections also occur and can be fatal. The major symptom of thrombocytopenia in MDS patients is bleeding: petechiae of the skin and mucous membranes, epistaxis and gum bleeding. Bleeding can be brisk in the presence of accompanying physical lesions related to the underlying MDS, or treatment with immunosuppression or hypomethylating agents, such as neutropenia-related fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage which is life threatening if not promptly treated. In the largest series of MDS patients reported to date, 20% of patients had hemorrhage as a contributory cause of death, and in 10% of patients hemorrhage was the sole cause of death.[2]

2.3 Management of cytopenias in MDS patients

Management of cytopenias associated with low to Int-1 risk MDS may be supportive or include therapies such as G-CSF +/- erythropoietin stimulating agents, lenalidomide in deletion 5q- patients, immunosuppression therapy, or in refractory cases, hypomethylating agents[9]. Patients with Int-2 and high risk disease are treated with hypomethylating agents and if appropriate, stem cell transplantation[9] Stem cell transplant is the only curative treatment for MDS, but many of these patients are older with co-morbidities which preclude them from receiving a transplant.

The current management of low to int-1 risk MDS patients with cytopenias either untreated or refractory to standard therapy is supportive. Red cell and platelet transfusions may be necessary and intravenous antibiotics are used to treat infections in patients with neutropenia. Patients with a platelet count less than 10,000/μL are routinely transfused to avoid significant bleeding. Due to the short half-life of platelets in the circulation, many MDS patients with severe thrombocytopenia require transfusions as frequently as two times per week. Platelet transfusions are associated with a number of side effects including febrile or allergic transfusion reactions, transmission of bacterial and viral infections, circulatory congestion, transfusion-related acute lung injury and allo-immunization. The possible increased demands on the blood supply in the future may further limit the feasibility of chronic platelets transfusions as therapy for MDS, particularly as the population ages there will be a concomitant increase in the prevalence of this disease. Red cell transfusion is similarly inconvenient for the patient and also has transfusion reactions as potential side effects. Iron overload is a common complication in heavily transfused patients and many require chelation therapy. Red cell alloimmunization may make selection of units difficult with repeated transfusions.

In patients responding to immunosuppression, lenalidomide, or hypomethylating agents, blood counts may improve weeks to months after treatment, allowing discontinuation of transfusions. However, none of these agents are curative and relapse rates are significant[10]. The management of MDS patients with persistent cytopenias remains unsatisfactory and novel therapy approaches are therefore needed.

It has been demonstrated that megakaryocytes in MDS patients exhibit defective signaling through the TPO receptor, *mpl*[4]. Specifically, there is impaired expression of the signaling proteins STAT-3 and STAT-5 after binding of the receptor to TPO[4]. Despite the fact that TPO levels are increased in MDS, we believe there is sufficient justification for a clinical trial testing the hypothesis that supraphysiologic pharmacologic levels of a TPO-R agonist could result in improved platelet production in patients with MDS by overcoming defective *mpl* signaling in MDS megakaryocytes. Despite elevated erythropoietin levels, some patients with MDS respond to combination therapy with erythropoietin and G-CSF[11]. In ITP, despite ongoing platelet production and normal TPO levels, pharmacologic dosing of TPO-R agonists can result in overcoming the impact of autoimmune platelet destruction. It is reasonable to ask whether TPO-R agonists could similarly overcome dysfunctional megakaryopoiesis and dysfunction or loss of more primitive hematopoietic stem and progenitor cells in MDS. Furthermore, we have studied eltrombopag in an ongoing clinical trial in patients with severe aplastic anemia, and thus far, several patients have achieved hematologic improvements in all 3 cell lines (Olnes NEJM 2012). These results suggest that eltrombopag stimulates hematopoiesis in patients with marrow failure and that this agent may be a useful approach in the treatment of any cytopenia in MDS Recently eltrombopag was shown to stimulate colony formation in megakaryocytes isolated from low risk MDS patients *in vitro*[12], and both eltrombopag and the *Mpl* agonist SB-559457 do not exert any proliferative effects on leukemia and lymphoma cell lines, or in primary AML cells grown *in vitro*[13,14,15].

2.4 The Investigational Agent Eltrombopag (Promacta®)

2.4.1 Description of the drug

Eltrombopag (SB-497115-GR, Promacta®), the bis-monoethanolamine salt form, is an orally bioavailable, small molecule 2nd generation thrombopoietin receptor (TPO-R) agonist, developed for the treatment of thrombocytopenia.

2.4.2 Nonclinical pharmacology

Studies conducted in vitro have shown that eltrombopag is an effective agonist binding to *mpl*, the thrombopoietin receptor (TPO-R), to stimulate thrombopoiesis. It binds *mpl* at a position distinct from the ligand binding site, and thus does not compete with TPO for binding to its receptor[16]. In vivo, eltrombopag increases platelet number in the chimpanzee (the only nonclinical species which is pharmacologically responsive to eltrombopag). These findings, coupled with supporting clinical efficacy data, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous thrombopoietin (TPO). Additionally, in vitro electrophysiology studies have been performed and in vivo safety pharmacology studies assessed the effects of eltrombopag on cardiovascular, respiratory and central nervous systems.

2.4.3 Nonclinical pharmacokinetics (distribution, metabolism and excretion in animal models)

Comprehensive nonclinical pharmacokinetic, distribution, metabolism and excretion studies were conducted in the mouse, rat and dog with eltrombopag. Absorption of eltrombopag was low to moderate and plasma clearance was generally low with moderate to long half-lives. Eltrombopag-related material was widely distributed into peripheral tissues in the mouse and rat but the concentrations in most tissues were lower than in the blood. Drug-related material did not extensively penetrate into the central nervous system or the lens of the eye, nor was it selectively retained in melanin containing tissues. There was no evidence of tissue accumulation of drug-related material in mice, including eyes, kidneys and skin. Eltrombopag was highly bound to plasma proteins in nonclinical species as well as in human plasma (>99%), with low association with blood cells. Eltrombopag was the predominant circulating component in all species. Minor metabolites in circulation included products of oxidation or glucuronidation. Eltrombopag was primarily eliminated as unchanged drug in the feces with renal elimination of cleavage products contributing a minor route. Qualitatively, all of the major metabolites of eltrombopag observed in humans were observed in the nonclinical species. In vitro, eltrombopag inhibited cytochrome P450 (CYP) enzymes CYP2C8 and CYP2C9 and several uridine diphosphate glucuronosyl transferase (UGT) enzymes at potentially clinically relevant concentrations. Eltrombopag was neither an inhibitor nor a substrate of human P-glycoprotein (Pgp) and was not a substrate of human organic anion transporting polypeptide (OATP1B1), although it was an inhibitor of this transporter with the potential for such an interaction confirmed clinically.

2.4.4 Nonclinical toxicology

There were no clinically-relevant findings in toxicity studies examining the potential effects of eltrombopag on the cardiovascular, respiratory and central nervous systems. In vitro, eltrombopag was shown to inhibit hERG (*human Ether-à-go-go Related Gene)*, the alpha subunit of a voltage-gated potassium (K^+) channel tail current. In an *in vitro* study using isolated dog Purkinje fibers, eltrombopag was not associated with action potential prolongation, but did cause decreases in the upstroke amplitude, maximum rate of depolarization and action potential durations. In a definitive clinical QTc study with a supratherapeutic dose of eltrombopag, there was no effect on cardiac repolarization.

The toxicity profile of eltrombopag has been defined in a single dose study in dogs and repeat dose toxicity studies of up to 13 weeks in mice, 28 weeks in rats and 52 weeks in dogs. In addition, repeat dose toxicity was assessed in 2 year carcinogenicity studies in mice and rats. The principal nonclinical toxicology findings associated with eltrombopag treatment include:

Cataracts (mice and rats): In vitro phototoxicity (3T3 and CHO cells) was observed. In mice and rats, the development of cataracts was dose- and time-dependent and the rapidly developing lenses of young mice were shown to be more susceptible. Cataract development was not associated with drug accumulation in ocular tissues. No treatment-related ocular abnormalities were evident in dogs given the maximum tolerated dose of 30 mg/kg/day (418 μg.h/mL) for 52 weeks based on detailed ophthalmologic and histologic examinations. There was no evidence of acute photo-ocular toxicity in albino or pigmented rats. An apparent delay in onset and a lower incidence of cataracts in albino mice housed in subdued versus ambient light was observed suggesting that light may contribute to cataract development in young mice. However, there was no evidence of ocular phototoxicity in young albino or pigmented mice given eltrombopag and exposed to repeated doses of solar-simulated ultraviolet radiation (UVR). B6C3F1 mice (a pigmented strain) given eltrombopag with or without UVR exposure appeared to be more susceptible than albino mice to eltrombopag-induced cataractogenesis. However, given that eltrombopag has not been shown to be selectively retained in melanin-containing tissues, this likely represents a strain difference in sensitivity to cataract induction.

Renal toxicity (mice and rats). In mice, renal proximal tubular toxicity was observed following repeated oral administration of eltrombopag in a 2 year carcinogenicity study at 1.4-fold clinical exposure in ITP patients. Renal toxicity was not observed in mice in a 13 week study at a greater exposure (4.5 -fold clinical exposure in ITP patients, respectively) than that achieved at the lowest dose in the 2 year study, suggesting that the renal effects are time-dependent. In rats, an increase in the incidence or severity of spontaneous, age-related chronic progressive nephropathy was observed at a similar exposure level, but not at lower exposures. The relationship of this finding to the renal effects observed in mice is unknown. No renal toxicity was observed following repeated oral administration to rats for 28 weeks or to dogs for 52 weeks at exposures up to 4.5- and 2.9-fold clinical exposure in ITP patients.

Hepatotoxicity (mice, rats and dogs). In mice, rats and dogs, hepatocyte degeneration and/or necrosis, often accompanied by markedly increased serum liver enzymes, was observed following repeated oral administration of eltrombopag at exposures generally associated with morbidity and mortality. In rats and dogs, no treatment-related hepatic effects were observed after 28 or 52 weeks at exposures up to 4.5- or 2.9-fold clinical exposure in ITP patients.

Genotoxicity: The toxic potential of eltrombopag was also assessed in a battery of in vitro and in vivo genetic toxicology studies and the weight of evidence provided by these assessments suggests that eltrombopag does not pose a genotoxic risk in humans.

Carcinogenicity: Eltrombopag was not carcinogenic to mice or rats following 2 year carcinogenicity studies.

Teratogenicity: Eltrombopag was not teratogenic in rats or rabbits and did not affect fertility in male rats or fertility, early embryonic development, embryofetal development, maternal reproductive function, or development of offspring in female rats at non-maternally toxic doses. No effect on embryofetal development was observed in rabbits. At a maternally toxic dose in rats, treatment with eltrombopag was associated with embryolethality, a low incidence of cervical ribs (a non-teratogenic fetal variation) and reduced fetal body weight. In definitive juvenile toxicity studies in rats, eltrombopag was not associated with adverse effects. In vitro, eltrombopag was toxic in the presence of ultraviolet-A (UV-A) radiation, indicating a phototoxic response. However, there was no evidence of cutaneous phototoxicity in hairless mice or ocular phototoxicity in pigmented or albino mice or rats. Eltrombopag also showed evidence of photoclastogenicity in vitro that was

associated with cytotoxic drug concentrations (15 to 29 μg/mL) and high intensity UV exposure [30 minimal erythematous dose (MED)]. However, no evidence of photoclastogenicity was observed at a 2-fold higher concentration (58.4 μg/mL) and UV exposure of ~15 MED. Eltrombopag did not adversely affect immune function in an immunotoxicity study in rats.

2.4.5 Clinical Pharmacology (based on studies done in healthy subjects and subjects with hepatic impairment or renal impairment)

Absorption: Eltrombopag is absorbed with a peak concentration occurring 2 to 6 hours after oral administration. Based on urinary excretion and biotransformation products eliminated in feces, the oral absorption of drug-related material following administration of a single 75 mg solution dose was estimated to be at least 52%. In a clinical study, administration of a single 75 mg-dose of eltrombopag with a polyvalent cation-containing antacid (1,524 mg aluminum hydroxide, 1,425 mg magnesium carbonate, and sodium alginate) decreased plasma eltrombopag AUC0-∞ and Cmax by 70%. The contribution of sodium alginate to this interaction is not known. An open-label, randomized, crossover study was conducted to assess the effect of food on the bioavailability of eltrombopag. A standard high-fat breakfast significantly decreased plasma eltrombopag AUC0-∞ by approximately 59% and Cmax by 65% and delayed tmax by 1 hour. The calcium content of this meal may have also contributed to this decrease in exposure.

Distribution: The concentration of eltrombopag in blood cells is approximately 50-79% of plasma concentrations based on a radiolabel study. In vitro studies suggest that eltrombopag is highly bound to human plasma proteins (>99%). Eltrombopag is not a substrate for p-glycoprotein (Pgp) or OATP1B1.

Metabolism: Absorbed eltrombopag is extensively metabolized, predominantly through pathways including cleavage, oxidation, and conjugation with glucuronic acid, glutathione, or cysteine. In a human radiolabel study, eltrombopag accounted for approximately 64% of plasma radiocarbon AUC0-∞. Metabolites due to glucuronidation and oxidation were also detected. In vitro studies suggest that CYP 1A2 and 2C8 are responsible for the oxidative metabolism of eltrombopag. UGT1A1 and UGT1A3 are responsible for the glucuronidation of eltrombopag.

Elimination: The predominant route of eltrombopag excretion is via feces (59%) and urine (31%). Unchanged eltrombopag in feces accounts for approximately 20% of the dose; unchanged eltrombopag is not detectable in urine. The plasma elimination half-life of eltrombopag is approximately 21 to 32 hours in healthy subjects and 26-35 hours in ITP patients.

Race: Based on both non-compartment analysis and population pharmacokinetic analysis, plasma eltrombopag exposure was approximately 70% higher in some Asian subjects of Japanese, Chinese, Taiwanese, and Korean ancestry (i.e., East Asian) with ITP as compared to non-Asian subjects who were predominantly Caucasian in these trials. In addition, the pharmacodynamic (PD) response to eltrombopag was qualitatively similar in the Asian subjects, but the absolute PD response was somewhat greater. An approximately 40% higher systemic eltrombopag exposure in healthy African- American subjects was noted in at least one clinical pharmacology study. The effect of African-American ethnicity on exposure and related safety and efficacy of eltrombopag has not been established.

Gender: Results from a population pharmacokinetic model suggest that males have a 27% greater apparent eltrombopag clearance than females, after adjustment for the body weight difference.

Hepatic Impairment: Plasma eltrombopag pharmacokinetics in subjects with mild, moderate, and severe hepatic impairment compared to healthy subjects was investigated following administration of a single 50 mg dose of eltrombopag. The degree of hepatic impairment was based on Child-Pugh score. Plasma eltrombopag AUC0-∞ was 41% higher in subjects with mild hepatic impairment, and 80% to 93% higher in subjects with moderate to severe hepatic impairment compared with healthy subjects.

2.4.6 Safety findings from completed and ongoing studies in patients with thrombocytopenia

A comprehensive clinical program was designed to assess the clinical utility of eltrombopag in the treatment of chronic idiopathic thrombocytopenia purpura (ITP). On Nov 20, 2008, the United States Food and Drug Administration (FDA) granted accelerated approval for eltrombopag (Promacta®) for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The approved indication is based on data from two pivotal studies in the short term treatment (TRA100773A and B) and one ongoing long-term treatment study of patients with chronic ITP (EXTEND). Safety data from 462 eltrombopag-treated subjects in 8 completed or ongoing GSK sponsored clinical efficacy studies are as follows:

TRA100773A (chronic ITP Study): A double-blind randomized, placebo-controlled, Phase II, parallel group study designed to investigate the efficacy, safety, tolerability, pharmacokinetics and pharmacodynamics of eltrombopag administered at 30 mg, 50 mg and 75 mg as oral tablets compared with placebo once daily for 6 weeks in 117 subjects with previously treated, chronic ITP.

TRA100773B (chronic ITP study): A double-blind, randomized, placebo-controlled Phase III study to assess the safety and efficacy of 50 mg eltrombopag administered as an oral tablet once daily for up to 6 weeks in 114 subjects who were previously treated for chronic ITP and who had a platelet count of less than 30,000/μL. The key safety and efficacy findings in Studies TRA100773A and TRA100773B are summarized below:

- No dose-dependent pattern of adverse events (AEs) was observed across the eltrombopag 30 mg, 50 mg, and 75 mg treatment groups.
- No clinically meaningful differences in incidence or severity of the most common (≥5%) AEs were observed between subjects treated with eltrombopag 50 mg compared to placebo.
- Similar incidences of serious adverse events (SAEs) (12% and 11%) and discontinuations due to AEs (7% and 5%) were observed in the placebo and eltrombopag 50 mg treatment groups, respectively.
- Increases in hepatobiliary values (ALT, AST, bilirubin, alk phos) were seen in 16/164 subjects (9.7%) in the eltrombopag group (all doses), compared with 5/67 (7.5%) in the placebo group. These elevations in liver aminotransferase were generally asymptomatic and returned to baseline after discontinuation of therapy.
- One case of thromboembolism was observed (platelet count 108,000/uL) in the eltrombopag 50 mg treatment group in a subject who died from sepsis of pulmonary origin.
- Preclinical findings that indicated potential for phototoxicity, cataracts and renal tubular toxicity did not appear to translate to clinical consequences during short-term use.
- Transient decreases in platelet counts to levels below baseline were observed in both treatment groups after eltrombopag treatment ended. However, the decreases in platelet count were not accompanied by a clinically meaningful increase in bleeding symptoms.

TRA105325 (Extend) (chronic ITP Study): An open-label, dose-modification, Phase 3 extension study to evaluate the safety and efficacy of eltrombopag for the treatment of 302 subjects with ITP who were previously enrolled in an eltrombopag trial. Of the 302 subjects enrolled in the study, 186 (62%) achieved a platelet count ≥50 Gi/L in the absence of rescue therapy for ≥50% of on-treatment assessments. Response rate in subjects with and without concomitant ITP medication used as baseline was 54% and 65%, respectively, and in subjects who were or were not splenectomised at baseline was 51% and 68%, respectively. The incidence of any bleeding symptoms (WHO grades 1-4) decreased from 57% at baseline to 16% at Week 52, 19% at Week 104, 12% at Week 156, and 14% at Week 208. Clinically significant bleeding (WHO grades 2-4) decreased from 17% at baseline to 4%, 5%, 0%, and 0% at Weeks 52, 104, 156, and 208, respectively.

TRA108057 (Repeat) (chronic ITP study): An ongoing, Phase II, multi-center, open label single group repeat dose study to evaluate the efficacy, safety and tolerability of repeated, short term administration of eltrombopag initially administered as 50 mg tablets once daily in subjects with previously treated chronic ITP (66 subjects with ongoing enrollment). In general, the results from the ongoing REPEAT and EXTEND studies confirmed the safety and efficacy profile noted in the completed TRA100773A and TRA100773B and are summarized below:

- The incidence of SAEs was 0% and 14% in REPEAT and EXTEND, respectively and discontinuations due to AEs were ≤6% across the 2 studies
- 2/66 (3%) subjects in REPEAT and 8/109 (7%) subjects in EXTEND developed elevations of hepatobiliary laboratory values. The majority of events were asymptomatic and resolved following drug discontinuation.
- The proportion of Asians who had hepatobiliary laboratory abnormalities (transaminases >3x ULN, bilirubin ≥1.5x ULN or ALP ≥1.5x ULN) was 15.8%, 16.7%, and 20.8%, as compared to 10.2%, 7.5%, and 4.5% of White-Caucasian subjects, in TRA100773A, TRA100773B, and EXTEND, respectively. High plasma eltrombopag concentrations were noted in 2 subjects who had ALT and AST elevations (>3x ULN).
- Four eltrombopag treated subjects developed thromboembolic events (4 in EXTEND, none in REPEAT). Although risk factors were present in all subjects, a causal relationship with eltrombopag cannot be ruled out.
- With the exception of the hepatobiliary findings in Asian subjects, no clinically meaningful differences in the safety profile of eltrombopag were found with regard to age, sex and race.

TRA100773A (chronic ITP Study): A double blind randomized, placebo controlled, Phase II, parallel group study designed to investigate the efficacy, safety, tolerability, pharmacokinetics and pharmacodynamics of eltrombopag administered at 30 mg, 50 mg and 75 mg as oral tables compared with placebo once daily for 6 weeks in 117 subjects with previously treated, chronic ITP.

TRA100773B (chronic ITP study): A double-blind, randomized, placebo-controlled Phase III study to assess the safety and efficacy of 50 mg eltrombopag administered as an oral tablet once daily for up to 6 weeks in 114 subjects who were previously treated for chronic ITP and who had a platelet count of less than 30,000/μL.

The primary analysis of this endpoint was performed on a dataset which classified subjects as either responders or non-responders (primary dataset). For this primary analysis of response, only on-therapy platelet counts were included. Responders either achieved a platelet count of ≥50 K/μL

(from a baseline platelet count of <30 K/uL) at the Day 43 Visit, or achieved a platelet count >200 GK/uL and discontinued study medication prior to Day 43; and non-responders either did not achieve a platelet count ≥50 K/μL at Day 43 or discontinued treatment prior to Day 43 for any reason other than a platelet count >200 K/μL. Supportive data analyses were performed using a dataset of all platelet counts during the treatment and follow-up periods, whether or not the subject discontinued treatment prematurely (observed dataset).

The odds of responding were significantly greater for the eltrombopag 50 mg treatment groups compared to placebo in both TRA100773A and TRA100773B (Table 1-). The primary method of analysis was a logistic regression model adjusted for ITP medication use at randomization, splenectomy status and baseline platelet count ≤15 K/μL. Results using observed Data were similar.

Table 1. Primary Endpoint in Studies TRA100773A and TRA100773B

Day 43 Visit	TRA100773A		TRA100773B	
	PBO N=27	50 mg N=27	PBO N=38	50 mg N=74
N	27	27	37[a]	73[a]
Responders, n (%)	3 (11.1)	19 (70.4)	6 (16.2)	43 (58.9)
Odds ratio for Active/placebo Treatments[b]	21.96		9.61	
95% CI	(4.72,102.23)		(3.31, 27.86)	
p-value[c]	<0.001		<0.001	

a. Two subjects, one in each treatment group did not have platelet counts at the Day 43 Visit.
b. The odds ratio indicates the odds of responding to eltrombopag compared to placebo.
c. One-sided for TRA100773A, and two-sided for TRA100773B.

Median Platelet Counts: Median platelet counts in the eltrombopag 50 mg treatment groups in both studies show an elevation of platelet counts as early as Day 8 and continue to rise to Day 15. A slight decrease in the median platelet count was observed after Day 15 in the eltrombopag 50 mg treatment groups in both studies. This decrease is explained by the number of subjects withdrawn after Day 15 from the 50 mg treatment groups due to a platelet response >200 K/μL. The median platelet levels remain elevated (>47 K/μL) throughout daily administration of 50 mg eltrombopag (Days 15-43) in both studies (TRA100773A, Figure 1; TRA100773B, Figure 2).

Figure 1

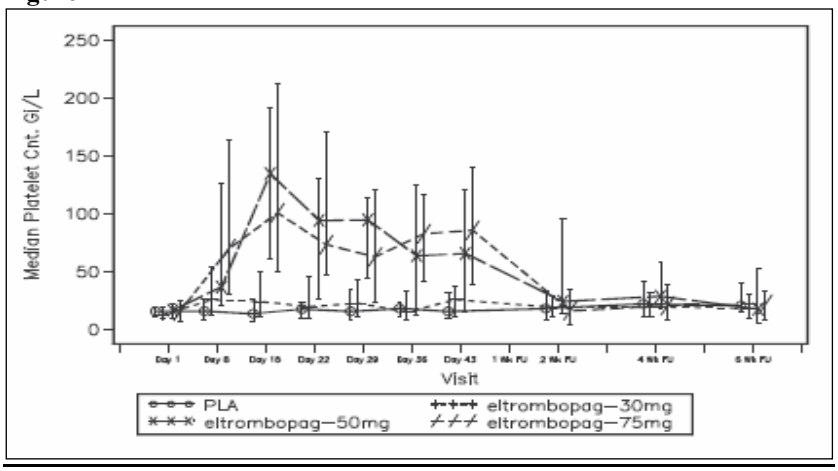
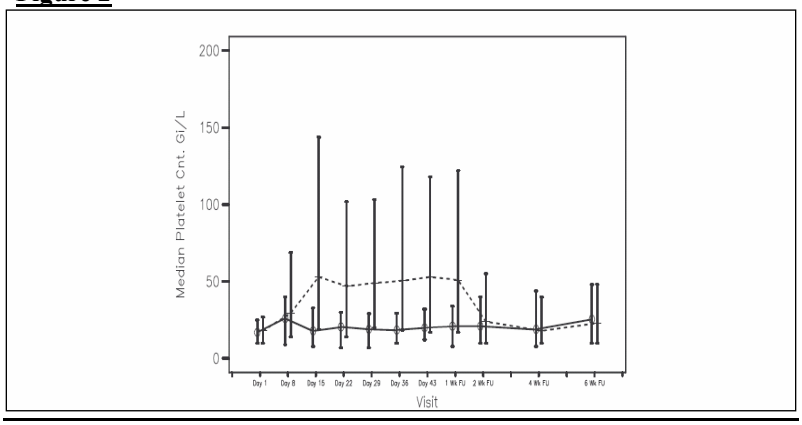


Figure 2



Primary Endpoint by Baseline Disease Characteristics: Data presented in this section are pooled analyses of the TRA100773A and TRA100773B placebo and eltrombopag 50 mg treatment groups. Eltrombopag increased platelet counts after up to 6 weeks of dosing both for subjects who had baseline platelet counts of ≤15 K/μL and for those who had baseline platelet counts >15 K/μL. A higher percentage of subjects in both treatment groups with baseline platelet counts >15 K/uL achieved a platelet count ≥50 K/μL compared to subjects with a baseline platelet count ≤15 K/μL. No significant interaction between response and baseline platelet count status was observed (p=0.443). Analysis of responders at the Day 43 Visit demonstrated that eltrombopag increased platelet counts after up to 6 weeks of dosing for subjects who used ITP medication at randomization and for those who did not. No significant interaction between the response to treatment and the use of ITP medication at randomization was observed (p=0.893).

Analysis of responders at the Day 43 Visit demonstrated that eltrombopag increased platelet counts after up to 6 weeks of dosing for subjects regardless of splenectomy status. The percentage of subjects in the eltrombopag treatment group who achieved a platelet count ≥50 K/μL was similar regardless of splenectomy status. No significant interaction between response and splenectomy status was observed (p=0.661).

Analysis of Bleeding: Results of bleeding signs and symptoms reported via the World Health Organization (WHO) Bleeding Scale during the TRA100773A and TRA100773B are presented.

The WHO Bleeding Scale has 5 grades: Grade 0 - no bleeding; Grade 1 – petechiae; Grade 2 - mild blood loss; Grade 3 - gross blood loss; and Grade 4 - debilitating blood loss. To analyze the data, subjects' assessments were summarized into categories: no bleeding (Grade 0), any bleeding (Grade 1 to Grade 4) and clinically significant bleeding (Grade 2 to Grade 4) (Table 2).

There was a decreased incidence of any bleeding (Grade 1 to Grade 4) on treatment relative to baseline in subjects who received eltrombopag. At the baseline visit, 61%-63% of subjects in each eltrombopag 50 mg treatment group and 56%-66% of subjects in the placebo treatment groups reported any bleeding. At the Day 43 Visit, 50% and 60% of subjects in the placebo treatment groups in TRA100773A and TRA100773B had bleeding compared with 25% in the eltrombopag treatment groups in TRA100773A and 39% in TRA100773B (Table 2).

These data indicate a reduction in the percentage of subjects with any bleeding compared to baseline in the eltrombopag treatment groups. This reduction was not statistically significant in Study TRA100773A. However, in TRA100773B, the odds of any bleeding in the eltrombopag arm were significantly lower than that of placebo at Day 43 (Odds Ratio [OR]=0.27, p=0.029). In addition, a lower proportion of eltrombopag subjects had any bleeding (as indicated by WHO Bleeding Grade 1-4) at any point in time over the course of their treatment (Day 8 up to Day 43) compared to subjects in the placebo group (OR=0.49, p=0.021).

Table 2	**WHO Bleeding Scale Assessment**			
Assessment Visit	**TRA100773A**		**TRA100773B**	
	PBO N=27	**50 mg N=27**	**PBO N=38**	**50 mg N=74**
Day 1, n (%)	27	27	35	70
No bleeding[a]	12 (44.4)	10 (37.0)	12 (34.3)	27 (38.6)
Any bleeding[b]	15 (55.6)	17 (63.0)	23 (65.7)	43 (61.4)
Clinically significant bleeding[c]	3 (11.1)	4 (14.8)	9 (25.7)	15 (21.3)
Day 43 Visit, n (%)	22	16	30	51
No bleeding[a]	11 (50.0)	12 (75.0)	12 (40.0)	31 (60.8)
Any bleeding[b]	11 (50.0)	4 (25.0)	18 (60.0)	20 (39.2)
Clinically significant bleeding[c]	3 (13.6)	1 (6.3)	4 (13.3)	5 (9.8)
Day 57 Visit, n (%)	25	26	34	72
No bleeding[a]	11 (44.0)	14 (53.8)	14 (41.2)	43 (59.7)
Any bleeding[b]	14 (56.0)	12 (46.2)	20 (58.8)	29 (40.3)
Clinically significant bleeding[c]	2 (8.0)	2 (7.7)	6 (17.6)	5 (6.9)

a. WHO Bleeding Scale Grade 0
b WHO Bleeding Scale Grade 1 to Grade 4
c. WHO Bleeding Scale Grade 2 to Grade 4

TRA108057 (Repeat) (chronic ITP study): An ongoing, Phase II, multi-center, open label single group repeat dose study to evaluate the efficacy, safety and tolerability of repeated, short term administration of eltrombopag initially administered as 50 mg tablets once daily in subjects with previously treated chronic ITP (66 subjects with ongoing enrollment). Across all three cycles, the median platelet counts at baseline of each cycle were below 35 K/μL. Elevation in median platelet counts was observed by Day 8 of each cycle, with the median platelet counts of 74, 110 and 102.5 K/μL observed in Cycles 1, 2 and 3, respectively. By Day 15, median platelet counts were 124, 132

and 156 K/μL in each cycle, respectively. One week after discontinuation of eltrombopag, median platelet counts remained >100 K/μL across all three cycles of treatment. Two weeks after discontinuation, platelet counts in each cycle returned to near baseline levels. These results are similar to those from TRA100773A and TRA100773B in which median platelet counts in the eltrombopag treatment groups showed an elevation of platelet counts as early as Day 8 and continued to rise to Day 15, and in which the median platelet levels remain elevated.

2.5 FDA approval

On November 20, 2008 GlaxoSmithKline announced that the United States Food and Drug Administration (FDA) granted accelerated approval for eltrombopag (Promacta®) for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The new drug application for eltrombopag was supported by the largest database of randomized clinical trial information on investigational therapies for chronic ITP patients. The approval of eltrombopag was supported by a unanimous decision by the FDA's Oncology Drugs Advisory Committee (ODAC) on May 30, 2008, in which the panel voted, 16-0 that eltrombopag demonstrated a favorable risk-benefit profile for the short-term treatment of patients with chronic ITP. The indication is based on data from the two pivotal studies (detailed above) in the short-term treatment and one ongoing long-term treatment study of patients with chronic ITP (EXTEND). Eltrombopag is the first oral thrombopoietin (TPO) receptor agonist approved for adult patients with chronic ITP.

2.6 Rationale for dose selection

Eltrombopag 50 mg once daily has been selected as the starting dose for this study because this regimen has been safe and effective in increasing platelet counts in patients with ITP and this was the FDA's recommended starting dose for this patient population. A starting dose of 25 mg once daily in East Asian patients will be used. Modified dosing for subjects of East Asian heritage (i.e., Japanese, Chinese, Taiwanese and Korean) has been implemented for the following reasons. In healthy Japanese subjects, plasma eltrombopag $AUC_{(0-\tau)}$ was approximately 80% higher when compared to non-Japanese healthy subjects who were predominantly Caucasian. Similarly, in patients with ITP, plasma eltrombopag exposure was approximately 70% higher in East Asian (i.e., Japanese, Chinese, Taiwanese and Korean) subjects as compared to non–East Asian subjects who were predominantly Caucasian as higher drug exposure in East-Asian subjects has been observed. After two weeks the dose can be increased by 25 mg per day every 2 weeks in incremental doses up to a maximum dose of 150 mg (East Asians 75 mg) once daily as detailed in the treatment plan (Section 5) based on the following considerations:

- NCT00903422; The effective dose in MDS subjects is unknown.
- In an ongoing open label study (NCT00358540), eltrombopag doses of 75 mg (n=10), 100 mg (n=6) and 150mg (n=2) have also been given to patients receiving adriamycin and ifosfamide (AI) for the treatment of advanced soft tissue sarcoma. Dose escalations to 300 mg are planned in this ongoing study. Eltrombopag is being dosed daily for 5 days before and 5 days after AI chemotherapy starting in Cycle 2. There were 4 patients who were dosed at 100mg. Two of the four had improved numeric platelet counts at the nadir in the cycle that they received eltrombopag compared to the cycle without eltrombopag. All had higher prechemotherapy platelet counts in Cycle 2 (with eltrombopag) compared to Cycle 1 (no eltrombopag). The one subject dose at 150mg did not take the tablets according to instructions.
- In healthy subjects, a clear dose and exposure response was seen for eltrombopag doses of 10 mg to 200 mg once daily for 5 days, with geometric mean $AUC_{(0-\tau)}$ values of 302 μg.h/mL

for the 200 mg once daily regimen. Eltrombopag was well tolerated in healthy subjects at all dose levels.

- In ITP subjects, a dose response was seen for eltrombopag doses of 30 mg to 75 mg once daily, with geometric mean $AUC_{(0-\tau)}$ values of 169 μg.h/mL for the 75 mg once daily regimen. There was no significant difference between the safety profile of ITP subjects receiving 30, 50 or 75 mg of eltrombopag. Eltrombopag has been examined in a placebo-controlled Phase II study (NCT00102726) in 183 cancer patients receiving carboplatin and paclitaxel. Eltrombopag, 50 mg, 75 mg and 100 mg and placebo (1:1:1:1) was dosed for 10 days after carboplatin and paclitaxel administration for up to 8 cycles in this study; eltrombopag was generally well tolerated as described in the Clinical Investigator Brochure (CIB)]. The eltrombopag geometric mean $AUC_{(0-\tau)}$ values of 191 μg.h/mL was observed for subjects at 100 mg dose group. The study results for the 100 mg group demonstrated that there was evidence for increased platelet production seen in all three eltrombopag arms after the nadir, with a gradual rise in platelet counts from Day 8 to 18 of chemotherapy. No apparent safety issues at 100 mg were identified.
- Thrombocytosis is a theoretical risk of eltrombopag treatment when high dosages are administered. Thrombocytosis has been observed in healthy volunteers as well as in subjects with ITP. None of these subjects experienced an AE related to thrombocytosis. The likelihood that MDS patients would develop thrombocytosis, given the underlying pathophysiology of their marrow disease, is likely to be low.
- There is evidence that higher doses of growth factors are required in MDS subjects: the effective erythropoietin (EPO) dose in MDS is several times higher than the dose used in renal anemia [(11)]
- To ensure subject safety, the current study uses a dose escalation scheme in which subjects are exposed to the lowest dose necessary to achieve the desired platelet count target or decrease in bone marrow blast count. Only subjects who have tolerated the previous dose will be considered for the next highest dose, dependent on their last bone marrow blast and platelet count. This approach minimizes potential risks while allowing the subject the maximum potential for benefit.

2.7 Rationale for use of Eltrombopag in MDS

Eltrombopag is not currently approved by the FDA for the treatment of thrombocytopenia due to causes of thrombocytopenia (e.g. myelodysplasia or chemotherapy) other than chronic ITP. Eltrombopag stimulation of the TPO receptor on the surface of hematopoietic cells may theoretically increase the risk for hematologic malignancies and progression of MDS to acute myeloid leukemia (AML).

In the controlled clinical studies in chronic ITP, patients were treated with eltrombopag for a maximum of 6 weeks and during this period no hematologic malignancies were reported. One hematologic malignancy (non- Hodgkin's lymphoma) was reported in the ITP extension study.

A similar theoretical risk exists for MDS patients treated with erythropoietin-stimulating agents. However, giving erythropoietin-stimulating agents (Procrit) and myeloid stimulating agents (G-CSF) are now considered standard of care in MDS. The combination of G-CSF and erythropoietin has recently been reported to improve survival in MDS patients, including patients with RAEB-2 and high risk disease[(17)]. Moreover, in the same study they reported no increase in rate of progression to AML.

Romiplostim (Nplate) is another TPO-agonist approved for refractory ITP that is administered by intravenous or subcutaneous administration. A-trial using romiplostim in MDS patients was recently reported[18]. The trial is a placebo-controlled study combining romiplostim with Vidaza versus Vidaza alone, and they reported that 2 of 27 MDS patients treated with the combination progressed to AML, which not beyond the expected rate of progression[18]. The warning on the label for Nplate® (romiplostim) states that Nplate increases blast cell counts and increases the risk of progression to acute myelogenous leukemia in patients with myelodysplastic syndromes.

While we believe that the theoretical risk of AML progression is present, it is low, and the potential benefit of treating life-threatening cytopenias outweighs this risk. We will carefully monitor our patients for progression to AML with serial bone marrow biopsy/aspirates, and weekly CBCs. If a patient progresses to AML, it will trigger a stopping rule for the trial.

2.8 Rationale for permitting dose interruption:

The effect of dose interruption is unknown in the MDS population. In the pooled data from ITP studies TRA100773A and TRA100773B, a total of 11 subjects (10%) treated with eltrombopag and 6 subjects (9%) treated with placebo had a transient decrease in platelet counts (platelet counts <10 Gi/L and at least 10 Gi/L less than baseline platelet count within 4 weeks of eltrombopag discontinuation); generally, the decreases in platelet counts were not associated with clinically meaningful bleeding events. We anticipate some patients on the current trial will be hospitalized for other disease-related issues such as fever and neutropenia during the study, and may require suspension of the study drug temporarily.

2.9 Rationale for extended access to study medication

In patients with refractory cytopenias due to MDS, there is little evidence for spontaneous recovery. There is also little evidence that cytokine drugs such as erythropoietin, G-CSF, or TPO-R agonists have efficacy sustained beyond the treatment period[20]. As this class of agents is cleared from the circulation and metabolized or excreted, new hematopoietic progenitor cells are being produced in the bone marrow and are not exposed to the drug. The impact on production of end-stage cells with life-spans in the circulation, such as red cells, platelets or neutrophils, therefore does not last more than days to weeks beyond cessation of therapy. GSK study TRA105325 is an open label dose modification extension study evaluating the safety and efficacy of extended therapy of eltrombopag in ITP subjects. As of 2/8/2008, the extent of exposure in this population was as follows: the median daily dose was 50 mg, the median number of days on treatment was 194 days (6.5 months) and the median cumulative dose was 6725 mg. ITP patients have return of their platelet counts to baseline within 1-2 weeks of discontinuation of drug.

We will continue treatment beyond the primary and secondary endpoint at 16 or 20 weeks in the current study, in patients meeting the response criteria. Toxicity and efficacy data will continue to be collected during extended access in order to help identify the secondary endpoints of efficacy, duration of response and toxicities with extended duration of therapy.

We hypothesize, based on interim results in patients on the extension phase of other eltrombopag trials conducted at NHLBI that once hematopoietic stem and primitive progenitor cells are normalized in number by exposure to eltrombopag, this increase in number may be able to maintain more normal hematopoiesis without continued exposure to drug, or with exposure to lower doses of drug. We have written parameters to taper and discontinue eltrombopag in the extension studies targeting the lowest dose or duration able to sustain blood counts in a safe and non-symptomatic range.

2.10 Scientific and Clinical Justification of the Protocol

The current management of MDS is suboptimal. Current therapies include hypomethylating agents, lenalidomide and immunosuppressive therapy [9]. None of these are curative and remissions are short-lived. Patients with MDS relapsed or refractory to standard therapy, and not eligible or suitable for allogeneic stem cell transplantation receive supportive care with transfusions and antibiotics in the setting of infection. Quality of life is severely impacted by the necessity to come to a treatment center as frequently as several times every week to receive transfusions. Thus, decreasing or abrogating the need for transfusions could significantly improve the quality of life and ability to carry out normal daily activities. New treatment modalities are needed for this population.

Thrombopoietin (TPO) is a potent endogenous cytokine and the principal regulator of platelet production. On binding to TPO receptors on megakaryocyte progenitors, TPO initiates a number of signal transduction events to increase the production of mature megakaryocytes and platelets. The non-peptide mimetic eltrombopag, a 2nd generation TPO-agonist, has been shown to increase platelets in healthy subjects and in patients with chronic immune thrombocytopenic purpura (ITP). Eltrombopag is administered orally, well tolerated and does not induce auto-antibodies, in contrast to first-generation TPO-R agonists such as megakaryocyte growth and development factor.

In efficacy studies in subjects with ITP, more than 59% of subjects responded with a clinically meaningful increase in platelet counts, regardless of baseline platelet counts, use of concomitant medication and/or splenectomy status. Eltrombopag induced elevations in platelet counts ≥50 K/μL. Clinically significant bleeding (WHO Bleeding Grades 2 to 4) in the eltrombopag 50 mg treatment groups was nearly one-half that observed in the placebo–treatment groups. Summary data indicate in the 269 subjects with ITP who received at least one dose of eltrombopag (from 30 to 75 mg) in either a short-term study (Studies TRA100773A and TRA100773B) for up to 6 weeks or an ongoing open-label study (Studies TRA105325/EXTEND and TRA108057/REPEAT): A dose-dependent increase in platelet count was observed after 5 to 10 day repeat dosing with eltrombopag. Maximum platelet counts were observed approximately 2 weeks after initiating dosing, and returned to within normal limits within 2 weeks after discontinuation of eltrombopag dosing in healthy adult subjects. Transient decreases in platelet counts to levels below baseline were observed in subjects after eltrombopag treatment cycles in REPEAT. However, the decreases in platelet count were not accompanied by clinically meaningful increases in bleeding symptoms. Consistent response to eltrombopag was observed based upon analysis of the primary endpoint in the REPEAT study. Eighty-eight percent of subjects who responded in Cycle 1, responded again in Cycle 2 or 3, with a similar pharmacodynamic response to eltrombopag and a decrease in bleeding symptoms as observed in Studies TRA100773A and TRA100773B. Efficacy data from the EXTEND study show clinically meaningful continuous platelet count elevations ≥50,000/μL for at least 10 consecutive weeks in the majority of subjects, with 24% achieving continuous elevation of platelet counts >50,000/μL for more than 6 months and a decrease in bleeding symptoms.

Because, severe dysfunction of megakaryocytes is the cause of thrombocytopenia in MDS patients, and because of the efficacy demonstrated in ongoing ITP clinical trials and subsequent FDA approval for use in ITP, we now propose this Phase 2, non-randomized pilot study of eltrombopag in patients with MDS. We hypothesize that the drug will stimulate more robust platelet production from the dysplastic megakaryocyte pool, and also potentially help drive primitive hematopoietic stem and progenitor cells to produce more megakaryocytes. In addition, this agent is likely to produce trilineage responses as it acts at the level of the hematopoietic stem cell. Ancillary studies will address questions such as identification of clinical and laboratory predictors of response, as well as cellular and molecular mechanism of eltrombopag action and the mechanism by which

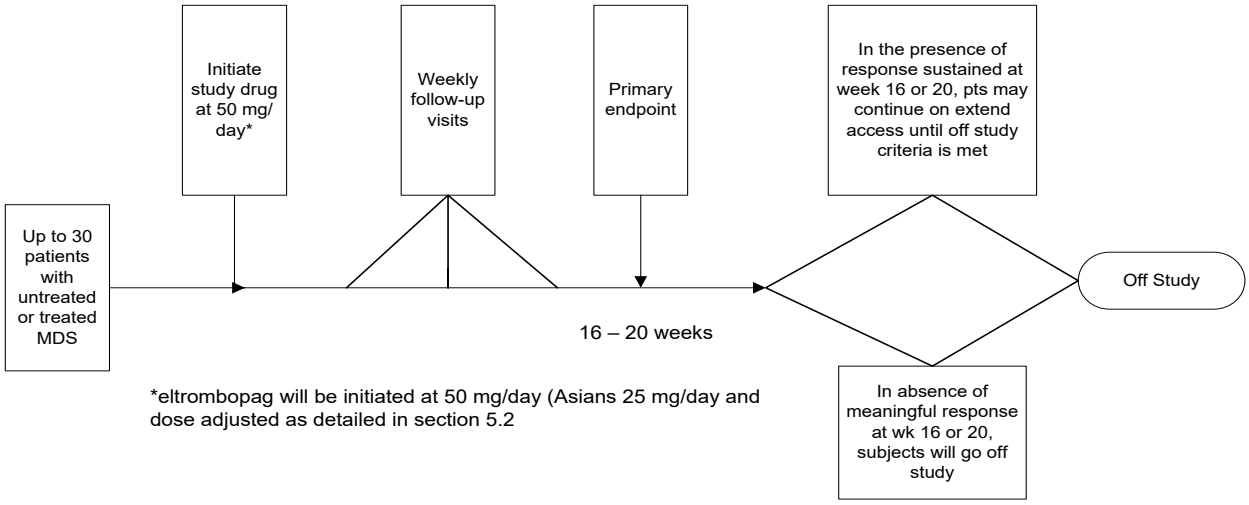
megakaryocyte function is impaired in MDS.

We therefore propose this non-randomized, pilot, phase II study of eltrombopag in low to Int-2 risk MDS patients with cytopenias that persists despite treatment with standard therapies to assess its effectiveness in this population.

3. STUDY DESIGN

The study is designed as a non-randomized, Phase II, dose modification study of the oral TPO-R agonist eltrombopag in subjects with MDS with any cytopenia. The primary endpoint is measured at 16 or 20 weeks. Subjects who cannot tolerate the medication or fail to respond will go off study medication. In the event that a subject is transfused platelets for a count >10,000/μL without a medical indication during the study period, the subject may continue on study drug and the response assessment may be extended for an additional 4 weeks at the discretion of the principal investigator to ensure eligibility to enter the extended access portion of the trial. Subjects with evidence for a clinical response in any lineage at 16 weeks but not yet meeting full primary endpoint response criteria, and who are tolerating investigational treatment, may receive an additional 4 weeks of eltrombopag and be reassessed after 20 weeks. At that time, if they meet primary endpoint response criteria, they will be eligible to enter the extended access part of the study. If they do not meet primary endpoint response criteria, eltrombopag will be discontinued.

Subjects with response may continue study medication (extended access) at the lowest dosage that maintains stable counts or taken off drug for robust response until they meet off study criteria. If subjects are taken off drug for robust response and maintain response, they will be taken off study after 2 years.



4. ELIGIBILITY ASSESSMENT

4.1 Inclusion criteria

4.1.1 Diagnosis of MDS**,** with WHO classification of refractory anemia, refractory cytopenia with unilineage dysplasia (RCUD), RARS, RCMD-RS, or RCMD

4.1.2 IPSS risk scores of low, intermediate-1, or intermediate-2.

4.1.3 Platelet count ≤ 30,000/μL or platelet-transfusion-dependence (requiring at least 4 platelet transfusions in the 8 weeks prior to study entry); OR hemoglobin less than 9.0 gr/dL or red cell

transfusion-dependence (requiring at least 4 units of PRBCs in the eight weeks prior to study entry) OR ANC≤500

4.1.4 Age ≥ 18 years old

4.1.5 Treatment naïve or off all other treatments for MDS (except stable dosing of filgrastim (G-CSF), erythropoietin, and transfusion support) for at least four weeks. G-CSF can be used before, during and after the protocol treatment for subjects with documented neutropenia (<500/Ul) as long as they meet the criteria for other cytopenias as stated above. G-CSF must be held for 3 weeks prior to enrollment bone marrow biopsy and prior to each study assessment bone marrow biopsy, unless clinically indicated to avoid infections per PI discretion.

4.1.6 Adequate liver function, as evidenced by total serum bilirubin ≤ 1.5 times the upper limit of normal (patients with Gilbert's disease are eligible, provided intermittent indirect hyperbilirubinemia, AST or ALT ≤ 5 times the upper limit of normal).

4.1.7 A serum creatinine concentration ≤ 2 x ULN

4.2 Exclusion criteria

4.2.1 WHO classification of chronic myelomonocytic leukemia (CMML), RAEB-1, RAEB-2, AML

4.2.2 Treatment with horse or rabbit ATG or Campath within 6 months of study entry

4.2.3 Subjects with liver cirrhosis including subjects infected with Hepatitis B or C

4.2.4 Subjects with HIV

4.2.5 Infection not adequately responding to appropriate therapy

4.2.6 History of malignancy treated with chemotherapy and cytogenetic abnormalities suggestive of secondary myelodysplasia.

4.2.7 Moribund status or concurrent hepatic, renal, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy

4.2.8 Life expectancy of less than 3 months

4.2.9 Hypersensitivity to eltrombopag or its components

4.2.10 Female subjects who are nursing or pregnant or are unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential

4.2.11 Unable to understand the investigational nature of the study or give informed consent or does not have a legally authorized representative or surrogate that can provide informed consent per section 10.5

5. TREATMENT PLAN

5.1 Administration of study drug (eltrombopag)

Subjects will initiate study drug at 50 mg orally once a day, taken on an empty stomach one hour before or at least two hours after a meal as detailed in section 5.8. Subjects of East Asian ancestry such as Japanese, Chinese, Taiwanese and Korean subjects will initiate study drug at 25 mg orally once a day.

5.2 Dose Adjustments (increases/decreases) of Eltrombopag (See section 2.6)

Patients will be enrolled on study because of anemia and/or thrombocytopenia. The majority of patients are expected to have cytopenias in both cell lines lineages, however some patients will have normal or close to normal counts in one or the other lineage at baseline. Therefore, dose adjustment criteria will vary depending on whether one or both lineages meet inclusion criteria, however dose adjustment will occur via the tables below and continue to the maximum dose until the inclusion criterion lineage or lineages responds, according to the schema set out below, unless toxicity-related stopping or dose reduction lab values occur. Note that patients entering the study based on anemia, with normal or near normal platelet count, will be expected to have an increased

in their platelet count on eltrombopag, but dose escalation for anemia will not be halted unless the platelet count goes above 400,000/μL. For patients entering meeting both anemia and thrombocytopenia eligibility criteria, dose escalation will not stop until both lineages reach the thresholds given below to stay on the current dose, unless platelets go over 400,000/μL or hemoglobin goes over 16.0 gr/dL, in which case drug will be discontinued for one week and the dose decreased by 50%. If the stopping platelet count or hemoglobin is reached regardless of entry criteria eltrombopag will be discontinued for one week and restarted at the next lowest dose.

Dose Adjustments for Patients Entering with Platelets ≤ 30,000/μL or transfusion-dependence:
Depending on response and/or tolerability, the daily dose may be increased or decreased according to the following rules:

Platelet Count ≤ 30,000/μL or transfusion-dependent at baseline	**Dose Adjustment or Response**
<20,000/μL above base line or platelet transfusion requirement has not decreased following at least 2 weeks of eltrombopag	Increase daily dose by 25 mg (25 mg for East Asians) every 2 weeks to 150 mg/day for non East Asians (75mg/day for East Asians).
≥20,000/μL above baseline but ≤200,000/μL following at least 2 weeks of eltrombopag	Keep at current dosage.
>200,000/μL (untransfused) at any time on study	Decrease dosage by 25 mg (25 mg for East Asians) every 2 weeks to lowest dosage that maintains platelet count ≥20,000/μL above baseline.
>400,000/μL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets < 20,000; restart at 50% of current dose.

Dose Adjustments for Patients Entering with Anemia (Hb < 9.0gr/dL or transfusion-dependence)
Depending on response and/or tolerability, the daily dose may be increased or decreased according to the following rules.

Hemoglobin < 9.0 gr/dL or transfusion dependent at baseline	**Dose Adjustment or Response**
hemoglobin rise of < 1.5 g/dL.	Increase daily dose by 25 mg (25 mg for East Asians) every 2 weeks to maximum 150mg/day for non- East Asians (75mg/day for East Asians).
≥1.5g/dL above baseline but ≤13g/dL following at least 2 weeks of-eltrombopag	Keep at current dosage.
>13g/dL (untransfused) at any time on study	Decrease dosage by 50% to lowest dosage that maintains Hb ≥1.5g/dL above baseline.
>15g/uL (untransfused) at any time on study	Discontinue eltrombopag for one week, if Hb<13g/dL restart at 50% of current dose. Phlebotomy may be performed if clinically indicated as determined by the investigator.

If after dose escalation to 150 mg (or a maximum tolerated dose below 150 mg) there is no response within the study period, treatment will be discontinued and subjects will go off study per section 8.6. In the event that a subject is transfused platelets for a count >10,000/μL without a medical indication during the study period, the subject may continue on study drug and the response assessment may extended for an additional 4 weeks at the discretion of the principal investigator.

5.3 Dose delays, modifications or discontinuation for non-hematologic side effects

5.3.1 Infection: Subjects who experience an infection requiring intravenous antibiotics will not have eltrombopag discontinued. If the subject experiences infection severe enough to require vasopressors or intubation, the drug will be withheld until the subject is stable.

5.3.2 Liver function abnormalities: In the event of an increase in the ALT level to > 6 times the ULN, -subjects will return to clinic or have blood tests drawn by their home physician every 3-4 days. If the ALT remains > 6 times the ULN on a second blood test, eltrombopag will be discontinued until ALT is < 5 times the ULN. Eltrombopag will be restarted at a dose level 25mg/day lower than the prior dose. If the toxicity appeared on a dose of 25 mg/day, eltrombopag will be discontinued permanently. If liver tests abnormalities return to and ALT of > 6 times ULN on this reduced dose, eltrombopag will be permanently discontinued.

5.4 Dose delays, modifications or discontinuation for hematologic side effects

5.4.1 Thrombosis/Embolism: Subjects who experience a deep venous thrombosis or a pulmonary embolus, a TIA or stroke, or a myocardial infarction at any time while on eltrombopag will discontinue the drug and go off study. Subjects with platelet counts of > 50,000/μL at the time of thrombosis will be treated with enoxaparin or another appropriate anticoagulant as clinically indicated until the platelet count drops below 20,000/μL with discontinuation of eltrombopag. They will be treated for the thrombotic event as otherwise clinically-indicated.

5.4.2 Peripheral blood smear shows new morphological abnormalities: The presence of persistent morphologic abnormalities (red cell teardrop forms or nucleated red blood cells; immature white blood cells) or the development of significant worsening of anemia or neutropenia while on study will require discontinuation of eltrombopag and performance of a bone marrow examination to assess for development of abnormal fibrosis or progression to RAEB-2 MDS or AML.

5.4.3 Thrombocytosis or erythrocytosis. Patients will have eltrombopag discontinued for one week if platelets increase to >400,000/ul or Hb to > 16 gr/dL, and if counts have fallen below these cutoffs, drug can be restarted at 50% dosage.

5.5 Extended access to study drug

Subjects with response at week 16 or week 20 may continue study medication (extended access) per dosing criteria given in section 5.2.

Since there are currently no guidelines for length of therapy with eltrombopag in this subject population, drug administration for each responding subject may be continued for as long as the study remains open if there is clinical benefit to the subject and no contraindication to the

continuation of therapy or new information available from the literature to suggest that a shorter duration of therapy would be effective.

While on extended access, participants may have drug tapered, stopped, and/or re-started. The criteria for dose adjustments during the extended access phase are:

> Once platelets >50,000/μL, Hb > 10 g/dL in the absence of transfusions, and neutrophils > 1,000 for more than 8 weeks, eltrombopag will be discontinued. If platelets drop to <30,000/μL, Hb to <9g/dL, or ANC to <500/μL the dose can be re-initiated back to the full dose. Once count stabilization again occurs, a slow dose reduction by 50 mg increments can be performed to identify the lowest dose necessary to keep counts over these thresholds.

If subjects have drug stopped on extended access due to robust response, and maintain the response, they will be taken off study after two years.

After the study is closed, the principal investigator will not provide additional doses of eltrombopag. The research team will help arrange follow-up care with the referring physician.

5.6 Permitted Supportive care

- Transfusion supportive care (e.g., blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF or erythropoietin) as clinically indicated. Filgrastim (G-CSF) must be held for 3 weeks prior to enrollment bone marrow biopsy and prior to each study assessment bone marrow biopsy.
- Estrogens or combination oral contraceptive pills (OCPs) as indicated for uterine bleeding.
- Prophylactic antibiotics and antivirals as clinically indicated.
- Romiplostim (N-Plate) or IL-11 (Neumega) should not be administered.

5.7 Concurrent Medications:

Rosuvastatin (Crestor®**):** In vitro studies demonstrated that eltrombopag is not a substrate for the organic anion transporter polypeptide, OATP1B1, but is an inhibitor of this transporter in vitro and as evidenced by increased plasma rosuvastatin levels when eltrombopag and rosuvastatin were co-administered in a clinical drug interaction study. When co-administered with eltrombopag, a reduced dose of rosuvastatin should be considered and careful monitoring should be undertaken. In clinical trials with eltrombopag, a dose reduction of rosuvastatin by 50% was recommended for co-administration of rosuvastatin and eltrombopag. Concomitant administration of eltrombopag and other OATP1B1 substrates (including pravastatin, simvastatin, and lovastatin) should be undertaken with caution.

Inhibitors of Cytochrome p450: In vitro studies demonstrate that CYP1A2 and CYP2C8 are involved in the oxidative metabolism of eltrombopag. Trimethoprim, gemfibrozil, ciprofloxacin, fluvoxamine and other moderate or strong inhibitors of CYPs may therefore theoretically result enhanced activity of eltrombopag, however these interactions have not yet been established in clinical studies. Subjects on cyclosporine requiring prophylaxis against PCP should be given inhaled pentamidine instead of TMP/SULF. NIH SAA patients are routinely placed on pentamidine instead of TMP/SULF for PCP prophylaxis to avoid potential marrow-suppressive effects of TMP/SULF anyway. Other CYP inhibitors can be used concomitantly but with careful attention to possible increased eltrombopag activity and toxicity.

Other medications: Subjects may continue on any of the medications that they were prescribed prior to study enrollment for co-morbid conditions, and standard anti-infectious prophylaxis medications including valacyclovir, and voriconazole.

5.8 Instructions to subjects

Timing in relation to food: Administration of a single 50 mg-dose of eltrombopag with a standard high-calorie, high-fat breakfast that included dairy products reduced plasma eltrombopag $AUC_{[0-\infty]}$ by 59% (90% CI: 54%, 64%) and C_{max} by 65% (90% CI: 59%, 70%). Food low in calcium [<50 mg calcium] including fruit, lean ham, beef, and unfortified (no added calcium, magnesium, iron) fruit juice, unfortified soy milk, and unfortified grain did not significantly impact plasma eltrombopag exposure, regardless of calorie and fat content. Subjects will be advised to take eltrombopag on an empty stomach (1 hour before or at least 2 hours after a meal).

Timing in relation to antacids: Eltrombopag chelates with polyvalent cations such as aluminum, calcium, iron, magnesium, selenium, and zinc. Antacids, dairy products and other products containing polyvalent cations such as mineral supplements should not be administered less than 4 hours before, or after eltrombopag dosing to avoid significant reduction in eltrombopag absorption.

Vigorous activities: should be avoided, as mild trauma could result in bleeding.

6. CLINICAL MONITORING

6.1 Pre-study Evaluation

Patients will be screened for participation by signing consent to participate in a NHLBI screening protocol. Their eligibility to participate in this protocol will be determined based on the Inclusion and Exclusion criteria (Section 4) and data collected on the screening protocol. The time between determination of eligibility for this protocol and signing informed consent for this protocol should be 4 weeks or less.

Baseline status will be evaluated as follows:

- Medical History and physical examination
- Concurrent medication review
- Baseline assessments (done at screening or diagnostic workup, not repeated on study)
  - Folate level
  - B12 level
  - Iron panel (ferritin, transferrin, % saturation)
- Baseline laboratory studies (evaluations designated with an * must be repeated within 72 hours of the first dose of study drug, all others within 6 months of study entry)
  - Complete blood count with differential*
  - Reticulocyte count*
  - Acute care, mineral and hepatic panels, CK, LDH, total protein and uric acid*
  - Pregnancy test (urine or blood HCG in women of child bearing potential)*
  - Thyroid function tests
  - Peripheral blood smear
  - Viral serologies for HIV, hepatitis B, C, HSV, EBV and CMV
  - HLA typing (if not already available)
  - DAT (direct antiglobulin test)
  - Type and screen (as clinically indicated)

Flow cytometry of the peripheral blood for GPI-cells
Lymphocyte phenotyping (TBNK flow cytometry)

- Bone marrow aspirate and biopsy with reticulin and collagen fiber staining and cytogenetic analysis (morphology, cellularity, percentage of blast cells on aspirate), flow cytometry (at clinician discretion),) within four months of first dose of study drug

6.2 Monitoring study drug initiation through Week 16 or 20

Subjects will be monitored weekly as long as they remain on study drug through week 16. At a minimum, subjects must be evaluated at the NIH Clinical Center at week 16 (+/- 4 days). If subjects are kept on drug an additional 4 weeks to assess response, they must be evaluated at the NIH clinical center at week 20 (+/- 4 days). *The week 20 evaluations will only need to be completed for subjects that are kept on drug an additional 4 weeks after week 16 to assess response.

Subjects must have weekly blood tests at the NIH or drawn by their referring health care provider. If subjects are to be followed at home for interim visits, progress notes and laboratory results from their health care provider and laboratory must be faxed to the study research nurse, at 301- 402-3088. The following assessments will be done:

- Clinical assessment (weeks 4, 8,12, 16, and 20* +/- 4 days, if seen at the NIH CC only)
- Concurrent medication review (with each clinical assessment)
- CBC with differential (weekly +/- 4 days)
- Acute care, mineral and hepatic panels, CK, LDH, total protein and uric acid (weekly +/- 4 days) (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine clearance, total bilirubin)
- Reticulocyte count (weekly +/- 4 days) (If seen at home, only if available)
- DAT (direct antiglobulin test) (as clinically indicated)
- Type and screen (as clinically indicated)
- Pregnancy test (urine or blood HCG) in woman of childbearing potential (4, 8, 12, 16, and 20* weeks +/- 4 days)
- Bone marrow aspirate and biopsy with reticulin and collagen fiber staining and cytogenetic analysis at primary end point (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis by PCR) (week 16 +/- 4 days or if clinical indicated)
- Flow cytometry of bone marrow aspirate (week 16 and 20* +/- 4 days) (at clinician discretion)
- Flow cytometry of the peripheral blood for GPI-cells
- Lymphocyte phenotyping (TBNK flow cytometry 16 and 20* weeks +/- 4 days)

6.3 Monitoring during extended access

Responding subjects who opt to remain on study drug (extended access) will be monitored every 1-3 months so long as they remain on study drug. At a minimum, subjects must be evaluated at the NIH Clinical Center every 12 months (+/-30 days) for up to 5 years. After 5 years, subjects will return to the NIH every other year with phone contact recorded in the medical record during the alternating year in lieu of a visit to the NIH. Subjects may be seen for interim visits at the NIH or at their referring home health care provider. Per the principal investigator's discretion, subjects will be seen by the healthcare provider for a clinical assessment every 1 – 3 months while on extended access, but will continue to have laboratory monitoring performed per the below outline. Subjects that are taken off drug due to response, but remain on study, will have the below procedures performed. Subjects who have had drug discontinued because of robust counts

as per section 5.5 will be followed for a period of 2 years unless drug is restarted as permitted in section 5.5. At the clinical investigator's discretion, participants may be evaluated more frequently if medically indicated based on disease status. If subjects are to be followed at home, progress notes and laboratory results from the home health care provider and laboratory must be faxed to the study research nurse, at 301-402- 3088.

- Interim clinical assessment, if seen at NIH
- Concurrent medication review (with each NIH clinical assessment)
- CBC with differential (every 1 – 3 months +/- 30 days)
- Acute care, mineral and hepatic panels, CK, LDH, total protein and uric acid (every 1 – 3 months +/- 30 days) (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin)
- Reticulocyte count (every 1 – 3 months +/- 30 days) (If seen at home, only if available)
- Pregnancy test (urine or blood HCG) in woman of childbearing potential (every 1 – 3 months +/- 30 days)
- DAT (direct antiglobulin test) (as clinically indicated)
- Type and screen (as clinically indicated)
- Bone marrow examination with reticulin and collagen staining and aspiration with cytogenetics and flow cytometry (flow cytometry at clinician discretion) (with each NIH clinical assessment)
- Flow cytometry of peripheral blood for GPI (with each NIH clinical assessment)
- Lymphocyte phenotyping (TBNK flow cytometry, with each NIH clinical assessment)

6.4 Off study assessment four weeks and 6 months after last dose of study drug.

Subjects who go off study drug due for any reason listed in section 8.6 will be monitored according to the following schedule. At a minimum, subjects must be evaluated at the NIH Clinical Center at 1 month (+/- 1 week) and 6 months (+/- 2 weeks) after the last dose of study medication. The following studies will be performed:

- Clinical Assessment (1 and 6 months)
- CBC with differential (1 and 6 months at NIH and weeks 1, 2 and 3 at home doctor or NIH)
- Acute care, mineral and hepatic panels, CK, LDH, total protein and uric acid (1 and 6 months at NIH and weeks 1, 2 and 3 at home doctor or NIH)
- Reticulocyte count (1 and 6 months at NIH and weeks 1, 2 and 3 at home doctor or NIH)
- Peripheral blood smear (1 and 6 months at NIH and monthly at home doctor if available till seen at NIH)
- Coagulation and thrombosis screens (PT, PTT) (1 month)
- Bone marrow biopsy with reticulin and collagen staining and aspiration with cytogenetics and flow cytometry (flow cytometry at clinician discretion) (6 months)
- Flow cytometry of peripheral blood for GPI (6 months)
- Lymphocyte phenotyping (TBNK flow cytometry, six months +/- 1 week)
- DAT (direct antiglobulin test) at NIH (1 and 6 months, as clinically indicated)
- Type and screen at NIH (1 and 6 months, as clinically indicated)

7. ANCILLARY LABORATORY RESEARCH STUDIES

7.1 Collection of samples

During the course of participating on this study, an additional 10 cc of blood (NIH visits only) and 5 cc of bone marrow aspirate each time a subject has a bone marrow examination may be

requested.

7.2 Intended use

These specimens will not be read by a pathologist nor be used for diagnostic purposes. Studies will not be used in assessing the primary endpoint but will be undertaken for descriptive or exploratory ancillary research. The following laboratory research studies may be done and if done, may be correlated with the presence or absence of response:

- Hematopoietic progenitor colony formation and long term-culture-initiating cell assays
- Assay for cytokines/chemokines and their receptors
- Flow cytometry for hematopoietic progenitor cell phenotyping
- Serum (or plasma) and cells for DNA/RNA extraction, protein analysis, apoptosis assessment
- Additional studies which are approved by the NIH IRB and listed in Appendix D of the protocol may be done on stored samples.
- Thrombopoietin (TPO) level

7.3 Tracking

Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use requires an executed Material Transfer Agreement (MTA) and may require IRB approval if results will be returned and re-identified.

7.4 Storage

Research samples will be stored with patient identifiers in the secure laboratory of the principal investigator. Research samples will be stored and tracked in accordance with the NHLBI DIR BSI Policy.

7.5 End of study procedures

Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

7.6 Loss or destruction of samples

Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

8. BIOSTATISTICAL CONSIDERATIONS

8.1 Objectives

Primary objective is to assess the efficacy of eltrombopag in low to Int-2 risk MDS subjects with cytopenias. Safety of eltrombopag in this subject population will be assessed concurrently.

Secondary objectives include the toxicity profile of extended treatment with eltrombopag (treatment longer than 4 months), reduction in incidence and severity of bleeding episodes, erythroid and neutrophil response, and response following extended access to study drug (treatment longer than 4 months).

Criteria for response to the primary endpoint: A treatment response will be any increase in a cytopenia in the lineage that fulfilled eligibility criteria for enrollment, which is defined as follows. Platelet response is defined as platelet count increases to 20,000/μL above baseline at 16 or 20 weeks, or stable platelet counts with transfusion independence for a minimum of 8 weeks in subjects who were previously transfusion dependent. In the event that a subject is transfused platelets for a count >10,000/μL without a medical indication during the study period, the subject may continue on study drug and the response assessment may extended for an additional 4 weeks, to week 20, at the discretion of the principal investigator. Subject being evaluated for platelet, erythroid, or neutrophil responses, may have the response assessment extended an additional 4 weeks, to week 20, at the discretion of the principal investigator. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment -compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L. Subjects with a platelet, erythroid, and/or neutrophil response at 16 to 20 weeks may continue study medication (extended access) until they meet an off study criteria. Subjects with erythroid, or neutrophil response at 16 weeks may continue study medication for an additional 4 weeks (to ensure eligibility) prior to being consented for entry into the extended access part of the trial. Patients may remain on the extended access until they met an off study criteria.

Transfusion Units: Single donor apheresis platelets have become the primary source of platelets in the US. Therefore, one transfused, single donor platelet apheresis product is considered 1 unit for protocol purposes. In the rare case that a patient received pooled platelet products, each completed platelet transfusion (1 bag) independent of donor units pooled, will be counted as 1 unit transfused.

8.2 Endpoints

The ***primary endpoint*** will be the portion of drug responders as defined by changes in the platelet count and/or platelet transfusion requirements or the proportion of subjects who meet erythroid response or neutrophil response criteria[1] Platelet *response is defined as platelet count increases to 20,000/μL above baseline at 16 to 20 weeks, or stable platelet counts* with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment - compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L. Subjects with a platelet, erythroid, and/or neutrophil response at 16 to 20 weeks may continue study medication (extended access) until they meet an off study criteria. Subjects with erythroid, or neutrophil response at 16 weeks may continue study medication for an additional 4 weeks (to ensure eligibility) prior to being consented for entry into the extended access part of the trial. Patients may remain on the extended access until they met an off study criteria.

The toxicity profile will be measured using the CTCAE Version 4.0 criteria.

Secondary endpoints will include incidence of grade 2 or higher bleeding events as measured by

CTCAE v. 4.0; changes in serum thrombopoietin level, measured at16 to 20 weeks; progression to higher risk MDS as measured by IWG criteria[1]; and discontinuation of drug in robust responders enrolled in extended access.

8.3 Sample Size

Because the efficacy of eltrombopag in this subject population is unknown, we would like to reject the treatment as quickly as possible with a small number of subjects if the treatment is not effective. In our past experience with this patient population, a novel treatment with a response rate 30% or more would generally warrant further investigation, while the treatment would be rendered non-effective if it has a response rate of 10% or less. We will use Simon's Two-Stage Minimax Design ([21]) with a response probability of 10% or less to terminate the treatment and the hypothesized actual response probability of 30% or more. The sample size is determined by testing the null hypothesis H_0: $p \leq 10\%$ versus the alternative H_1: $p \geq 30\%$ at a significance level of 0.05 and a power of 0.8. At the first stage, 15 subjects will be accrued. Further accrual will be halted until the all 15 subject reach the 16 to 20 weeks response time point. The null hypothesis will not be rejected if no more than 1 subject responds to the treatment within16 to 20 weeks. If 2 or more subjects respond to the treatment within 16 to 20 weeks at the first stage, then an additional 10 subjects will be accrued, bringing the total number of subjects to n = 25. The null hypothesis of $p \leq 10\%$ will be accepted if the total number of responders within 4 months is 5 or less.

Subjects who discontinue the study drug prematurely (before20 weeks): Platelet count measurement will be attempted even if a subject discontinues study drug. Important covariates for this study include age, karyotype, IPSS score, duration of platelet transfusion dependence, marrow cellularity and TPO level. The Cox proportional hazard model will be applied to time-to-event variables, such as time-to-death, time-to-relapse and time-to-progression. Since the sample size is relatively small, we will mostly consider Cox models with a single continuous or categorical covariate to ensure reasonable statistical accuracy. Such analysis is exploratory in nature and aimed at generating statistical hypothesis for future studies. Subjects who withdraw from the study for reasons other than lack of efficacy or toxicity (rendering platelet count missing) may be replaced. All other subjects should be evaluable for efficacy (i.e. all subjects who withdraw for treatment related concerns prior to end of week 16 or 20 assessment for toxicity or failure to respond will be counted as non-responders at the primary time points (intent to treat paradigm)). Based on the assumed dropout rate of 15-20% and the goal of having 25 evaluable subjects, 5 additional subjects may be enrolled.

8.4 Statistical Methods

The time to responses will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate and Cox regression type analysis for covariates. Important covariates for this study include age, karyotype, IPSS score, duration of platelet transfusion dependence, marrow cellularity and TPO level. The Cox proportional hazard model will be applied to time-to-event variables, such as time-to-death, time-to-relapse and time-to-progression. Since the sample size is relatively small, we will mostly consider Cox models with a single continuous or categorical covariate to ensure reasonable statistical accuracy. Such analysis is exploratory in nature and aimed at generating statistical hypothesis for future studies. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their corresponding 95% confidence intervals. Methods based on univariate and/or multivariable regression, analysis of variance, and logistic regression with the above covariates will also be employed if deemed appropriate. For example, a logistic regression model will be used to evaluate which patients will have a higher probability of response at 16 to 20 weeks or at an extended time period. Again, since the sample size is small, it is likely that categorical covariates

specified by certain threshold values will be employed, and exploratory data analysis techniques, such as classification and regression trees, will be used to evaluate the threshold choices.

The 5 patients accrued to date will not be included in the primary analysis of the Two-Stage Minimax Design, but will be included as an exploratory analysis for the endpoints considered. Amendment L submitted on 2/13/2013, included revisions to the eligibility criteria to include MDS patients with anemia and revisions to the primary response criteria to also include erythroid and neutrophil responses (which prior to Amendment L were secondary response criteria). Given the significant changes to the protocol it was decided it would not be appropriate to include the first 5 patients in the primary analysis. The accrual ceiling for the protocol is 30 subjects, so this will allow for the goal of having 25 evaluable subjects. The review of Amendment of L included both a Scientific and Statistical review, both of which were approved, and the IRB approved Amendment L on 5/13/2013.

8.5 Study Stopping Rules

The study will be monitored to ensure that the occurrence of a specified set of treatment related serious adverse events (TRSAEs) that occur during the treatment period does not substantially exceed an anticipated rate. The following specified TRSAEs determined to be probably or definitely related to eltrombopag will be considered for early stopping of the study:

1. Death
2. Any Grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities
3. Grade IV thrombosis/embolism
4. Progression to acute myeloid leukemia by WHO criteria (20% or greater bone marrow or peripheral blood blasts), or other marrow morphology changes deemed concerning by the investigators
5. Increase in reticulin fibrosis grade by 3 points above baseline

We anticipate the rate of these specified TRSAEs within the 16 to 20 weeks study period to be 20% or less. Following Geller et al. (Advances in Clinical Trial Biostatistics, 2004), our stopping rule is determined by a Bayesian approach. The stopping boundary for an experiment is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAE's exceeds this benchmark rate of 20% is at least 90%. We take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (0.6, 2.4)$. The parameter are chosen so that the mean $\alpha / (\alpha + \beta) = 0.2$ as the expected proportion of specified TRSAE's and the sum $\alpha + \beta = 3$ as the "worth" we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is 3/30=10% of the weight we will place on the results of the new study. Hence when we make decisions about stopping the study, the data from this study will dominate over the prior opinion. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 or more subjects have developed a TRSAE. The following table summarizes the threshold numbers for stopping the study.

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 6	3
≤ 9	4
≤ 13	5
≤ 17	6

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 21	7
≤ 25	8

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 25 independent Bernoulli trials, each had a probability p for having TRSAE and q=1-p for not having TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for p:

Probability of TRSAE = p	0.10	0.20	0.25	0.30	0.40
Proportion of Stopped Studies	2.4%	22.7%	41.5%	61.5%	89.7%
Average number of subjects	24.6	21.8	19.3	16.4	11.0
Average number TRSAEs	2.5	4.4	4.8	4.9	4.4

These results suggest that our stopping rule has a low probability stopping a study when the proportion of specified TRSAE is below the benchmark value of 20%, and the probability of stopping a study is high when the true proportion of TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rule has satisfactory statistical properties.

8.6 Off Study Criteria

8.6.1 Per Subject choice: Subjects may withdraw from study at their request. The risks of withdrawing will be discussed, as will alternative treatment options. Those subjects who choose to withdraw while taking eltrombopag will be strongly encouraged to continue to have labs monitored until he/she initiates alternative therapy.

8.6.2 Per principal investigator decision: Should any of the following adverse events occur during the 16 to 20 week study period, or in the extension treatment arm in responders, eltrombopag will be discontinued. The subject will be followed until resolution of the event. Labs will be monitored through 30 days off study drug time point or until he/she initiates alternative disease directed therapy at which time the subject's participation on this study will be considered complete and the subject will go off study.

- Intolerance of eltrombopag not resolved by dose reduction
- Life threatening acute hypersensitivity reaction
- Thrombosis/embolism (DVT, PE, stroke or TIA, myocardial infarction) other than central line thrombosis
- Persistent hepatotoxicity as defined in section 5.3.2
- New or worsening morphological abnormalities or cytopenia(s) (see section 5.4.2)
- No treatment response by week 16, (20 weeks at P.I. discretion)
- Any Grade IV toxicity considered related to the study medication excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities
- Significant progression of disease or a concomitant condition that would make the subject ineligible for further protocol participation

- Pregnancy or unwillingness to use acceptable forms of contraception
- Initiation of non-protocol therapy for MDS
- Non-compliance with protocol
- Lost to follow-up
- Study completion

Once off study (either by per subject choice or per PI decision), subjects will be referred back to his or her referring physician or consented to the NHLBI evaluation and treatment protocol (94-H-0010) for consideration for standard therapy or evaluation for eligibility for another Branch protocol, depending on what is considered to be in the best interest of the subject.

9 DATA AND SAFETY MONITORING

9.1 Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Neal S. Young, M.D.

NIH Intramural IRB. Accrual and safety data will be monitored and reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient consent will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to Title 45 CFR 46. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects or data and sample analysis is ongoing.

NHLBI DSMB*:* The NHLBI Data Safety and Monitoring Board will review the protocol at 6 to 12 month intervals, as determined by the DSMB. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

FDA: An annual progress report, protocol amendments, and changes in the status of the protocol will be forwarded to the FDA using the following address:

Division of Drug Oncology Products, Office of Drug Products
Center for Drug Evaluation and Research, FDA
5901 B Ammendale Road, Beltsville, MD 20705-1266
301-796-2192

9.2 Event Characterization and Reporting

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance.

The principal investigator will review all events (AEs, protocol deviations, UPs, SAEs) to determine the seriousness, expectedness, and reportability of the event. As required and/or needed, the principal investigator will review the events with the Sponsor to make the final determination of seriousness and reportability.

9.2.1 Definitions

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all of the following criteria:

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. related or possibly related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. **Serious non-compliance**: Non-compliance that:
    a. Increases risks, or causes harm, to participants.
    b. Decreases potential benefits to participants.
    c. Compromises the integrity of the NIH HRPP.
    d. Invalidates the study data.

2. **Continuing non-compliance**: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. **Minor (non-serious) non-compliance:** Non-compliance that, is neither serious nor continuing.

9.2.2 Adverse Events Management:

All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. The AEs will be graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

Eltrombopag has known and unknown toxicity profiles, thus, any observed or volunteered adverse events that are as listed in the Package Insert and/or Investigator's Brochure will not be reported unless (1) the adverse event was not present at baseline exam; (2) the adverse event is previously unknown (not on the label); (3) the adverse event is more severe than on the label; (4) the frequency of the adverse events increases above the listed frequency; or (5) meets the criteria for a serious adverse event.

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will be collected to include any change from laboratory assessments done prior to first dose of eltrombopag that result in a progression to a grade 3 or 4 laboratory toxicity and/or are characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

In view of the underlying illness (bone marrow failure), many patients may enter the study with compromised hematologic indices that would be classified as toxicities. Therefore, we will look

at the relative decline of hematologic parameters and length of time to recovery to the patient's baseline as more significant than absolute values of these parameters. The laboratory toxicities will be graded by severity utilizing CTCAE version 4.0 or as mild, moderate, severe or life threatening according to the tables below:

Attribution of Adverse Events:

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment		
1	**Not related**	This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)
2	**Unlikely (must have two)**	In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug. An adverse event may be considered unlikely if or when: 1. It does not follow a reasonable temporal sequence from administration of the test drug. 2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It does not follow a known pattern of response to the test drug. 4. It does not reappear or worsen when the drug is re-administered.
3	**Possibly (must have two)**	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known pattern of response to the test drug.
4	**Probably (must have three)**	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug. An adverse event may be considered probably related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia). 4. It follows a known pattern of response to the test drug.
5	**Definitely (must have all)**	This category applies to those adverse events which, the Investigator feels are incontrovertibly related to test drug. An adverse event may be assigned an attribution of definitely related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug. 2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: this is not to be construed as requiring re-exposure of the subject, however, a category of definitely related can only be used when a recurrence is observed.) 4. It follows a known pattern of response to the test drug.

Grading of Adverse events:

Grade	Severity	Description
1	Mild	asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
3	Severe or medically significant but not immediately life-threatening	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden)
4	Life-threatening consequences	urgent intervention indicated.
5	Death	Death related to AE.

Duration of adverse event collecting and reporting: Thirty days after the last dose of study drug, adverse event reporting will be limited to those events considered possibly, probably, or definitely related to study drug.

9.2.3 Serious Adverse Events Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clearly not related to the research).

Treatment related SAEs (TRSAEs) are those attributed as definitely, probably, or possibly. As detailed in section 8.5 that will be monitored and considered for early stopping the study according to statistically determined criteria include death and any grade 4 toxicity considered to be probably or definitely related to study medication. John Tisdale, MD, will serve as the independent monitor who reviews the attribution of TRSAEs.

Hospitalizations for administrative issues (to receive a transfusion) or upgrading to ICU for routine monitoring will not be reported as an SAE.

Duration of Serious Adverse Event collecting and reporting: The collection of SAEs will begin on the first day of initiation of the study drug and will continue along as the subject is on study. SAE reporting will continue as long as the subject remains on study.

9.2.4 Reporting Events

Principal Investigator*:* All events will be reported to Principal Investigator of this study

Neal S. Young, M.D., NHLBI, NIH, Clinical Center
10 Center Dr. Building 10, Room CRC 3-5142 Bethesda, MD 20892-1452
Tel: 301-496-5093
E-mail: youngns@nhlbi.nih.gov

9.2.4.1 Reporting Timeframes to Clinical Director, and/or NIH Intramural IRB

Serious Events
Reports to the IRB and CD: The PI must report Serious UPs, and Serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event via the NIH Problem Report Form.

Reports to the CD: The PI must report all SAEs that do not meet the definition of UP to the CD not more than 14 days after the PI first learns of the event.

Non-serious Events
Reports to the IRB and CD: The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event via the NIH Problem Report Form.

Deaths
The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

9.2.4.2 At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs
- All AEs (except for those granted a waiver of reporting)
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above and summarized at the time of continuing review.

Exclusions to data reporting:
The following Adverse Events will be captured only in the source documents and will not be reported to the IRB at the time of continuing review.

- Laboratory values that do not meet the definition of AE listed in Section 9.2.2.
- All grade 1 events listed as expected in the investigator's brochure, package insert, and/or anticipated events.
- In view of the underlying illness, MDS, all patients will enter the study with abnormally low blood counts that would meet criteria as grade 3 or more commonly grade 4 toxicity, and requiring frequent platelet and/or red cell transfusions, and thus AEs regarding hematologic lab values including thrombocytopenia or platelet-transfusion dependence, anemia or red cell transfusion dependence, neutropenia, lymphopenia, or leukopenia will not be evaluable. Thus, we will collect hematologic laboratory values in the subject's source documents, but will not record or report these abnormalities as adverse events.

In addition, the following non-hematologic AEs will be captured only in the source documents and will not be reported to the IRB at the time of continuing review:

- Because eltrombopag (Promacta®) is an FDA approved drug with known toxicity profiles, any observed or volunteered adverse events that are listed on the package insert will not be reported unless (1) the adverse event is previously unknown (not on the package insert); (2) the adverse event is more severe than on the package insert; or (3) meets the criteria for a serious adverse event. The collection of AEs information will begin on the first day of initiation of therapy.

9.2.4.3 NHLBI DSMB Reporting:
Reports of serious adverse events that are unexpected and thought to be related to the experimental drug will also be forwarded as soon as possible but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse event to the Data and Safety Monitoring Board (DSMB). A summary of events will be included in DSMB reports for review by the DSMB.

9.2.4.4 Sponsor and FDA Reporting

IND # 105,207
Sponsor: NHLBI OCD

A summary of all SAEs, non-serious AEs, and other events will be recorded and submitted to the Sponsor and FDA in annual progress reports (21 CFR 312.64(b)).

The PI will report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The Sponsor (or designee) will determine the reportability of the event to the FDA and IND safety report will be submitted to the FDA as required. The PI or designee will report SAEs to the Sponsor within 24 to 72 hours of discover.

IND Safety Reports to the FDA (Refer to 21 CFR 312.32)
The sponsor must notify FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. The sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

15-day reporting
The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group

The sponsor must submit each IND safety report in a narrative format or on FDA Form 3500A.

FDA contact:

Division of Drug Oncology Products, Office of Drug Product
CDER Therapeutic Biological Products
Document Control Center
Center for Drug Evaluation and Research, Food and Drug Administration
5901 B Ammendale Road, Beltsville, MD 20705-1266
(301) 796-2192

9.2.4.4 Drug Manufacture Reporting

As needed, the IND Sponsor (or designee) will forward IND safety reports and SAEs to Novartis.

Kelly Haines
Clinical Research Manager
Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080
USA

Phone +1 862 778 3640
Mobile +1 201 452 8479
Fax +1 973 781 2116
kelly.haines@novartis.com

9.3 Reporting of pregnancy

Subjects who become pregnant during the study should discontinue the study immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her designee, will submit pregnancy information to the DSMB, IRB, FDA and Novartis within two weeks of learning of a subject's pregnancy. Information on the status of the mother and child will be forwarded to the DSMB, IRB, FDA and Novartis. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded and reported to the DSMB, IRB, FDA and Novartis as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported to the DSMB, IRB, FDA and Novartis. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported to the DSMB, IRB, FDA and Novartis. While the investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting.

9.4 Data management

Data management
The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from the home physician. Laboratory values from referring home physicians will be entered into the system. All human subjects personally identifiable information (PII) eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., unique code or minimum PII required for subject identification.

In order to maintain patient confidentiality, all communications relating to the study will identify participants by assigned subject study numbers. No personally identifiable information will be sent to the DSMB, IRB, FDA or Novartis. The US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

Publication Policy: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate institutional approval.

No identifiable data will be sent outside NIH without IRB notification and an executed MTA or CTA.

A Clinical Trials Agreement (CTA) with Novartis is in place to support this study.

9.5 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records) readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

10. HUMAN SUBJECT PROTECTION

10.1 Rationale for Subject Selection

MDS is not known to show a racial or gender preference in incidence, as such, there will be no restrictions based on ethnicity or gender in patient selection. One of the difficulties clinicians face is distinguishing between hypoplastic MDS and aplastic anemia when faced with a hypoplastic marrow. For the purposes of this protocol, patients with hypocellular marrows (<30%) will be classified as MDS. A cytogenetic abnormality in the setting of a hypoplastic marrow makes a diagnosis of MDS. When there are no cytogenetic abnormalities the distinction between MDS and aplastic anemia needs to be made on morphologic grounds. However, dyserythropoiesis, which is a feature of both AA and MDS will not be used as a sole diagnostic criterion.

The Hematology branch has a robust source of patients with bone marrow failure and we do not anticipate recruitment to be problematic. Since 1997, we have screened over 550 bone marrow failure patients for the actively enrolling protocols to include 250 patients with MDS (about 35/year). We expect a similar distribution by race, gender and age as described below:

- By gender: 99 females (36%), and 160 males (64%).
- By age: 12-85 (median 61), subjects under 18: 3 (1%)
- By race: 16 Asian (6.4%), 14 Black (5.6%), 30 Hispanic (12%), and 190 white (76%).

For subjects of Asian heritage: Plasma eltrombopag exposure was approximately 70% higher in East Asian (i.e. Japanese, Chinese, Taiwanese and Korean) subjects as compared to non-East Asian

subjects who were predominantly Caucasian. Therefore, subjects of Asian heritage will be included but they will be initiated at a lowered dose and monitored closely.

For subjects with renal impairment: The pharmacokinetics of eltrombopag has been studied after administration of eltrombopag to adult patients with renal impairment. Following administration of a single 50 mg dose, there was a trend for reduced plasma eltrombopag exposure in patients with renal impairment, but there was substantial variability and significant overlap in exposures between patients with renal impairment and healthy volunteers. Therefore, patients with impaired renal function will be included but participation will be monitored closely.

For subjects with hepatic impairment: The pharmacokinetics of eltrombopag has been studied after administration of eltrombopag to adult patients with hepatic impairment. Following the administration of a single 50 mg dose, the AUC0-∞ of eltrombopag was increased by 41% in subjects with mild hepatic impairment and by 80% to 90% in subjects with moderate or severe hepatic impairment compared with healthy volunteers. Therefore, patients with minimally impaired hepatic function will be included but participation will be monitored closely.

For pregnant and nursing mothers: Eltrombopag was not teratogenic when studied in pregnant rats and rabbits but caused a low incidence of cervical ribs (a fetal variation) and reduced fetal body weight at doses that were maternally toxic. There are no adequate and well-controlled studies of eltrombopag in pregnant women. The effect of eltrombopag on human pregnancy is unknown. Therefore, women of childbearing potential must agree to use adequate contraception prior to (hormonal or barrier method of birth control; abstinence) and for the duration of study participation. If a woman becomes pregnant or suspects she is pregnant while on study, her treating physician should be informed immediately.

Recruitment efforts: The study will be listed on the clinicaltrials.gov, Clinical Center research studies, The MDS Foundation, and the National Heart, Lung and Blood Institute patient recruitment websites. If recruitment goals are not met, recruitment plan will be developed by the Clinical Center Office of Patient Recruitment. Hematologists and Oncologists throughout the country will be informed about the protocol by letter.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population. The ability to offer patients who fail to have an optimal response to initial immunosuppression protocols another option will be a very positive addition to our MDS program.

Reimbursement for protocol, travel, food, and lodging will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.

10.2 Participation of Pediatric Patients

MDS is a rarely seen in children therefore, we will not be including pediatric subjects in this study.

10.3 Risks and Discomforts:

10.3.1 Promacta® (eltrombopag)

The following information is excerpted from Promacta product label, dated 09/2015 and the Investigator Brochure updated Edition 13.0 dated 13-Apr-2016.

10.3.1.1 Boxed warnings related to Promacta® (eltrombopag):

WARNING: RISK FOR HEPATIC DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C

RISK OF HEPATOTOXICITY

See full prescribing information for complete boxed warning.
In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation.

PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Monitor hepatic function and discontinue dosing as recommended.

10.3.1.2 Warnings and Precautions:

Hepatic Decompensation in Patients with Chronic Hepatitis C
In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation. In two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, ascites and encephalopathy occurred more frequently on the arm receiving treatment with PROMACTA plus antivirals (7%) than the placebo plus antivirals arm (4%). Patients with low albumin levels (less than 3.5 g/dL) or Model for End-Stage Liver Disease (MELD) score greater than or equal to 10 at baseline had a greater risk for hepatic decompensation on the arm receiving treatment with PROMACTA plus antivirals. Discontinue PROMACTA if antiviral therapy is discontinued.

Hepatotoxicity
PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Measure serum ALT, AST, and bilirubin prior to initiation of PROMACTA, every 2 weeks during the dose adjustment phase, and monthly following establishment of a stable dose. PROMACTA inhibits UDP-glucuronosyltransferase (UGT)1A1 and organic anion-transporting polypeptide (OATP)1B1, which may lead to indirect hyperbilirubinemia. If bilirubin is elevated, perform fractionation. Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until resolved or stabilized. Discontinue PROMACTA if ALT levels increase to greater than or equal to 3 x ULN in patients with normal liver function or greater than or equal to 3 x baseline (or greater than 5 x ULN, whichever is the lower) in patients with pre-treatment elevations in transaminases and are:

- progressively increasing, or
- persistent for greater than or equal to 4 weeks, or
- accompanied by increased direct bilirubin, or
- accompanied by clinical symptoms of liver injury or evidence for hepatic decompensation.

If the potential benefit for reinitiating treatment with PROMACTA is considered to outweigh the risk for hepatotoxicity, then consider cautiously reintroducing PROMACTA and measure serum liver tests weekly during the dose adjustment phase. Hepatotoxicity may reoccur if PROMACTA is reinitiated. If liver test abnormalities persist, worsen, or recur, then permanently discontinue PROMACTA.

Isolated cases of severe liver injury were identified in clinical trials. The elevation of liver laboratory values occurred approximately three months after initiation of PROMACTA. In all cases, the event resolved following PROMACTA discontinuation.

Thrombotic/Thromboembolic Complications
Thrombotic/thromboembolic complications may result from increases in platelet counts with PROMACTA. Reported thrombotic/thromboembolic complications included both venous and arterial events and were observed at low and at normal platelet counts.

Consider the potential for an increased risk of thromboembolism when administering PROMACTA to patients with known risk factors for thromboembolism (e.g., Factor V Leiden, ATIII deficiency, antiphospholipid syndrome, chronic liver disease). To minimize the risk for thrombotic/thromboembolic complications, do not use PROMACTA in an attempt to normalize platelet counts. Follow the dose adjustment guidelines to achieve and maintain target platelet counts.

In two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, 3% (31/955) treated with PROMACTA experienced a thrombotic event compared with 1% (5/484) on placebo. The majority of events were of the portal venous system (1% in patients treated with PROMACTA versus less than 1% for placebo).

In a controlled trial in patients with chronic liver disease and thrombocytopenia not related to ITP undergoing elective invasive procedures (N = 292), the risk of thrombotic events was increased in patients treated with 75 mg of PROMACTA once daily. Seven thrombotic complications (six patients) were reported in the group that received PROMACTA and three thrombotic complications were reported in the placebo group (two patients). All of the thrombotic complications reported in the group that received PROMACTA were portal vein thrombosis (PVT). Symptoms of PVT included abdominal pain, nausea, vomiting, and diarrhea. Five of the six patients in the group that received PROMACTA experienced a thrombotic complication within 30 days of completing treatment with PROMACTA and at a platelet count above 200 x 10^9/L. The risk of portal venous thrombosis was increased in thrombocytopenic patients with chronic liver disease treated with 75 mg of PROMACTA once daily for 2 weeks in preparation for invasive procedures.

Cataracts
In the three controlled clinical trials in adults with chronic ITP, cataracts developed or worsened in 15 (7%) patients who received 50 mg of PROMACTA daily and 8 (7%) placebo-group patients. In the extension trial, cataracts developed or worsened in 11% of patients who underwent ocular examination prior to therapy with PROMACTA. In the two controlled clinical trials in patients with chronic hepatitis C and thrombocytopenia, cataracts developed or worsened in 8% of patients treated with PROMACTA and 5% of patients treated with placebo.

Cataracts were observed in toxicology studies of eltrombopag in rodents. Perform a baseline ocular examination prior to administration of PROMACTA and, during therapy with PROMACTA, regularly monitor patients for signs and symptoms of cataracts.

Clinical Experience:
For full information on clinical experience with eltrombopag in for the treatment of all approved indications, see PACKAGE INSERT.

Severe Aplastic Anemia: In the single-arm, open-label trial, 43 patients with severe aplastic anemia received PROMACTA. Eleven patients (26%) were treated for greater than 6 months and 7 patients (16%) were treated for greater than 1 year. The most common adverse reactions (greater than or equal to 20%) were nausea, fatigue, cough, diarrhea, and headache.

Adverse Reactions (≥10%) from One Open-label Trial in Adults with Severe Aplastic Anemia

Adverse Reaction	PROMACTA (n = 43) (%)
Nausea	33
Fatigue	28
Cough	23
Diarrhea	21
Headache	21
Pain in extremity	19
Dyspnea	14
Pyrexia	14
Dizziness	14
Oropharyngeal pain	14
Febrile neutropenia	14
Abdominal pain	12
Ecchymosis	12
Muscle spasms	12
Transaminases increased	12
Arthralgia	12
Rhinorrhea	12

Rash was reported in 7% of patients.

In this trial, patients had bone marrow aspirates evaluated for cytogenetic abnormalities. Eight patients had a new cytogenetic abnormality reported on therapy, including 5 patients who had complex changes in chromosome 7.

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of eltrombopag use in pregnancy. In animal reproduction and developmental toxicity studies, there was evidence of embryo lethality and reduced fetal weights at maternally toxic doses. PROMACTA should be used in pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

In an early embryonic development study, female rats received oral eltrombopag at doses of 10, 20, or 60 mg/kg/day (0.8, 2, and 6 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.3, 1, and 3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Increased pre- and post-implantation loss and reduced fetal weight were observed at the highest dose which also caused maternal toxicity.

Eltrombopag was administered orally to pregnant rats at 10, 20, or 60 mg/kg/day (0.8, 2, and 6 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.3, 1, and 3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Decreased fetal weights (6% to 7%) and a slight increase in the presence of cervical ribs were observed at the highest dose which also caused maternal toxicity. However, no evidence of major structural malformations was observed.

Pregnant rabbits were treated with oral eltrombopag doses of 30, 80, or 150 mg/kg/day (0.04, 0.3, and 0.5 times, respectively, the human clinical exposure based on AUC in patients with ITP at 75 mg/day and 0.02, 0.1, and 0.3 times, respectively, the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). No evidence of fetotoxicity, embryo lethality, or teratogenicity was observed.

In a pre- and post-natal developmental toxicity study in pregnant rats (F0), no adverse effects on maternal reproductive function or on the development of the offspring (F1) were observed at doses up to 20 mg/kg/day (2 times the human clinical exposure based on AUC in patients with ITP at 75 mg/day and similar to the human clinical exposure based on AUC in patients with chronic hepatitis C at 100 mg/day). Eltrombopag was detected in the plasma of offspring (F1). The plasma concentrations in pups increased with dose following administration of drug to the F0 dams.

Nursing Mothers
It is not known whether eltrombopag is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from PROMACTA, a decision should be made whether to discontinue nursing or to discontinue PROMACTA taking into account the importance of PROMACTA to the mother.

Pediatric Use
The safety and efficacy of PROMACTA in pediatric patients 1 year and older with chronic ITP were evaluated in two double-blind, placebo-controlled trials. The pharmacokinetics of eltrombopag have been evaluated in 168 pediatric patients 1 year and older with ITP dosed once daily. The safety and efficacy of PROMACTA in pediatric patients younger than 1 year with ITP have not yet been established.
The safety and efficacy of PROMACTA in pediatric patients with thrombocytopenia associated with chronic hepatitis C and severe aplastic anemia have not been established.

Investigator Brochure, version 13, dated 4/13/2016 - "Adverse Events considered to be Expected for Reporting Purposes"

Below are lists of "Adverse Events considered to be Expected for Reporting Purposes" for each chronic ITP and SAA. This list is based upon evaluation of the available clinical safety information, including data from all global clinical trials (phase I-III) and the Novartis safety database, Argus (cut-off date of 29 February 2016).

Adverse reactions are listed below for each indication by MedDRA body system organ class and by frequency. Frequency category for each adverse drug reaction is based on the following convention (CIOMS III). The frequency categories used are:
Very common: ≥1 in 10 (≥10%)
Common: ≥1 in 100 and <1 in 10 (≥1% and <10%)
Uncommon: ≥1 in 1,000 and <1 in 100 (≥0.1% and <1%)
Rare: ≥1 in 10,000 and <1 in 1,000 (≥0.01% and <0.1%)

Adverse Events considered to be Expected for Reporting Purposes in cITP adults

Infections and infestations
Common: Pharyngitis Urinary tract infection
Gastrointestinal disorders

Very Common:	Nausea
	Diarrhea
Common:	Dry mouth
	Vomiting
Hepatobiliary disorders	
Common:	Increased aspartate aminotransferase
	Increased alanine aminotransferase
	Blood bilirubin unconjugated increased
Uncommon:	Drug-induced liver injury
Skin and subcutaneous tissue disorders	
Common:	Alopecia
	Rash
Musculoskeletal and connective tissue disorders	
Common:	Back pain
	Musculoskeletal chest pain
	Musculoskeletal pain
Myalgia	
Vascular disorders	
Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously	

Additional adverse Events considered to be expected for Reporting Purposes in cITP pediatric Patients (Aged 1 to 17 years) in addition to those seen in cITP in adults

Infections and infestations
Very common: Nasopharyngitis, upper respiratory tract infection
Common: Rhinitis
Gastrointestinal disorders
Common: Abdominal pain, toothache
General disorders and administration site conditions
Common: Pyrexia
Respiratory, thoracic and mediastinal disorders
Common: Cough, oropharyngeal pain, rhinorrhea
Vascular disorders
Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously

Adverse Events considered to be expected for Reporting Purposes in SAA

Blood and lymphatic system disorders
Very common: Anemia
Gastrointestinal disorders
Very common: Abdominal pain, diarrhea, nausea
General disorders and administrative conditions
Very common: Dizziness, fatigue, febrile neutropenia, pyrexia
Hepatobiliary disorders
Very common: Transaminases increased
Musculoskeletal and connective tissue disorders
Very common: Arthralgia, muscle spasms, pain in extremity
Nervous systems disorders
Very common: Headache
Respiratory, thoracic and mediastinal disorders
Common: Cough, dyspnea, oropharyngeal pain, rhinorrhea
Skin and subcutaneous tissue disorders
Very common: Ecchymosis
Vascular disorders

Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously

Adverse Events considered to be expected for Reporting Purposes in MDS/AML

Blood and lymphatic system disorders Very common: Leukocytosis**, white blood cell count increased
Gastrointestinal disorders Very common: Nausea, diarrhea, vomiting, constipation, abdominal pain
General disorders and administrative conditions Very common: Fatigue, pyrexia
Hepatobiliary disorders Uncommon: Drug-induced liver injury
Investigations Rare: Serum discoloration***
Nervous systems disorders Very common: Dizziness, Headache
Respiratory, thoracic and mediastinal disorders Very common: Cough
Skin and subcutaneous tissue disorders Common: Skin discoloration
Vascular disorders Very common: Hematoma

** Leukocytosis and white blood cell count increased occur individually with a frequency of common, however the terms were grouped as they represent the same medical concept, giving a revised frequency of very common.

*** Serum discoloration has been reported in investigator sponsored studies in MDS/AML, and can lead to analytical interference with some colorimetric analytical methods

10.3.1.3 Related to pregnancy and nursing mothers: The effects of eltrombopag on the developing human fetus are unknown. For this reason and because it is unknown whether eltrombopag is teratogenic, women of childbearing potential must agree to use adequate contraception prior to (hormonal or barrier method of birth control; abstinence) and for the duration of study participation. If a woman becomes pregnant or suspects she is pregnant while on study, the research team must be informed immediately. Study drug will be discontinued and the pregnancy followed and outcome reported. (see section 2.4.4, teratogenicity in animals)

10.3.2 Related to bone marrow aspirate and biopsy: No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

10.3.3 Related to blood draws: No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws or infections may rarely occur.

10.4 Risks in Relation to Benefit

10.4.1 For adult subjects:

The benefits to the subjects could be improvement of cytopenias resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents. Potentially, bleeding complications and treatment with other more toxic therapies could also be avoided or postponed.

Therefore, this research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

10.5 Informed Consent Processes and Procedures

Note: Effective January 21, 2019, a witness to the signature of the written long form research consent at an NIH site (whether initially approved by an IRB before or after January 21, 2019) is no longer a requirement.

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient during the initial clinic evaluation. An associate, principal, or medically responsible investigator on this protocol delegated to obtain the informed consent will lead the discussion. The consent form will be signed in the presence of the investigator and a witness prior to commencement of the treatment plan. The treatment plan and risks will be discussed again and in detail during their hospital visit for treatment.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

If it is anticipated that a potential research participant previously enrolled in the screening protocol, may not be able to be physically present at the NIH at the time of consent into this protocol, we will use the following telephone consent process in accordance with the NIH Policy: MAS 77-2:

- Ideally, a copy of the consent document will be provided at the time of screening so in the event the subject is found eligible, there is sufficient time to make an informed decision or come up with questions to bring up during the telephone consent process.
- Informed consent will be obtained by the Principal Investigator or an Associate Investigator approved to obtain informed consent. If not already done, a copy of the consent document will be sent to the potential subject via fax or e-mail or the U.S. Postal Service, if fax & e-mail options are not available.
- Either the investigator or the potential subject may initiate the phone call for discussion of the study after a reasonable amount of time is given to participants to review the consent document prior to telephone consent. A conference call is recommended and both parties will properly identify themselves and the purpose of the telephone call followed by a thorough explanation of the protocol by the investigator with ample time for questions related to participation.
- The potential subject will be instructed to sign and date the consent document along with the signature of an adult witness during the conference call.
- The original signed informed consent document may be faxed back (301-402-3088) or e-mailed to the PI followed by delivery of the original signed document via the US Postal Service or FedEx to the Research Nurse, Hematology Branch, NHLBI, NIH, Building 10, CRC Room 4-5362, MSC 1607, Rockville Pike, Bethesda, MD, 20892.

The telephone informed consent process will be documented in the progress note by the investigator obtaining consent and a copy of the note and the original fully signed consent document will be filed in the subject's medical records with a copy provided to the subject.

Informed Consent of Non-English Speaking Research Participants:
If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in NIH HRPP SOP 12. The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form for up to a maximum of 5 participants in a given language and we will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

Informed Consent for adult research participants unable to provide consent:
If there is an unexpected enrollment of a research participant unable to provide informed consent, we will follow the procedures for obtaining informed consent from a legally authorized representative (LAR) (Category B) per NIH HRPP SOP 14E. See Appendix D for details.

Justification for inclusion: This research provides the prospect of direct benefit, therefore inclusion is justified. The benefits to the participants could be improvement of cytopenias resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents. Potentially, bleeding complications and treatment with other more toxic therapies could also be avoided or postponed. Not allowing participants who cannot provide consent would deny them the potential benefits this protocol offers for their MDS. There are no plans to include institutionalized participants.

Risk/Benefit Assessment:
This research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

Consent and Assent:
Procedures to determine capacity: If documentation of decision making capacity is not present in the medical record or the investigator questions the decision making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

Procedures to obtain assent and documentation of assent or dissent: The informed consent discussion will include the individual unable to provide informed consent along with LAR. The individual unable to provided informed consent will be asked if they agree to participate in the research and this will be documented in the medical record.

10.6 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the NHLBI Clinical Director.

10.7 FWA Coverage Agreement

Dr. Winkler is currently working at Agios Pharmaceutical and will be analyzing identifiable data

as a Non-NIH Engaged Investigator in this protocol. Dr. Winkler's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity has been executed by Dr. Winkler and Dr. Young.

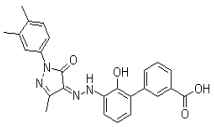
11 PHARMACEUTICALS

11.1 Eltrombopag (Promacta®):

Chemical Name: The chemical name for eltrombopag olamine is 3'-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazino}-2'-hydroxy-3-biphenylcarboxylic acid - 2-aminoethanol (1:2).

Molecular formula: $C_{25}H_{22}N_4O_4.2(C_2H_7NO)$.

Molecular weight is 564.65 for eltrombopag olamine and 442.5 for eltrombopag free acid

Chemical and structural formula: 

Physical form: red/brown solid

Solubility: Eltrombopag olamine is practically insoluble in aqueous buffer across a pH range of 1 to 7.4, and is sparingly soluble in water

Supply: The drug Novartis is providing for this study may be either investigational or commercial material, based on their supply, and is available in tablets and as a powder for oral suspension. Each sachet contains eltrombopag olamine equivalent to 25 mg of eltrombopag. The tablets are available as 12.5, 25, 50, and 75 tablets.

- **Tablets:** White, round, film-coated tablets without debossing are provided, containing eltrombopag olamine equivalent to 12.5 mg, 25 mg, 50 mg, or 75 mg of eltrombopag free acid. Tablets are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps.

  Commercial image actives (12.5 mg - white, 25 mg- orange or white, 50 mg blue or brown, 75 mg – pink,) which are equivalent to the clinical forms with the exception of the film coated color may also be provided for clinical use. These forms are also packed in white HDPE bottles with white plastic, induction -seal, child-resistant caps. Desiccant may be included. Additionally, the commercial image tablets may be provided in aluminum foil blister packages.

- **Powder for Oral Suspension (Sachets):**
  The powder for oral suspension is a reddish brown to yellow powder in a sachet. A 25 mg PfOS strength is available, containing eltrombopag olamine equivalent to 25 mg of eltrombopag free acid. The powder blend composition contains eltrombopag olamine equivalent to 20 mg of eltrombopag free acid. The powder fill weight is 1.25 gram for PfOS 25 mg.

  The entire content of the sachet is added to a specified amount of water to produce a

suspension equivalent to 2 mg of eltrombopag per mL. Enough sachets will be provided for a few days of overage in case a patient is delayed returning to clinic.

Stability: Store at 25°C (77°F); excursions permitted to 15º to 30°C (59º to 86°F) *[see USP Controlled Room Temperature]*.

Shipping: The NIH Investigational Drug Management and Research Section (IMDRS) will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is:

National Institutes of Health
/CC/PHARM/IMDRS, Room 1C230
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196
Shipping Designee Name: Jihyun Esther Jeon
Shipping Designee Phone No: (301) 496-4363
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: jihyunesther.jeon@NIH.gov

Accountability Procedures: Drug accountability records will be maintained for all clinical supplies by the Pharmacy department.

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Promacta (eltrombopag) full prescribing information for compound sB-497115 (elthrobopag olamine) Nplate® (romiplostim) full prescribing information

APPENDIX A: The World Health Organization (WHO) classification of myelodysplastic syndromes (MDS)

Disease	Blood Findings	Bone Marrow Findings
Refractory anemia (RA)	Anemia No or rare blasts < 1 x 10^9/L monocytes	Erythroid dysplasia only < 10% grans or megas dysplastic < 5% blasts < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only < 10% grans or megas dysplastic ≥15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1 x 10^9/L monocytes	Dysplasia in ≥10% of cells in two or more myeloid cell lines < 5% blasts in marrow No Auer rods < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1 x 10^9/L monocytes	Dysplasia in ≥10% of cells in two or more myeloid cell lines ≥15% ringed sideroblasts < 5% blasts No Auer rods
Refractory anemia with excess blasts – 1 (RAEB-1)	Cytopenias < 5% blasts No Auer rods < 1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 5%–9% blasts No Auer rods
Refractory anemia with excess blasts – 2 (RAEB-2)	Cytopenias 5%–19% blasts Auer rods +/– < 1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 10%–19% blasts Auer rods +/–
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Unilineage gran or mega dysplasia < 5% blasts No Auer rods
MDS associated with isolated del (5q)	Anemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobulated nuclei < 5% blasts No Auer rods Isolated del (5q)

APPENDIX B: International Prognosis Scoring System (IPSS) in MDS

Score					
Variable	0	0.5	1.0	1.5	2.0
BM blasts %	<5	5-10	-	11-20	21-30
Karyotype†	Good	Intermediate	Poor	-	-
Cytopenias‡	0 or 1	2 or 3	-	-	-

†Karyotype definitions:
Good: Normal, -Y, del (5q) only, del (20q) only
Poor: Complex (≥3 abnormalities); abnormal chromosome 7
Intermediate: All others
‡Cytopenia definitions:
Red blood cells: Hemoglobin < 10g/dL
White blood cells: Absolute neutrophil count < 1800/uL
Platelets: Platelet count < 100,000/uL

A score from zero to two is determined for each of the three variables; the three values are added to obtain the IPSS score.
Median Survival and Evolution to AML in MDS Patients Based on IPSS Score

Risk group	IPSS score	Median survival	AML evolution†
Low	0	5.7 years	9.4 years
Intermediate-1	0.5-1.0	3.5 years	3.3 years
Intermediate-2	1.5-2.0	1.2 year	1.1 years
High	2.5-3.5	0.4 year	0.2 years

†The time for 25 percent of the patients in each of the four risk groups to evolve into acute leukemia
Survival was also stratified for patient age (table 4). While survival of low risk patients was strongly dependent on age, survival of high risk patients was independent of age:
Median Survival (in Years) in MDS According to IPSS and Age

IPSS risk group	Age ≤ 60	Age > 60	Age > 70
Low	11.8	4.8	3.9
Intermediate-1	5.2	2.7	2.4
Intermediate-2	1.8	1.1	1.2
High	0.3	0.5	0.4

APPENDIX C: NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES- 2/5/2013

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett) – No longer active as of July 1, 2018**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA, protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Micro assay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No

B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyl transferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectra typing for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelodysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No

C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Nucleotide sequencing of genes reportedly associated with bone marrow failure disorders, MDS or AML.	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inoculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No

E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Laser capture microdissection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

APPENDIX D: SOP 14E, (APPENDIX B, TABLE 1)

REQUIREMENTS FOR THE DETERMINATION OF AN LAR'S APPROPRIATENESS TO CONSENT TO RESEARCH NOT INVOLVING GREATER THAN MINIMAL RISK (CATEGORY A) AND FOR RESEARCH INVOLVING GREATER THAN MINIMAL RISK BUT PRESENTING THE PROSPECT OF DIRECT BENEFIT TO THE INDIVIDUAL SUBJECTS (CATEGORY B)

First preference is #1. If not possible, go to option #2. If #2 is not possible, go to option #3.)

Cognitively Impaired Adults and Identification of a LAR	**Requirements for Determining Appropriateness of LAR to Consent to Research at Clinical Center (CC) and non- CC sites**	**Role of the LAR at all sites**
1. Adults who cannot consent and have a court-appointed guardian from a state that allows it[i] or a DPA[ii] for healthcare and/or research participation.	PI/designee[iii], unless the IRB designates an independent person(s) to perform this role (e.g., ACAT[iv] if the protocol is taking place at the CC), must assess appropriateness of LAR to consent to research. An appropriate LAR is one who at least: (a) Understands that the protocol involves research; (b) Understands the risks, potential benefits (if any), and alternatives to the study; and (c) Has sufficient reason to believe participation in the study is consistent with the subject's preferences and values.	LAR may give permission for the research and sign the consent form for the protocol on behalf of the subject.
2. Adults who cannot consent and who do not have a DPA or court-appointed guardian, but who are capable of understanding the DPA process and can assign a DPA[v].		
3. Adults who cannot consent, who do not have a DPA or court-appointed guardian, and cannot appoint a DPA: **At the CC**: A person at the highest level of the following may consent to research participation if found to be appropriate: 1) spouse or domestic partnervi, 2) adult child, 3) parent, 4) sibling, 5) other close relative **At non-CC sites:** Consult with OGC to identify applicable state law.		

[i] A court appointed guardian may only consent to enroll a subject in research if the guardian has authority to do so under the laws of the state that issued the guardianship order and the terms of the guardianship order. The Office of the General Counsel (OGC) should be asked to review guardianship orders to determine if the guardian has legal authority to consent to the subject's participation in the research. PIs are encouraged to seek an OGC consultation in advance of a potential subject with a guardianship order coming to an NIH research site to enroll on a study.

[ii] DPA means the individual holding the durable power of attorney for healthcare. Consult with OGC if concerned about the authority provided in a DPA.

[iii] If the protocol is taking place at the CC, the PI's designee may be someone on the research team or a member of ACAT. If not at the CC, the PI's designee may be someone on the research team or an independent person outside of the research team if it is felt that the team does not have the required competencies to undertake the evaluation.

[iv] NIH Ability to Consent Team. For definition please see **14E.4**.