

CLINICAL RESEARCH PROJECT

Protocol #11-H-0134

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Title: A Pilot Study of a Thrombopoietin-receptor Agonist (TPO-R agonist), Eltrombopag, in Moderate Aplastic Anemia Patients

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Subjects of Study:	<u>Number</u>	<u>Sex</u>	<u>Age-range</u>
	38	Either	≥ 2 years and weight >12 kg

Project Involves Ionizing Radiation? No (only when medically indicated)

Off-Site Project? No

Multi center trial? No

DSMB Involvement? Yes

11-H-0134

Cynthia E. Dunbar, M.D.

01/02/2019 (Amendment HH)

PRECIS

Moderate aplastic anemia (MAA) is a blood disease which can be effectively treated with immunosuppressive drug regimens. However, a significant number of patients have persistent cytopenias. Currently, the treatment of these patients is regular transfusion, which are expensive, inconvenient, and associated with serious side effects related to iron overload, or cytokines such as erythropoietin or G-CSF, which are expensive, and not effective in many patients.

Thrombopoietin (TPO) is a protein made by the body that is important for normal production of platelets by the bone marrow. TPO may also be able to stimulate bone marrow stem cells to produce red cells and white cells. TPO cannot be given by mouth, and as an alternative, a drug, eltrombopag, has been designed that acts in the same way as TPO but is stable and active when given by mouth. Eltrombopag has been shown to safely increase platelet numbers in healthy volunteers and in patients with chronic immune thrombocytopenic purpura (ITP). It has been recently granted accelerated approval by FDA on November 20, 2008 for the treatment of patients with chronic immune thrombocytopenic purpura (ITP) who have had an insufficient response to standard therapies.

We have previously shown encouraging results when eltrombopag is used to treat patients with severe aplastic anemia, with some patients responding with increases in platelets, red cells and white cells. Given these encouraging early preliminary results in our clinical trial using eltrombopag in SAA, and low toxicity and ease of administration of this drug, we now propose a non-randomized pilot phase II study of eltrombopag in moderate aplastic anemia patients with clinically significant thrombocytopenia or anemia. Patients with MAA may not reach criteria for SAA, but none the less may be transfusion-dependent or have significant symptoms from cytopenias. We hypothesize that patients with MAA as compared to SAA may have a better chance of response, due to better residual marrow function in MAA patients compared to SAA.

Eligible patients can have treated or untreated MAA, as well as counts meeting criteria for MAA following a partial response to treatment with immunosuppression for SAA. We will also include patients with bone marrow failure and unilineage cytopenia. Treatment response for the platelet lineage is defined as platelet count increases to 20,000/uL above baseline at 16 to 20 weeks, or freedom from platelet transfusions for ≥ 8 weeks in transfusion-dependent patients. For patients with anemia (untransfused hemoglobin ≤ 8.5 g/dL), a treatment response will be an increase in Hb by ≥ 1.5 g/dl at four months, measured on at least 2 serial measurements and sustained for 1 month or more without transfusion support **OR** for transfusion dependent patients, reduction of units of RCC transfused by 50%/8 weeks compared with the pretreatment transfusion number in the previous 8 weeks or transfusion independence (no transfusions for ≥ 8 weeks). 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.

The **primary objective** is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in moderate aplastic anemia patients or patients with bone marrow failure and unilineage cytopenia. **Secondary objectives** include the analysis of the incidence and severity of bleeding, clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia and the impact on quality of life.

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1.0 OBJECTIVES

The **primary objective** is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in patients with moderate aplastic anemia or bone marrow failure with unilineage cytopenia.

Secondary objectives include the analysis of the incidence and severity of bleeding episodes, clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia and the impact on quality of life.

2.0 BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 Pathophysiology of aplastic anemia

Aplastic anemia is characterized by a reduction in or a total absence of hematopoietic precursors in the bone marrow. Evidence suggests that the likely mechanism is autoimmune attack^{1,2} and this is supported by the observation that a majority of these patients will respond to immunosuppressive therapy. Perturbations of the immune system of patients with aplastic anemia such have been observed and are similar to those associated with other autoimmune disorders³. Furthermore, the presence of antibodies to human cellular antigens has been detected in some patients with aplastic anemia⁴. Interferon gamma may mediate the apoptotic death of hematopoietic stem cells and increased concentrations of FAS antigen are found on CD34+ cells of patients with this disorder⁵. In aplastic anemia, TPO levels are significantly increased, in contrast to TPO levels in ITP, which are generally within the normal range or only moderately increased (^{6, 7}).

2.2 Clinical consequences of aplastic anemia

Cytopenias in at least two of three cell lines defines aplastic anemia. Common presenting symptoms are recurrent infections as a result of neutropenia, fatigue secondary to anemia, and bruising secondary to thrombocytopenia. Infections are usually bacterial in origin but fungal infections can also occur and can be fatal. The major symptom of thrombocytopenia in aplastic anemia 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 aplastic anemia or treatment with immunosuppression, such as corticosteroid-related gastritis, or 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 long-term follow-up studies in the modern era, up to 10% of patients presenting with aplastic anemia still eventually die of bleeding, and in patients that do not respond to immunosuppression, more than half of subsequent deaths are due to bleeding (⁸).

2.3 Treatment and prevention of complications of aplastic anemia patients

Patients with aplastic anemia often require prophylactic or therapeutic transfusions of blood products. Platelet transfusions are given usually at a threshold platelet count of 10,000/uL. Due to the short half-life of platelets in the circulation, many aplastic anemia patients with severe thrombocytopenia require transfusions as frequently as two or three 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 platelet transfusions as therapy for aplastic anemia.

The majority of patients presenting with aplastic anemia are anemic and will require blood transfusion at some stage in their clinical course. Red cell transfusions are similarly costly and not without potentially serious complications, including iron overload and transmission of infectious agents. Infections as a result of neutropenia are common, typically bacterial and often cause pneumonia or urinary tract infection. Invasive fungal infection is a common cause of death, especially in patients with prolonged neutropenia. Prompt treatment with antibiotics is essential in these patients. All of these problems have a significant impact on the quality of life of aplastic anemia patients.

Despite the fact that TPO levels are generally already increased in aplastic anemia, 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 counts in patients with moderate aplastic anemia. Despite elevated erythropoietin levels, some patients with persistent anemia following immunosuppressive therapy for aplastic anemia respond to combination therapy with erythropoietin and G-CSF⁹. 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 autoimmune destruction or loss of more primitive hematopoietic stem and progenitor cells in aplastic anemia.

Our group conducted a pilot phase I/II study of eltrombopag in patients with severe aplastic anemia. A total of 44 patients were enrolled in the protocol and 43 had received study drug. The overall response rate in this heavily pre-treated patient population was 17 of 43 patients (40%) at 3 to 4 months, including trilineage and bilineage responses. Most patients (14/17) continued to show trilineage improvement and 5 patients had drug discontinued for near normalization of blood counts without relapse. Eight patients developed new cytogenetic abnormalities on eltrombopag, including 5 with chromosome 7 loss or partial deletion, but none evolved to leukemia. Eltrombopag was immediately discontinued when cytogenetic abnormalities were observed. In January 2014 eltrombopag (Promacta) gained Breakthrough Therapy designation status from the FDA and Priority Review in April 2014. On August 26, 2014, the FDA approved the additional use of eltrombopag in patients with severe aplastic anemia who have had an insufficient response to immunosuppressive therapy. The approval was based on results from the clinical trial done at the NIH described above, 09-H-0154 (NCT00922883) (14,15). We hypothesize that patients with MAA as compared to SAA may have a better chance of response, due to increased residual marrow stem cell reserve compared to SAA.

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 suprathreshold 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 embryoletality, 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 AUC_{0-∞} and C_{max} 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 AUC_{0-∞} by approximately 59% and C_{max} by 65% and delayed t_{max} 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 AUC_{0-∞}. 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 AUC_{0-∞} 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 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 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/uL. 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 ($\geq 5\%$) 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 $\geq 50\%$ 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 $\leq 6\%$ 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 \geq 1.5x ULN or ALP \geq 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/uL.

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 \geq 50 K/uL (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 \geq 50 K/uL at Day 43 or discontinued treatment prior to Day 43 for any reason other than a platelet count >200 K/uL.

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 46). The primary method of analysis was a logistic regression model adjusted for ITP medication use at randomization, splenectomy status and baseline platelet count \leq 15 K/uL. 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 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/uL. The median platelet levels remain elevated (>47 K/uL) throughout daily administration of 50 mg eltrombopag (Days 15-43) in both studies (TRA100773A, Figure 1; TRA100773B, Figure 2).

Figure 1

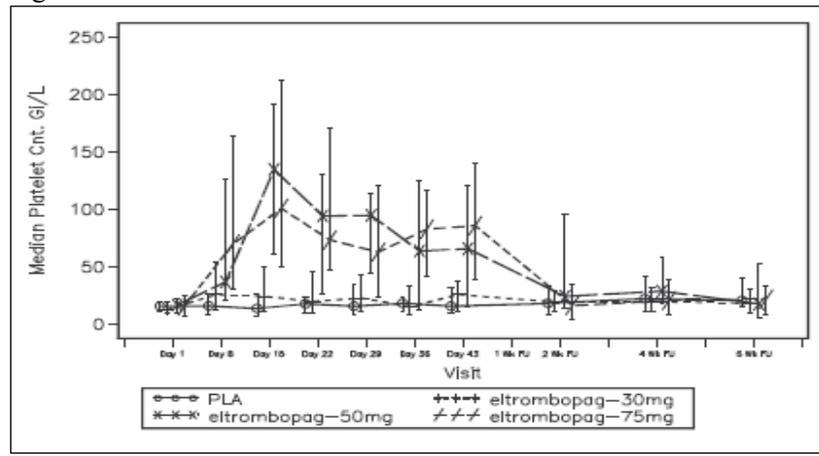
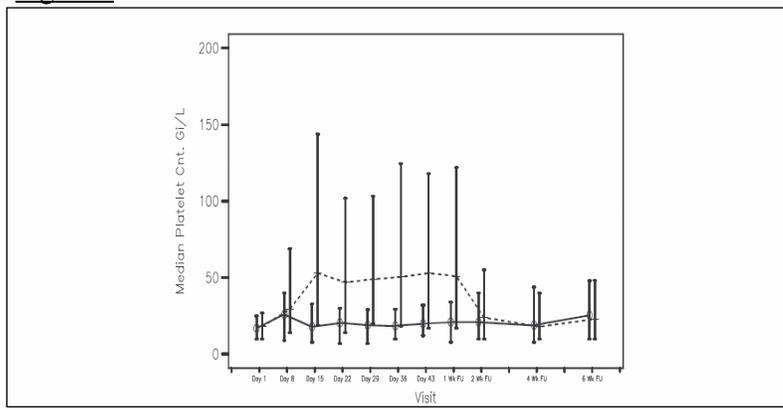


Figure 2



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/uL and for those who had baseline platelet counts > 15 K/uL. A higher percentage of subjects in both treatment groups with baseline platelet counts > 15 K/uL achieved a platelet count ≥ 50 K/uL compared to subjects with a baseline platelet count ≤ 15 K/uL. 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/uL 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/uL. 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/uL observed in Cycles 1, 2 and 3, respectively. By Day 15, median platelet counts were 124, 132 and 156 K/uL in each cycle, respectively. One week after discontinuation of eltrombopag, median platelet counts remained >100 K/uL 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, it was 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.

In January 2014 eltrombopag (Promacta) gained Breakthrough Therapy designation status from the FDA and Priority Review in April 2014. On August 26, 2014 the FDA approved the additional use of eltrombopag in patients with severe aplastic anemia who have had an insufficient response to immunosuppressive therapy. The approval was based on results from the clinical trial done at the NIH described above, 09-H-0154 (NCT00922883).

Eltrombopag has also received FDA approval for the treatment of thrombocytopenia in patients with chronic hepatitis C to allow the initiation and maintenance of interferon-based therapy.

2.6 Rationale for dose selection

Dose selection prior to January 8, 2015:

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 300 mg (East Asians 125 mg) once daily as detailed in the treatment plan (Section 5) based on the following considerations:

- 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 $\mu\text{g}\cdot\text{h}/\text{mL}$ 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 $\mu\text{g}\cdot\text{h}/\text{mL}$ 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 CIB. The eltrombopag geometric mean $AUC_{(0-\tau)}$ values of 191 $\mu\text{g}\cdot\text{h}/\text{mL}$ 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 aplastic anemia patients would develop thrombocytosis, given the underlying pathophysiology of their marrow disease, is likely to be low.
- 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 or hemoglobin. Only subjects who have tolerated the previous dose will be considered for the next highest dose, dependent on their last platelet count or hemoglobin. This approach minimizes potential risks while allowing the subject the maximum potential for benefit.
- Our group is currently conducting a clinical trial in patients with severe aplastic anemia using a dosing scheme whereby subjects increase doses by 25mg every 2 weeks. Interim results have shown responses in all 3 lineages with low toxicity. All patients reached the maximum dose of 150mg without any reductions in dose necessary. It is anticipated that it would be similar in MAA population with this revised dosing schedule likely to improve responses.

Dose selection after January 8, 2015:

Starting dose will be 150 mg/day (75 mg/day in East Asian). The dose will be increased by 50 mg/day (25 mg/day Asian) every 4 weeks until subjects reach the maximum dose of 300 mg/day (150mg/day Asian). This will allow reaching the 300 mg dosing at the same time as the previous cohort and generates the least changes in the treatment schema. The rationale for using the half dose for East Asian patients is unchanged; the change in the starting Eltrombopag dose is based on our growing experience with dosing Eltrombopag in bone marrow failure patients (no hematologic response seen at doses lower than 150 mg/day and all patients were increased at least up to 200 mg/day without any side effects) for patients with bone marrow failure syndromes, as well as the recommendation from our DSMB reviewers in 2014. Also, the extended time for each dose, from 2 to 4 weeks will allow for better identification of patients that respond at lower doses than the maximal 300 mg/day.

Pediatric dosing:

For pediatric subjects, there is a predicted higher weight-adjusted drug clearance than older children and adults based upon studies of several drugs approved for use in children, such as anticonvulsants, proton pump inhibitors, theophylline, and HIV protease inhibitors, have routinely demonstrated that young children have higher weight-adjusted drug clearance than older children and adults [Lamictal Package Insert, 2007; Trileptal Package Insert, 2007; Keppra Package Insert, 2008; Prilosec Package Insert, 2008; Kaletra Package Insert, 2007; Viracept Package Insert, 2007; Grygiel, 1983]. The recently published results of a phase II pediatric chronic ITP study (PETIT), subjects between 1 and 5 years received a maximal mean dose of 2.9 mg/kg eltrombopag once daily, while subjects between 6 and 17 years of age received an average daily dose of 57.3 mg daily (13). The maximum dose used in the PETIT trial among all age groups is 75 mg daily dose. In the dose finding part of the study 5 patients was recruited to cohort 3 (ages 1 to 5 years). These subjects initiated dosing with 0.7 mg/kg once daily and increased to at least 1.4 mg/kg once daily by the Week 12 visit. Patients in this cohort received a median of 66mg eltrombopag daily (3mg/kg: range 34-75mg). Preliminary PK data collected for 3 subjects (ages ranging from 2 to 5 years) receiving eltrombopag 1.1 to 1.2 mg/kg once daily at Week 6 suggest that this regimen delivers

plasma eltrombopag exposure similar to a 37.5 to 50 mg once daily regimen in adults. No new pediatric specific safety signal has been identified thus far. The available platelet count, safety, and PK data available for subjects enrolled in the PETIT trial support a starting dose of 2.5 mg/kg once daily for non-Asian subjects aged 2-5 years.

2.7 Rationale for permitting dose interruption

The effect of dose interruption is unknown in the MAA 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.

2.8 Rationale for extended access to study medication

In patients with refractory cytopenias due to MAA, 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. 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. 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 endpoint at 16 or 20 weeks in the current study, in patients responding to the drug. 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 our 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.

2.9 Scientific and Clinical Justification of the Protocol

Management of moderate aplastic anemia is similar to that of severe disease and includes immune suppressive therapy (IST), supportive therapy with transfusions or cytokines, or allogeneic transplantation in IST refractory patients. Prophylactic platelets may be essential to prevent bleeding complications and transfusion may be required more than once a week. Red cell transfusion is often necessary up to once or twice every month. There are many potentially serious complications of blood product administration including alloimmunisation, transmission of infectious agents, iron overload, and transfusion reactions. Blood products are expensive and supply is often precarious. 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. A nonpeptide mimetic, eltrombopag, 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 (MDGF).

In 2 completed efficacy studies in subjects with ITP (TRA100779A/TRA100773B), 62% of subjects responded with a clinically meaningful increase in platelet counts. Eltrombopag induced elevations in platelet counts ≥ 50 K/uL. 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 $\geq 50,000$ /uL for at least 10 consecutive weeks in the majority of subjects, with 24% achieving continuous elevation of platelet counts $> 50,000$ /uL for more than 6 months and a decrease in bleeding symptoms.

TPO has significant effects on multipotent stem and progenitor cells in vitro and in mouse models. Our previous studies in patients with refractory severe aplastic anemia confirmed that eltrombopag is able to restore trilineage hematopoiesis likely due to its TPO agonistic effect on hematopoietic stem cells. Patients with MAA have less severe manifestations of their disease as a result of better hematopoietic reserve. Despite this, treatment options are limited and there is no licensed agent for specific use in this population. Eltrombopag has a favorable toxicity profile, is administered conveniently as an oral agent and is likely to be efficacious in patients with MAA because of functional albeit reduced hematopoietic reserve.

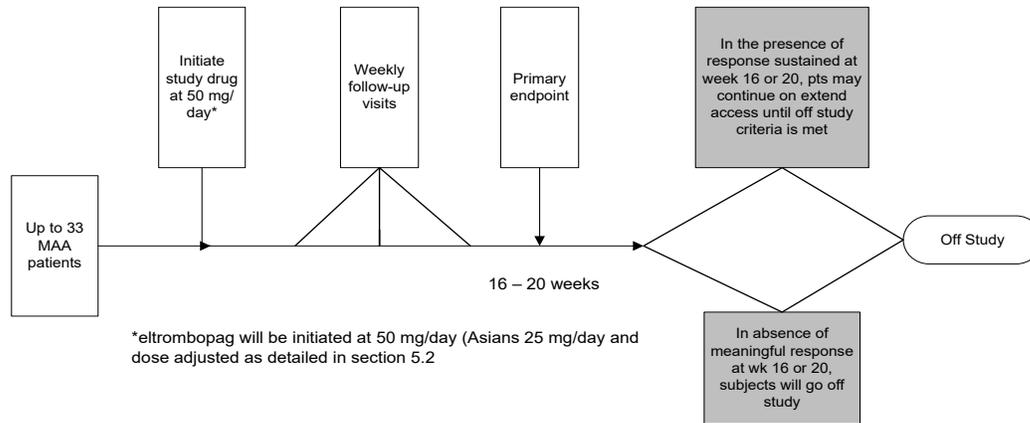
Because trilineage responses can be achieved with this agent bone marrow failure with unilineage cytopenias also have the potential to respond.

3.0 STUDY DESIGN

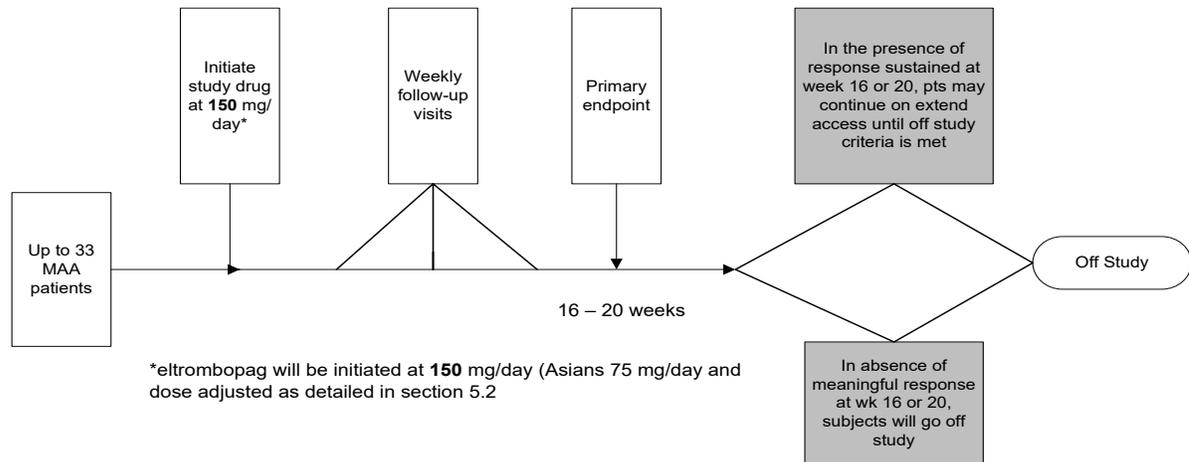
The study is designed as a non-randomized, Phase II, dose modification study of the oral TPO-R agonist eltrombopag in moderate aplastic anemia patients or those with bone marrow failure with unilineage cytopenia. The primary endpoint is measured at 16 to 20 weeks (+/- 7 days). Subjects with evidence 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 (+/- 7 days). 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 who cannot tolerate the medication or fail to respond by 16 to 20 weeks (+/- 7 days) will go off study. Drug dose during extended access will be at the lowest dosage that maintains stable blood counts or taken off drug for robust response until they meet off study

criteria or the study is closed. If subjects are taken off drug for robust response during extended access and maintain response, they will be taken off study after 3years.

Protocol Schema prior to January 8, 2015:



Protocol Schema after January 8, 2015:



4.0 ELIGIBILITY ASSESSMENT

4.1 Inclusion criteria

4.1.1 Current diagnosis of moderate aplastic anemia **OR** unilineage bone marrow failure disorders.

- **Moderate aplastic anemia is defined** as aplastic anemia (hypocellular bone marrow for age) with no evidence for other disease processes causing marrow failure, and depression of at least two out of three blood counts below the normal values:

- $ANC \leq 1200/mm^3$
- platelet count $\leq 70,000/mm^3$
- anemia with hemoglobin ≤ 8.5 g/dL and absolute reticulocyte count $\leq 60,000/mm^3$ in transfusion-dependent patients

but not fulfilling the criteria for severe disease defined by depression of two of the three peripheral counts:

- $ANC \leq 500/mm^3$

- platelet count $\leq 20,000/\text{mm}^3$
 - reticulocyte count $\leq 60,000/\text{mm}^3$)
 - **Unilineage bone marrow failure disorders are defined:**
 - Hemoglobin < 8.5 g/dL and reticulocyte count $< 60,000$ or red cell transfusion dependent and hypocellular to normocellular bone marrow for age with significantly reduced erythroid precursors.
 - **OR** thrombocytopenia $\leq 30,000/\text{uL}$ or platelet transfusion dependent and hypocellular to normocellular bone marrow for age with reduced megakaryocytes.
 - No evidence of viral or drug suppression of the marrow, dysplasia, or underproduction anemias secondary to B12, folate, iron or other reversible causes.
- 4.1.2 Platelet transfusion dependent is defined as the need for platelet transfusion due to platelet counts of $< 10,000$ /uL with no bleeding (prophylactic transfusion) or $< 20,000/\text{uL}$ with bleeding (therapeutic transfusion). Red cell transfusion dependent is defined as transfusion of greater than 4 units of blood in the 8 weeks prior to study entry.
- 4.1.3 Age ≥ 2 years old
- 4.1.4 Weight > 12 kg

4.2 Exclusion criteria

- 4.2.1 Known Diagnosis of Fanconi anemia
- 4.2.2 Counts that meet criteria for severe aplastic anemia
- 4.2.3 Infection not adequately responding to appropriate therapy
- 4.2.4 HIV positivity
- 4.2.5 Creatinine > 2.5 mg/ dL
- 4.2.6 Bilirubin > 2.0 mg/ dL, including congenital abnormalities in the bilirubin count
- 4.2.7 SGOT or SGPT > 5 times the upper limit of normal
- 4.2.8 Hypersensitivity to eltrombopag or its components
- 4.2.9 Female subjects who are nursing or pregnant or are unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential
- 4.2.10 Evidence of an active malignant hematological or clonal disorder, or abnormal cytogenetic studies of the bone marrow performed within 12 weeks of study entry
- 4.2.11 Unable to understand the investigational nature of the study or give informed consent and does not have a legally authorized representative or surrogate that can provide informed consent per section 10.5
- 4.2.12 Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy, or that death within 7-10 days is likely
- 4.2.13 Treatment with horse or rabbit ATG or Campath within 6 months of study entry
- 4.2.14 Treatment with cytokines such as G-CSF or Erythropoietin
- 4.2.15 Subjects with known liver cirrhosis in severity that would preclude tolerability of eltrombopag as evidenced by albumin $< 35\text{g/L}$
- 4.2.16 Life expectancy of less than 3 months
- 4.2.17 Patients with an active diagnosis of cancer who have received chemotherapeutic treatment or other specific antineoplastic drugs or radiation therapy within 6 months of study entry
- 4.2.18 Unable to take investigational drug

5.0 TREATMENT PLAN

5.1 Administration of study drug (eltrombopag)

Note: Currently sachets are not available, and the manufacturer is working on a new formulation. However, information regarding the use of sachets for children 2-5 years of age or older children unable to take pills is no longer applicable, because the study did not enroll any subjects that required use of the sachets.

Prior to January 8, 2015

Subjects will initiate study drug at 50mg orally once a day, taken on an empty stomach one hour before or at least two hours after a meal as detailed in section 5.9. Subjects of East Asian ancestry (Japanese, Chinese, Taiwanese and Korean) will initiate study drug at 25 mg orally once a day.

After January 8, 2015

Subjects will initiate study drug at 150 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 5.9. Subjects of East Asian ancestry (Japanese, Chinese, Taiwanese and Korean) will initiate study drug at 75 mg orally once a day. Pediatric patients will be treated in a separate cohort with a fixed dose (see Pediatric Dosing table below in section 5.2 and section 10.2).

5.2 Dose Escalation of Eltrombopag Through Primary Endpoint at 16-20 Weeks (See section 2.5)

Patients will be enrolled on study because of anemia and/or thrombocytopenia. The majority of patients are expected to have cytopenias in both lineages, however some patients will have normal or close to normal counts in one or the other lineage at baseline. Therefore, dose escalation criteria will vary depending on whether one or both lineages meet inclusion criteria. Escalation 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/uL. 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/uL or hemoglobin goes over 16.0 gr/dL, in which case drug will be discontinued for one week and the dose decreased by 50%.

Dose Escalation for Patients Entering with Platelets <30,000/uL or transfusion-dependence:

Platelet Count < 30,000/uL or transfusion-dependent at baseline	Dose Adjustment or Response
<20,000/uL above baseline	Increase daily dose by 50 mg (25 mg for East Asians) every 4 weeks to maximum 300 mg/day for non- East Asians (150 mg/day for East Asians).
≥20,000/uL above baseline but ≤200,000/uL following at least 2 weeks of eltrombopag	Keep at current dosage.
>200,000/uL (untransfused) at any time on	Decrease dosage by 50 mg (25

Platelet Count < 30,000/uL or transfusion-dependent at baseline	Dose Adjustment or Response
study	mg for East Asians) every 4 weeks to lowest dosage that maintains platelet count $\geq 20,000/uL$ above baseline.
>400,000/uL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets < 20,000/uL; restart at 50% of current dose.

Dose Escalation for Patients Entering with Anemia (Hb < 8.5gr/dL or transfusion-dependence)

Hemoglobin < 8.5 gr/dL or transfusion dependent at baseline	Dose Adjustment or Response
Hemoglobin rise of < 1.5 g/dL.	Increase daily dose by 50 mg (25 mg for East Asians) every 4 weeks to maximum 300mg/day for non- East Asians (150mg/day for East Asians).
$\geq 1.5g/dL$ above baseline but $\leq 15g/dL$ following at least 2 weeks of-eltrombopag	Keep at current dosage.
>15g/dL (untransfused) at any time on study	Decrease dosage by 50% to lowest dosage that maintains Hb $\geq 1.5g/dL$ above baseline.
>16g/uL (untransfused) at any time on study	Discontinue eltrombopag for one week, if Hb<15g/dL restart at 50% of current dose. Phlebotomy may be performed if clinically indicated as determined by the investigator.

If after 16 to 20 weeks of treatment there is no response, treatment will be discontinued and subjects will go off study per section 8.6.

Dosing for pediatric patients:

Age groups	Daily dose
Non-Asian	
12-18	150 mg
6-11	75 mg
2-5	2.5 mg/kg
East Asian, South East Asian	
12-18	75 mg
6-11	37.5 mg
2-5	1.25 mg/kg

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

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Cynthia E. Dunbar, M.D.

01/02/2019 (Amendment HH)

eltrombopag discontinued. If the subject experiences infection severe enough to require vasopressors or intubation, the drug will be withheld until the patient is stable.

5.3.2 Liver function abnormalities: In the event of an increase in the ALT level to > 6 times the ULN, patients 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 50 mg/day lower than the prior dose. If the toxicity appeared on a dose of 25 mg/day, eltrombopag will be discontinued permanently. If liver test abnormalities return to an 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. Patients with platelet counts of > 50,000/uL 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/uL with discontinuation of eltrombopag. They will be treated for the thrombotic event as otherwise clinically-indicated.

5.4.2 Evidence for disease progression: The presence of persistent morphologic abnormalities (red cell teardrop forms; immature blast cells) or the development of significant worsening of anemia (requiring transfusions) 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 MDS or AML.

Any morphological changes in the bone marrow fulfilling current WHO criteria for MDS or cytogenetic abnormalities indicating clonal hematopoiesis will require discontinuation of eltrombopag.

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 improvement in blood counts or transfusion requirements but not yet meeting response criteria 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. Subjects that have their response assessment extended an additional 4 weeks and have a response will be able to go on the extended access part of the trial after signing the consent. Per dosing criteria given in section 5.2, patients may remain on the extended access as long as they maintain a treatment response.

Dose adjustments during extended access: Eltrombopag will be discontinued in patients that reach robust hematologic response (platelets >50,000/ μ L and Hb > 10 g/dL in the absence of transfusions, and neutrophils > 1,000) for more than 8 weeks. In patients who maintain initial response criteria (but do not meet robust response criteria in all three lineages) and show no further clinically-significant improvement in their counts over a 6 month period eltrombopag can be stopped at PI's discretion. Eltrombopag can be re-initiated at PI's discretion if peripheral cell counts drop below numbers measured when the patient entered the study or if platelets drop to <30,000/ μ L, Hb to <9g/dL, or ANC to <500/ μ L. 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.

5.6 Permitted Supportive care

- Transfusional supportive care (e.g., blood and platelets) as clinically indicated. Estrogens or combination OCPs as indicated for uterine bleeding
- Prophylactic antibiotics and antivirals as clinically indicated
- The PI should be informed about all medication changes

5.7. Non-permitted medication

Romiplostim (N-Plate) or IL-11 (Neumega) should not be administered.

5.8 Concurrent Medications:

Rosuvastatin: 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 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. Patients 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: Patients may continue on any dietary supplements or medications that they were prescribed prior to study enrollment for co-morbid conditions, and standard anti-infectious prophylaxis medications including pentamidine, valacyclovir, and voriconazole.

5.9 Instructions to patients

Timing in relation to food: Patients will be advised to take eltrombopag on an empty stomach (1 hour before or 2 hours after a meal)

Timing in relation to antacids: Because co-administration of eltrombopag with antacids decreased plasma AUC of eltrombopag by 70%, patients will be advised to take the eltrombopag at least 4 hours apart from antacids and other products containing polyvalent cations (i.e. aluminum, calcium, magnesium, iron, selenium and zinc) such as mineral supplements and dairy products.

Vigorous activities: You should avoid vigorous activities, as mild trauma could result in bleeding.

6.0 CLINICAL MONITORING

6.1 Pre-study Evaluation

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 done within 7 days of the first dose of study drug, all others should be within 6 months of study entry or as indicated)
 - Complete blood count with differential*
 - Reticulocyte count*
 - Chem 20 panel (Acute care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LDH) panel)*
 - Pregnancy test (urine or blood HCG in women of child bearing potential)*
 - Coagulation screens (PT, PTT) (within 12 weeks prior to consent).
 - Thyroid function tests (within 12 weeks prior to consent).
 - Peripheral blood smear (at baseline and at response evaluation)
 - Viral serologies for HIV, hepatitis B, C, HSV, EBV and CMV (within 12 weeks prior to consent).
 - HLA typing (if not already available) (within 12 weeks prior to consent).
 - DAT (direct antiglobulin test) (within 12 weeks prior to consent).
 - Flow cytometry of the peripheral blood for GPI-cells (within 12 weeks prior to consent).
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies
- Bone marrow aspirate and biopsy with reticulin and collagen fiber staining and cytogenetic analysis (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis) within 12 weeks of first dose of study drug
- FACT questionnaire within 7 days prior to start of first dose of study drug

6.2 Monitoring study drug initiation through primary endpoint (response assessment at week 16 or week 20 +/- 7 days)

Subjects will be monitored so long as they remain on study drug through week 16 or week 20. Subjects may be followed by their home physician or at the Clinical Center. Progress notes and lab tests not done at the NIH will be faxed to the Research Nurse. Subjects will be evaluated for response at week 16 (+/- 7 days) and week 20 (+/- 7 days) if needed at the clinical center. Evaluations designated by the “***” below will only need to be completed for subjects at response assessment either at week 16 or 20. At the clinical investigator's discretion, participants may be evaluated more frequently if medically indicated based on disease status. Subjects must have interim weekly blood tests drawn by their referring health care provider or at the NIH. Women of childbearing potential will also have pregnancy tests by their referring health care provider or at the NIH at the time points listed below. If subjects are to be followed at home, progress notes (if available) and laboratory results from their health care provider and laboratory must be faxed to the study research nurse and if this requirement is not fulfilled strictly subjects will be taken off study. Subjects may have the testing performed at the NIH. The following assessments will be done:

During 16 or 20 weeks (+/- 7 days) landmark visits

- Clinical assessment and vital signs
- Medication review with attention to compliance with eltrombopag so that early discontinuation and subsequent rebound exacerbation is carefully monitored CBC with differential
- Acute care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LDH) panel
- Coagulation screens (PT, PTT)**
- DAT (direct antiglobulin test) (as clinically indicated)
- Type and screen (as clinically indicated)
- Pregnancy test (urine or blood HCG in women of child bearing potential) (after 16, 20** weeks of medication (+/- 7 days))
- Flow cytometry of the peripheral blood for GPI-cells**(+/- 3 weeks)
- 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)** (+/- 3 weeks)
- FACT questionnaire ** (+/- 3weeks)
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies
- In pediatric patients and selected adult patients blood for pharmacokinetic evaluation of eltrombopag levels with samples drawn pre-dose, and at 2,4,6, and 8 hours after the dose, with an optional final sample at 24 hours after the first dose.

On Study Monitoring (through 16 and 20 weeks +/- 5 days)

Subjects may be followed by their home physician or at the Clinical Center. Progress notes and lab tests not done at the NIH will be faxed to the Research Nurse. The PI will review outside test results and these will be uploaded into CRIS and filed in the research charts, and all lab data will be recorded.

- Complete blood counts with differential (every 2 weeks +/-5 days)
- ALT, AST, Total Bilirubin or Direct Bilirubin,(every two weeks +/- 5 days)

6.3 Monitoring during extended access

Patients fulfilling response criteria (per section 8.2) at the 16 or 20 week visit will be offered participation in the extended access cohort, and after signing consent will be permitted to continue on eltrombopag. Subjects taking eltrombopag must be evaluated at the Clinical Center every 6 months (+/- 30 days) while they remain on extended access and continue periodic laboratory monitoring in their home physician's office or the NIH every 2 weeks (+/- 7 days). Follow-up on this schedule will continue as long as they remain on eltrombopag on this clinical protocol.

Testing during NIH visit (every 6 months +/- 30 days) required for patients while on extended access and still being treated with eltrombopag:

- Acute care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LDH) panel
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
- Bone marrow chromosomal analysis via standard cytogenetic techniques

- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- FACT questionnaire
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies

On Study Monitoring every two weeks while on drug (+/- 7 days)

- Subjects may be followed by their home physician or at the Clinical Center. Progress notes and lab tests not done at the NIH will be faxed to the Research Nurse. The PI will review outside test results and these will be uploaded into CRIS and filed in the research charts, and all lab data will be recorded
- Complete blood counts with differential (every 2 weeks +/-7 days), Reticulocyte count
- ALT, AST, Total Bilirubin or Direct Bilirubin, (every two weeks +/- 7 days)

6.3.1 Subjects that have drug stopped for robust response

Subjects that have drug stopped for robust response or for a stable response with no significant improvement in their counts over a 6 month period will have blood counts monitored every three months be seen for up 3 years after the last dose of drug, and then be taken off study as long as the subject maintains response. Subjects off drug must be evaluated at the NIH Clinical Center every 12 months (+/- 30 days). Evaluations designated by the “***” below will only need to be completed for subjects at response assessment every 6 months. Subjects may be seen for monthly interim visits at the NIH or at their referring home health care provider. 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 (if available) and laboratory results from the home health care provider and laboratory must be faxed to the study research nurse.

Testing during NIH visit (every 12 months) required for patients while on extended access but off eltrombopag:

- Complete blood counts with differential (monthly +/- 7 days for 6 months off eltrombopag, then every 3 months for 3 years)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities. (every 12 months +/- 60 days for three years)
- Bone marrow chromosomal analysis via standard cytogenetic techniques (every 12 months +/- 60 days for three years)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells (every 12 months +/- 60 days for three years)
- FACT questionnaire (every 12 months +/- 60 days for three years)
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies (every 12 months +/- 60 days)
- Lymphocyte phenotyping (TBNK flow cytometry)
- Research blood as detailed in section 7
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 12 months +/- 60 days)

6.4 Off study assessment

Patients who fail to respond to eltrombopag after 16 or 20 weeks (+/- 10 days) of therapy or who are taken off treatment prior to the 6 months evaluation for any of the other reasons listed in section 8.6 will likely choose to pursue other treatments, including immunosuppressive therapy, related, unrelated donor or cord blood transplantation, or experimental therapies. We will offer patients a follow-up evaluation at the NIH 6 months following being taken off eltrombopag treatment either due to lack of response or for the other reasons listed in section 8.6, but this visit will not be required. If they do not return to the NIH, we will contact their primary hematologist for information on their current hematologic status 6 months following their final dose of eltrombopag, and then take them off study.

If patients do return to the NIH at 6 months (+/- 30 days) for the off study assessment, the following may be performed, and then the patient will be taken off study:

- History and physical examination
- CBC with differential
- Acute care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LDH) panel
- Reticulocyte count
- Peripheral blood smear
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Bone marrow biopsy with reticulin and collagen staining and aspiration with cytogenetics
- Lymphocyte phenotyping (TBNK flow cytometry)
- Research blood as detailed in section 7
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron

General Quality of Life FACT questionnaire

The FACT instrument is a health assessment instrument that was originally designed to measure multi-dimensional quality of life in cancer therapy. There are several different subscales for anemia (FACT-An)¹⁰, neutropenia (FACT-N) and thrombocytopenia (FACT-Th) allowing it to be adapted for the specific use in patients with hematological disorders. There are 28 core generic questions which examine physical, social, emotional and functional well-being. The FACT will be completed at baseline and the end of the study (after 16 weeks and 20 weeks (+/- 4 days) of treatment). Subjects who leave the study prematurely will also be administered the FACT questionnaire.

7.0 ANCILLARY LABORATORY RESEARCH STUDIES

7.1 Collection of samples

During the course of participating on this study, an additional 30 cc of blood (NIH visits only) and 5 cc of bone marrow aspirate each time a patient has a bone marrow examination may be requested. These samples will be stored with the subject's permission for other exploratory laboratory research studies reviewed and approved by the IRB and listed in Appendix A. Research samples will be coded and stored in the secure laboratory of the principal investigator.

7.2 Intended use: These specimens will not be read by a pathologist or 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. Additional studies which are approved by the IRB and listed in the Appendix of the protocol may be done on stored samples.

- T cell receptor V-beta profile in the marrow and peripheral blood
- Extended peripheral blood flow cytometric phenotyping for cell surface or intracellular proteins
- Evaluation for the presence of abnormalities of the telomere repair complex including telomere length and genetic testing of genes associated with the telomere repair complex
- Evaluation for the presence of abnormalities of genes associated with hematopoiesis, via genetic testing or gene expression analysis
- Serum cytokine, chemokines and soluble receptor levels
- Serum (or plasma) and cells for viral analyses
- Hematopoietic progenitor colony, long term-culture-initiating cell, and immunodeficient mouse engraftment assays for primitive cell content and function
- Pharmacokinetic studies of eltrombopag kinetics performed at PI's discretion
- 24 hour urine to investigate the influence of eltrombopag on iron metabolism and renal excretion.
- In the event there is any extra sample, these will be stored with the subject's permission for other exploratory laboratory research studies reviewed and approved by the IRB and listed in Appendix A.
- Serum thrombopoietin level at baseline, weeks 16 and 20, 6 months after last dose of study drug, and if on extended access, then every 6 months NIH visits

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. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

7.4 Storage: Research samples will be stored with identifiers in the secure laboratory in the Hematology Branch.

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 breach in our plan for tracking and storage of samples has occurred, the IRB will be notified.

8.0 BIOSTATISTICAL CONSIDERATIONS

8.1 Objectives

The *primary objective* is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in moderate aplastic anemia patients or patients with bone marrow failure and unilineage cytopenia.

Secondary objectives include the analysis of the incidence and severity of bleeding episodes, clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia and the impact on quality of life.

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 hemoglobin and/or PRBC transfusion requirements and the toxicity profile as measured using the CTCAE criteria. Treatment response for the platelet lineage is defined as an absolute increase of $\geq 20 \times 10^9/L$ above baseline at 16 or 20 weeks, measured on at least two serial measurements performed one week apart and sustained for 1 month or more without support of platelet transfusions, or for transfusion dependent patients stable platelet counts with transfusion independence for ≥ 8 weeks. For patients with anemia (untransfused hemoglobin ≤ 8.5 g/dL), a treatment response will be an increase in Hb by ≥ 1.5 g/dL at four months, measured on at least 2 serial measurements and sustained for 1 month or more without transfusion support **OR** for transfusion dependent patients, reduction of units of RCC transfused by 50%/8 weeks compared with the pretreatment transfusion number in the previous 8 weeks or transfusion independence (no transfusions for ≥ 8 weeks).

Criteria for response: Treatment response for the platelet lineage is defined as platelet count increases to 20,000/uL above baseline at 16 or 20 weeks, or freedom from platelet transfusions for ≥ 8 weeks in transfusion-dependent patients. For patients with anemia (untransfused hemoglobin ≤ 8.5 g/dL), a treatment response will be an increase in Hb by ≥ 1.5 g/dL at four months, measured on at least 2 serial measurements and sustained for 1 month or more without transfusion support **OR** for transfusion dependent patients, reduction of units of RCC transfused by 50%/8 weeks compared with the pretreatment transfusion number in the previous 8 weeks or transfusion independence (no transfusions for ≥ 8 weeks). We will analyze a composite of both lineage responses and the individual responses.

Secondary endpoints will include incidence of bleeding, changes in serum thrombopoietin level (as measured by enzyme-linked immunosorbent assay), clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia and health related *General Quality of Life (FACT questionnaire)* for Subjects ages 18 and older will be completed at baseline and 16 weeks.

8.3 Sample Size

We intend to accrual a maximum of 38 subjects for this study. Because the efficacy of eltrombopag in this patient population is unknown, we would like to reject the treatment as quickly as possible with a small number of patients if the treatment is not effective. We will use the Two-Stage Minimax Design outlined in Table 1 of Simon ⁽¹⁾ with a response probability of 20% or less to terminate the treatment and the hypothesized actual response probability of 40% or more. The sample size is determined by testing the null hypothesis $H_0: p \leq 20\%$ versus the alternative $H_1: p \geq 40\%$ at a significance level of 0.05 and a power of 0.8. At the first stage, 18 subjects will be accrued and the trial will be terminated by accepting the null hypothesis if 4 or fewer subjects respond to the treatment within 16 to 20 weeks. If 5 or more subjects respond to the treatment within 16 weeks at the first stage, then an additional 15 subjects will be accrued at the second stage. If needed, the replacement of 5 drop-out subjects will bring the total number of subjects to $n=38$. The null hypothesis of $p \leq 20\%$ will be accepted (i.e., the drug will be rejected) if the total number of responders within 16 to 20 weeks is 10 or less. This sample size is calculated by the PASS power analysis and sample size software (NCSS, LLC., 329 N 1000 E, Kaysville, UT 84037).

Subjects who discontinue the study drug prematurely (before 16 weeks):

Platelet count measurement will be attempted even if a patient discontinues study drug. Patients who withdraw from the study for reasons other than lack of efficacy or toxicity (rendering platelet count missing) may be replaced. All other patients should be evaluable for efficacy. Based on the assumed drop-out rate of 15-20% and the goal of having 33 evaluable patients, 5 additional patients may be enrolled if needed.

8.4 Statistical Methods

The change of quality of life measure from baseline will be examined by the paired t-test or the Wilcoxon signed-rank. The planned analyses will include descriptive statistics on the proportions of responses (i.e. % subjects with treatment response) and the time to response. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypothesis testing. Distributions of the sample proportions will be evaluated using Binomial distributions and their normal approximations.

The time to responses will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate and Cox regression analysis for covariates, which takes consideration of both death without the event of interest as a complete risk and random censoring due to loss of follow-up. 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. The Kaplan-Meier estimates and Cox regression will be used to evaluate the probability distributions of overall survival time.

8.5 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

We anticipate the rate of these specified TRSAEs within the 3 month study period to be 20% or less. Following Geller et al. (12), 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 $(\alpha, \beta) = (0.72, 2.88)$, so that $\alpha + \beta = 3.6$ is approximately 15% of the expected sample size. The parameter are chosen so that the mean $\alpha / (\alpha + \beta) = 0.2$ as the expected proportion of specified TRSAE's and the sum $\alpha + \beta = 3.6$ as the "worth" we place on our prior clinical opinion. Practical interpretations and justifications of the Bayesian approach and the choice of the prior distributions can be found in Geller et al. 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:
≤ 5	3
≤ 9	4
≤ 13	5
≤ 16	6
≤ 20	7
≤ 24	8
≤ 28	9
≤ 33	10

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 33 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	1.8%	22.4 %	43.9 %	66.7 %	93.9 %
Average number of subjects	24.7	22.4	20.3	17.6	12.0
Average number TRSAEs	3.2	5.7	6.2	6.1	5.0

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 Patient choice: Patients 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 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) as defined in section 5.4.2

- No treatment response after 16 to 20 weeks of treatment
- 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 aplastic anemia or bone marrow failure and unilineage cytopenia
- Cytogenetic abnormalities, new or worsening morphological abnormalities or cytopenia(s) as defined in section 5.4.2.
- Non-compliance with protocol procedures
- Lost to follow-up
- Study Completion

Once off study (either by per patient choice or per PI decision), subjects will be referred back to his or her referring physician or consented to the Hematology Branch 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 Data and Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Cynthia E. Dunbar, 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 and assent forms 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.

NHLBI DSMB: The NHLBI Data safety and Monitoring Board will review the protocol at 6 to 12 month intervals. 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.

Monitoring: As per ICH-GCP 5.18 and 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 Clinical Research Associates (CRAs)/Monitors employed 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) 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 site monitors, and the NHLBI staff for confirmation of the study data.

FDA: Under IND 104,877 an annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA to via the Project Manager or designee:



Novartis: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to the CRADA Sponsor.



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.

Eltrombopag dose may be interrupted when clinically indicated at the discretion of the investigator. These interruptions will not be reported as deviations; however, when the interruption is a consequence to a serious adverse event, the interruption will be included in the SAE NIH Problem Report.

Interruptions such as delays in request for medication refills or medication errors by subjects, unless they

result in a serious adverse event or impact the integrity of the research data, will not be reported as deviations to the IRB, but will be recorded in the medical record.

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 attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at <http://ctep.cancer.gov/reporting/ctc.html>.

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.

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 study medication 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, 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 as adverse events.

Abnormal laboratory values recorded in the database will be recorded at the highest grade and resolved in the database when the value is a grade 2 or lower.

Hypertension CTCAEv4 Grade 1 is not an applicable category for this study and therefore only hypertension greater than grade 2, that require medical intervention, will be documented.

Unscheduled laboratory results or patient reports that have been sent in addition to the protocol required ones will be reviewed for AE's and if not qualifying for an AE Grade 3 or 4 will be recorded in CRIS but not in the studies database. Grade 3 or 4 AE qualifying information will be reported in the database.

The laboratory toxicities will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 4.0.

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.

Grading of Adverse events:

1	Mild	Symptom barely noticeable to subject; does not influence performance or functioning. Prescription drug not ordinarily needed for relief of symptom but may be given because of personality of subject.
2	Moderate	Symptom of a sufficient severity to make subject uncomfortable; performance of daily activities influenced; subject is able to continue in study; treatment for symptom may be needed.
3	Severe	Symptom causes severe discomfort. May be of such severity that subject cannot continue. Severity may cause cessation of treatment with test drug; treatment for symptom may be given and/or subject hospitalized.
4	Life-threatening	Symptom(s) place the patient at immediate risk of death from the reaction as it occurred; it does not include a reaction that, had it occurred in a more serious form, might have caused death.

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: <ol style="list-style-type: none"> 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: <ol style="list-style-type: none"> 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)	<p>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:</p> <ol style="list-style-type: none"> 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)	<p>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:</p> <ol style="list-style-type: none"> 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.

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 related that will be monitored and considered for early stopping of the study according to statistically determined criteria. These include death and any grade IV toxicity considered to be probably or definitely related to study medication. Dr. John Tisdale 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.

9.2.4 Reporting of Events

Principal Investigator: All adverse events will be reported to the Principal Investigator

Cynthia E. Dunbar, M.D.
TSCBB, NHLBI, NIH, Clinical Center
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9.2.4.1 Reporting Timeframes to IRB Chair, Clinical Director, and/or 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 using the IRB system or other approved formats.

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 using via email.

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 using the IRB system or other approved formats.

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.

9.2.4.4 NHLBI DSMB:

All grade 4 TRSAEs and reports of serious adverse events that are unexpected and thought to be related to the experimental drug will also be forwarded immediately to the Data and Safety Monitoring Board (DSMB). A summary of events will be included in DSMB reports for review by the DSMB.

9.2.4.5 Sponsor and FDA Reporting

IND #: 104,877

Sponsor Representative: Cynthia E. Dunbar, M.D., TSCBB, NHLBI

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 Annual Report

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)). Annual progress reports will be submitted within 60 days after the anniversary date of the IND.

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: Mara Miller, M.A.
Regulatory Project Manager, Food and Drug Administration
Food & Drug Administration Document Room
Center for Drug Evaluation and Research
Division of Hematology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266
(301) 796-0683 (phone)

9.2.4.6 Reporting Serious Adverse Events to CRADA Sponsor Novartis:

All unexpected and possibly, probably or definitely related SAEs occurring during the study or within 30 days of the last administration of eltrombopag will be reported to Novartis within 24 hours of the research team learning of the event. A copy of the SAE report will 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 events. If the SAE is unexpected and determined possibly, probably or definitely related to study drug the SAE report will be forwarded to Novartis and FDA within 24 hours of learning of event. Follow-up reports regarding the patient's subsequent course will be submitted until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment) or the patient dies. The SAE report will contain a full written summary detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports will be included. The investigator will always provide an assessment of causality at the time of the initial report as described in 'Assessment of

Causality’.

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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 Novartis within two weeks of learning of a subject’s pregnancy. Information on the status of the mother and child will be forwarded to 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 Novartis as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported to 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 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 collection and distribution:

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. All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability, 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 or other unique code, or minimum PII required for subject identification.

Novartis will receive-quarterly accrual and toxicity information as detailed in the CRADA. 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 Novartis. In accordance with local and federal regulations, the Investigator will allow Novartis personnel or their designee, access to all pertinent medical records in order to verify the data gathered and to audit the data collection process.

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 breach 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 human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

10.0 HUMAN SUBJECT PROTECTION

10.1 Rationale for Subject Selection

The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on gender, race or ethnicity.

Epidemiologic studies suggest that an estimated 2-4 million cases of aplastic anemia will be diagnosed each year worldwide; the incidence occurs in three peaks: 2-5 years, 20-25 years and 55-60 years; cases are approximately evenly split between male and females. Based on previous experience at our institution, approximately 60 new patients with aplastic anemia per year will be evaluated for protocol participation and the distribution in this patient population will be:

Gender: 60% males and 40% females;

Race/ethnicity: approximately 55% White, 15% Black, 6% Asian and 24% Hispanic;

Age: will range between 2 and 82 (median age of 30) and that 15% of patients will be under the age of 18.

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 AUC_{0-∞} 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 Aplastic Anemia 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. Because many aplastic anemia patients may respond to initial immunosuppressive treatment with a response that is sufficient to prevent serious infections, but have persistent thrombocytopenia, we will also be able to rapidly recruit study patients who have completed other trials for aplastic anemia therapy within the Branch.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population. The ability to offer patients MAA a convenient alternative to standard therapy will be a very positive addition to our aplastic anemia 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.

Subjects will not receive payment for time and inconvenience for participating in the protocol.

10.2 Participation of Pediatric patients.

The initial protocol excluded pediatric MAA patients because of lack of safety information for this patient age group at that time. However, based on the PETIT trial (NCT00908037, see also section 2.6) and our own experience in pediatric patients with naïve or refractory aplastic anemia (12-H-0150 and 13-H-0133) we have now established a dosing schedule that was associated with very limited toxicities. As discussed in section 2.6. a higher weight-adjusted drug clearance is possible, particularly in younger children. Pharmacokinetic studies (PK) in pediatric AA patients treated with eltrombopag have not been performed. Therefore, we will treat pediatric AA patients in a separate cohort according to our established dosing scheme for refractory and naïve AA patients. PK-studies will be performed. If PK studies indicate significant faster drug clearance potentially affecting its efficacy we will amend the protocol and adjust the pediatric dosing accordingly.

10.3 Risks and Discomforts:

10.3.1 Promacta® (eltrombopag)

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.**

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 10⁹/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

<p>Infections and infestations Common: Pharyngitis Urinary tract infection</p>
<p>Gastrointestinal disorders Very Common: Nausea Diarrhea Common: Dry mouth Vomiting</p>
<p>Hepatobiliary disorders Common: Increased aspartate aminotransferase Increased alanine aminotransferase Blood bilirubin unconjugated increased Uncommon: Drug-induced liver injury</p>
<p>Skin and subcutaneous tissue disorders Common: Alopecia Rash</p>

<p>Musculoskeletal and connective tissue disorders</p> <p>Common: Back pain Musculoskeletal chest pain Musculoskeletal pain Myalgia</p>
<p>Vascular disorders</p> <p>Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously</p>

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.

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

Adverse Events considered to be expected for Reporting Purposes in SAA

<p>Blood and lymphatic system disorders</p> <p>Very common: Anemia</p>
<p>Gastrointestinal disorders</p> <p>Very common: Abdominal pain, diarrhea, nausea</p>
<p>General disorders and administrative conditions</p> <p>Very common: Dizziness, fatigue, febrile neutropenia, pyrexia</p>
<p>Hepatobiliary disorders</p> <p>Very common: Transaminases increased</p>
<p>Musculoskeletal and connective tissue disorders</p> <p>Very common: Arthralgia, muscle spasms, pain in extremity</p>
<p>Nervous systems disorders</p> <p>Very common: Headache</p>
<p>Respiratory, thoracic and mediastinal disorders</p> <p>Common: Cough, dyspnea, oropharyngeal pain, rhinorrhea</p>
<p>Skin and subcutaneous tissue disorders</p> <p>Very common: Ecchymosis</p>
<p>Vascular disorders</p> <p>Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously</p>

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

<p>Blood and lymphatic system disorders</p> <p>Very common: Leukocytosis**, white blood cell count increased</p>
<p>Gastrointestinal disorders</p>

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

10.3.2 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 also section 2.4.4, non clinical toxicology)

10.3.3 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.

10.3.4 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.

10.3.5 Related to General Quality of Life FACT questionnaire: The only anticipated adverse consequences associated with the SF36 will be the time required for the participants to complete the questionnaire.

10.4 Risks in Relation to Benefit

10.4.1 For adult subjects:

The benefits to the patients could be improvement of thrombocytopenia (increased platelet count) and/or reduction or even abolition of platelet transfusion requirements, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, and/or a susceptibility to infections. Potentially, 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).

10.4.2 For pediatric subjects:

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 as follows:

(a) the risk is justified by the anticipated benefit to the subjects: We are offering pediatric subjects, with a potentially progressive and thus in some cases lethal hematological disease, a treatment option.

(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. The benefits to the patients could be reduction or even abolition of transfusion requirements and/or improvement of low peripheral blood counts, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. Potentially, treatment with other more toxic therapies could also be avoided or postponed.

(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore, participation of pediatric subjects on this protocol involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects (45 CFR 46.405).

10.5 Informed Consent Processes and Procedures

Adult Subjects:

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. The PI, Dr. Dunbar or an associate investigator on this protocol with a "*" beside their name on the cover page will lead this discussion and obtain the informed consent. 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 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, page 8, section X.:

- 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 Dr. Dunbar or an AI 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 PI 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 Cynthia E. Dunbar, M.D., TSCBB, NHLBI, NIH, Building 10, Room CRC 4-5132, 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."

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.

We anticipate the enrollment of Spanish or Vietnamese speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2. 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 MAS Policy M77-2, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. We will request prospective IRB approval of the use of the short form for up to a maximum of 5 participants in a given language and 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 participants in a given language, we will notify the IRB of the need for an additional use of the Short Form and 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, the following justification and procedures per NIH HRPP SOP 14E (Category B) will be used to enrolled participants in the this protocol. See Appendix C 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 disease. 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.

Minor subjects:

If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison).

If the minor subject is a female of childbearing age, she will be informed about pregnancy testing and will be told that if her pregnancy test is positive, we will counsel her and help her tell her parents or we will tell her parents. If she does not agree she will be advised not to sign the assent.

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.

When a pediatric subject reaches age 18, continued participation will require re-consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

- (1) The research involves no more than minimal risk to the subjects
 - a. Analysis of samples and data from this study involves no additional risks to subjects.
- (2) The waiver or alteration will not adversely affect the rights and welfare of the subjects
 - a. Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.
- (3) The research could not practicably be carried out without the waiver or alteration
 - a. Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.
- (4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
 - a. We only plan to request a waiver of re-consent for those subjects who have been lost to follow-up.

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.

10.7 FWA Coverage Agreement

Dr. Winkler is currently working at Agios Pharmaceutical and will be analyzing identifiable data as a Non-NIH, Non-Enrolling 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. Dunbar.

11.0 PHARMACEUTICALS

11.1 Eltrombopag (Promacta®)

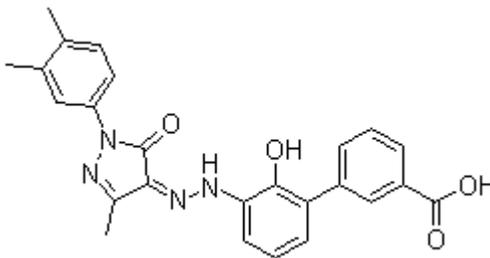
- will be supplied by Novartis as 75mg, 50 mg and 25 mg tablet.

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: C₂₅H₂₂N₄O₄.2(C₂H₇NO).

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. The tablets are available as 12.5, 25, 50, and 75 mg 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. Placebos to match the active tablets are available. Tablets are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps.

Green, oval, film-coated tablets debossed with 'SLC' on one side are provided, containing eltrombopag olamine equivalent to 200 mg or 300 mg of eltrombopag free acid. A placebo to match the active tablets is available. Tablets are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps. Desiccant may be included.

Commercial image actives (12.5 mg - white, 25 mg- orange or white, 50 mg blue or brown, 75 mg – pink, 200 mg – brown and 300 mg - blue) 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):**

Note: Currently sachets are not available, and the manufacturer is working on a new formulation. However, information regarding the use of sachets for children 2-5 years of age or older children unable to take pills is no longer applicable, because the study did not enroll any subjects that required use of the sachets.

The powder for oral suspension is a reddish brown to yellow powder in a sachet. Two PfOS strengths are available, containing eltrombopag olamine equivalent to 20 mg and 25 mg of eltrombopag free acid. Both strengths have identical powder blend composition and contains eltrombopag olamine equivalent to 20 mg of eltrombopag free acid per gram of powder. The difference in dosage strength is achieved by controlling the powder fill weight to 1.0 and 1.25 gram for PfOS 20 mg and PfOS 25 mg, respectively.

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. Doses for children between the ages 2 to 5 (2.5mg/kg non-East/South Asian participants, and 1.25mg/kg East/South Asian participants) will be provided in the form of sachets. If a child's dose is based on body weight and needs a dose of 23 mg, then dose only single sachet that provides 20 mg dose. However, if the child needs a dose of 24 mg or greater, then the suggestion is to start using the second sachet. This is mainly suggested to prevent the wastage of medicine by opening a second sachet to meet the additional 1-3 mg dose. Dosing 20 mg where a patient needs 23 mg should not have a significant impact on PD response. 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 15° to 30°C (59° 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 investigational drug may be shipped internationally to study subjects. The shipping address for Novartis supplied investigational agent is



Accountability Procedures: Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials which are destroyed, and copies of these documents will be provided to the Sponsor and Novartis. Disposition of all unused boxes of study drug

will be carried out according to instructions provided by the Sponsor and/or Novartis at the end of the study after drug accountability is performed by the study monitor.

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APPENDIX A: NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES v. 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)		
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	Microassay 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	No	No

	and function.		
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 phosphoribosyltransferase 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 spectratyping 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 serum chemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramer staining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-FISH, in situ hybridization and STELA	No	No

C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: <i>DKC1</i> , <i>TERC</i> , <i>TERT</i> , <i>SBDS</i> , <i>NOp10</i> , <i>NHP2</i> .	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 α 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	Lasar capture microdissection of cells from tumor biopsies and tissue samples to	No	No

	determine origin (donor vs patient).		
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 endovascular 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 membranous 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 B: SCHEDULE OF EVENTS

Schedule of Events for Initial Treatment Phase through weeks 16 or 20

Visit/ Time Point	Consent	Pre-study	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Wk 12 (+/-5 days)	Wk 14 (+/-5 days)	Week 16/ 20 (+/-7 days)	Wk 18 (+/-5 days)	Month 6-Off study (optional) (+/- 10 days)
Procedure												
Consent	X											
Medical history		X(†)								X		X
physical examination		X(†)								X		X
Concurrent medication review		X								X		
Complete blood count with differential		X(\$)	X	X	X	X	X	X	X	X	X	X
Reticulocyte count		X(\$)								X		X
DAT (direct antiglobulin test)		X(†)								X		
Acute care (Na, K, Cl, CO ₂ , Creatinine, Glucose, and Urea Nitrogen)		X(\$)								X		X
Mineral (Phosphorus, Magnesium, Albumin, and Calcium)		X(\$)								X		X
Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)		X(\$)	X	X	X	X	X	X	X	X	X	X
Total Protein		X(\$)								X		X
CK		X(\$)								X		X
Uric Acid		X(\$)								X		X
LDH		X(\$)								X		X
Coagulation and thrombosis screens (PT, PTT)		X(†)								X**		

Visit/ Time Point	Consent	Pre-study	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Wk 12 (+/-5 days)	Wk 14 (+/-5 days)	Week 16/ 20 (+/-7 days)	Wk 18 (+/-5 days)	Month 6-Off study (optional) (+/- 10 days)
Viral serologies for hepatitis A, B (including HBsAg, HBsAb and HB DNA PCR), C, HIV, HSV, EBV and CMV		X(†)										
Folate level		X(†)										
B12 level		X(†)										
24 hour urine collection to determine the total iron content(&)		X(†)								X**		X
Iron panel (ferritin, transferrin, % saturation) (&)		X(†)								X**		X
HLA typing (if not already performed & available)		X(†)										
Pregnancy test (blood or urine HCG in women of child bearing potential)		X(\$)		X		X		X		X**		
Research Bloods		X(†)								X		X
Bone marrow aspiration and core biopsy,*		X(†)								X**		X
Bone marrow chromosomal analysis via standard cytogenetic techniques		X(†)								X**		X
Flow cytometry of the peripheral blood to quantitate GPI-negative cells		X(†)								X**		X

Visit/ Time Point	Consent	Pre-study	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Wk 12 (+/-5 days)	Wk 14 (+/-5 days)	Week 16/20 (+/-7 days)	Wk 18 (+/-5 days)	Month 6-Off study (optional) (+/- 10 days)
Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)		X(†)								X**		X
Research Blood		X(†)								X**		X
FACT Questionnaire		X (‡)								X**		X
PK(&)										X**		

* stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities

(\$) Within 7 days of consent

(†) Within 12 weeks prior to consent

(‡) Only adults subjects 18 years and older who read English or Spanish will complete the survey

** Evaluations will only need to be completed for subjects at response assessment either at week 16 or 20.

(&) at PI's discretion

Schedule of events Extended Access phase

Visit/ Time Point	Extended Access - On eltrombopag		Extended Access - Off eltrombopag		
	Every 2 Weeks (+/- 7 days)	Every 6 Months (+/- 30 days)	monthly +/- 7 days for 6 months off eltrombopag, then every 3 months for 3 years	Every 12 months for 3 years (+/- 60 days)	Off study (optional) (+/- 30 days)
Procedure					
Complete blood count with differential	X	X	X	X	X
Reticulocyte count					X
Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)	X	X			X
Acute care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)		X			
Mineral (Phosphorus, Magnesium, Albumin, and Calcium)		X			
Total Protein, CK, Uric Acid, and LDH		X			
Research Blood				X	X
Bone marrow aspiration and core biopsy, stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities		X		X	X
Bone marrow chromosomal analysis via standard cytogenetic techniques		X		X	X
Flow cytometry of the peripheral blood to quantitate GPI-negative cells		X		X	X
Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)				X	X
Iron panel (ferritin, transferrin, % saturation) (&)		X		X	
24 hour urine collection to determine the total iron content (&)		X		X	
FACT questionnaire (‡)		X		X	

(%) only every 12 months +/- 60 days for robust responders that discontinue eltrombopag

(‡‡)not required for robust responders

(&) at PI's discretion

(‡) Only adults subjects 18 years and older who read English or Spanish will complete the survey

11-H-0134

Cynthia E. Dunbar, M.D.

01/02/2019 (Amendment HH)

APPENDIX C: 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 partner, 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**.