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<u>Subjects in study:</u>	<u>Number</u>	<u>Gender</u>	<u>Age range</u>
	60	M/F	≥2 years

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Ionizing Radiation for Research: No (Medically indicated only)

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Précis

Severe aplastic anemia (SAA) is a life-threatening blood disease that can be successfully treated with immunosuppressive drug regimens or allogeneic stem cell transplantation. However, 20-40% of patients are ineligible for transplant due to lack of an appropriate donor, age, or comorbidities. Immunosuppression can be more broadly utilized, but about 1/3 of patients do not respond to a single course of horse ATG and cyclosporine and have persistent severe cytopenias. Among patients who do respond to immunosuppression, responses may be partial, with persistent thrombocytopenia, neutropenia, and/or anemia. About 30% of responding patients either relapse or are dependent on continued cyclosporine administration. Patients with refractory severe cytopenias are at risk of dying from infection or bleeding, and they require regular platelet and/or red blood cell transfusions, which are expensive and inconvenient. Patients with refractory SAA are also at risk for progression to other hematologic disorders, including myelodysplasia and leukemia.

Thrombopoietin (TPO) was first identified as the principal protein regulating platelet production, and it stimulates the proliferation of megakaryocytes and release of platelets. TPO was later shown to stimulate proliferation of more primitive bone marrow stem and progenitor cells in vitro and in animal models, suggesting it could have an impact of production of red and white blood cells as well as platelets.

The 2nd generation oral small molecule TPO-agonist eltrombopag (Promacta®) has been shown to increase platelets in healthy subjects and in thrombocytopenic patients with chronic immune thrombocytopenic purpura (ITP) and hepatitis C virus (HCV)-infection. Eltrombopag has been well-tolerated in clinical trials, and unlike recombinant TPO, it does not induce autoantibodies. Eltrombopag received FDA accelerated approval on November 20, 2008 for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. In November 2012, FDA approval was received for hepatitis C associated thrombocytopenia.

We conducted a pilot dose finding study in patients with severe aplastic anemia who had refractory thrombocytopenia following standard immunosuppressive therapy. Patients began at a dose of 50 mg/day and escalated every two weeks to a maximum dose of 150 mg/day. We reported that 11 of 25 patients (44%) achieved hematological response in at least one lineage following 12 weeks of dose-escalating eltrombopag therapy, with minimal toxicity. Responding patients as assessed at 12 weeks were invited to continue on drug in an extension phase. With a median follow-up of 27 months on drug, 7 eventually became tri-lineage responders. Nine became transfusion-independent for platelets (median increase in platelet count 34,000/ μ l), six had improved hemoglobin levels (median increase of 3.8g/dL), including three previously dependent on red cell transfusions achieving transfusion-independence, and eight exhibiting increased neutrophil counts (median increase 590 cells/ μ L). Serial bone marrow biopsies demonstrated normalization of tri-lineage hematopoiesis in responders, without increased fibrosis.

In the previous study, response assessment occurred at 12 weeks, and patients not fulfilling response criteria at that time had the drug discontinued. Several patients began to have detectable changes in transfusion requirements or blood counts by 12 weeks, but did not fulfill response criteria by that time point and therefore had to discontinue eltrombopag. Other patients who barely met response criteria at 12 weeks showed very marked further improvements in blood counts in all lineages during the extension phase, in some cases not reaching maximal responses until one year after initiating eltrombopag. We hypothesize that a larger fraction of patients may respond if eltrombopag is continued for longer than 12 weeks.

We, therefore propose a follow-up Phase 2 study giving eltrombopag treatment for 24 weeks prior to definitive response assessment, and initiating study medication at a fixed dose of 150 mg/day (75 mg/day for individuals of East Asian ethnicity), given lack of toxicity at that dose in the prior study, and no evidence for response in any patient during dose escalation prior to reaching this dose. Responses will be assessed in all three lineages. Subjects

with platelet, red cell, and/or neutrophil responses at 24 weeks may continue study medication (extended access) until they meet off study criteria.

The **primary objective** is to assess the efficacy of 6 months of eltrombopag administration in improving bone marrow function in SAA patients with persistent severe cytopenias refractory to treatment with immunosuppressive treatment.

Secondary objectives include assessment of relapse or clonal evolution, pre-treatment characteristics predicting response, and the impact of treatment and treatment response on quality of life.

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1 Objectives

The *primary objective* is to assess the efficacy of 6 months of eltrombopag administration in improving bone marrow function in SAA patients with persistent severe cytopenias refractory to treatment with immunosuppressive treatment.

Secondary objectives include assessment of relapse or clonal evolution, pre-treatment characteristics predicting response, and the impact of treatment and treatment response on quality of life.

2 Background and Scientific Justification

2.1 Pathophysiology of Aplastic Anemia

Aplastic anemia is a serious hematologic disease characterized by pancytopenia and a hypocellular bone marrow. Although the exact etiology of aplastic anemia is not known, clinical experiences and laboratory data suggest that the primary mechanism leading to development of bone marrow failure is immune-mediated destruction of hematopoietic stem and progenitor cells.¹ Specific populations of effector T-cells are elevated and localized to the bone marrow in aplastic anemia, including activated cytotoxic T-cells expressing HLA-DR, the IL-2 receptor, and IFN- γ .²⁻⁴ The effects exerted by cytotoxic T-lymphocytes are mediated in part due to Fas ligand-induced apoptosis of hematopoietic progenitor cells; IFN- γ , in addition to its intrinsic inhibitory activity on hematopoietic progenitor and stem cells, can induce over-expression of Fas on target cells.⁵ High resolution VB CDR3 analysis in patients with aplastic anemia shows significantly increased nonrandom skewing of the VB-chain families of the T cell receptor, suggestive of disease specific clonal expansion.⁶ Immune-mediated marrow destruction with many similarities to the pathophysiology of human aplastic anemia can be modeled in the mouse.⁷

Despite its often acute presentation, aplastic anemia is now recognized as a chronic disease with frequent flares of the immune process and the need for long-term immunosuppression. There is evidence that depletion of primitive hematopoietic stem and progenitor cells is profound, demonstrating that immune attack against the most primitive stem cells is paramount.⁸ Even with recovery of blood counts following successful immunosuppressive therapy, a significant quantitative stem cell defect persists, suggesting either ongoing immune destruction or persistent depletion of stem cells even in the absence of an active immune process.⁹

2.2 Clinical consequences of aplastic anemia

Symptoms derive from low blood counts. Anemia leads to fatigue, weakness, lassitude, headaches, and in older patients dyspnea and chest pain, and these manifestations are most commonly responsible for the clinical presentation. Thrombocytopenia produces mucosal bleeding: petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage. Bacterial and fungal infections in the setting of neutropenia are a major cause of morbidity and mortality, and most often the cause of death in refractory or untreated aplastic anemia.

2.3 Management of SAA refractory to immunosuppression

Up to 40% of patients will not respond to an initial course of immunosuppression with ATG and cyclosporine (CSA). A significant number of these can be salvaged by a second course with rabbit ATG/CSA. Our group has shown alemtuzimab monotherapy to have equivalent efficacy in this setting and this may be desirable option in elderly patients who experienced toxicity with CSA¹⁰. In younger patients who have not responded to

immunosuppression an unrelated donor stem cell transplant is the best long-term option. Those who do not have a high resolution genetic match should be considered for alternative donor regimens especially in the setting of severe neutropenia.

The majority of patients achieve a hematological response with one or more courses of immunosuppression. However, a significant minority of SAA patients have persistent severe cytopenias, and in these patients a deficiency in stem cell numbers may explain failure to reconstitute adequate hematopoiesis despite adequate immunosuppression. These patients represent an unmet medical need with few options beyond supportive care with red cell and platelet transfusion available. Using a TPO-R agonist like eltrombopag to treat these patients is an alternative approach that has shown promise in a recent study from our group³⁸.

2.4 Thrombopoietin and Hematopoiesis

Thrombopoietin (TPO) was purified, identified and cloned by independent research groups in academia and industry in the mid 1990s, based on its activity as the primary factor stimulating maturation of megakaryocytes and platelet release, and its binding to the receptor c-mpl. TPO is a glycoprotein class 1 hematopoietic cytokine, produced primarily in the liver.

A number of lines of evidence support a pleiotropic role for TPO in hematopoiesis, beyond function as the primary endogenous factor controlling platelet production. The c-mpl receptor is expressed and functional on primitive hematopoietic stem and progenitor cells.¹¹ Animals and patients with genetic defects in either TPO or c-mpl have significant reduction in HSC numbers and activity, along with profound defects in platelet production.^{12,13} *In vitro* expansion of functional and phenotypic HSCs can be stimulated by TPO, either alone or in combination with other cytokines.¹⁴

The control of TPO levels and TPO production is complex, and involves sensing of c-mpl receptor occupancy, with levels generally inversely proportional to megakaryocyte mass. In early studies performed in our Branch, we demonstrated that TPO levels were extremely high in SAA and surprisingly low to normal in chronic ITP, comparing patients with these two conditions with equivalent platelet counts.¹⁵ More recent studies also confirm TPO levels to be high in SAA and moderately elevated in myelodysplastic syndromes compared to normal controls.¹⁶

A slightly modified form of recombinant TPO, termed megakaryocyte growth and development factor (MGDF), was in clinical development by Amgen in the late 1990s. It clearly stimulated platelet production *in vivo* in healthy control individuals and in chemotherapy patients, but its development came to a halt when several normal volunteers receiving MDGF prior to donating platelets developed neutralizing antibodies, which reacted not only to MDGF but also to endogenous TPO, causing profound persistent thrombocytopenia.

2.5 Eltrombopag

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 by scientists at GlaxoSmithKline.¹⁷ Studies conducted *in vitro* have shown that eltrombopag is an effective agonist binding to *mpl*, the TPO-R, to stimulate thrombopoiesis. It binds *mpl* at a position distinct from the ligand binding site, within the juxtamembrane domain of the receptor, and thus does not compete with TPO for binding to its receptor.¹⁸ The differences in binding to the receptor may theoretically result in activation of different signaling pathways from native thrombopoietin, however, to date, data indicates similar impact on megakaryocytes and stem cells to thrombopoietin.¹⁹

In vivo, eltrombopag increased platelet number in the chimpanzee (the only non-human species which is pharmacologically responsive to eltrombopag).¹⁹ These findings, coupled with supporting clinical efficacy data

in humans, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous thrombopoietin (TPO). Initial clinical trials were carried out in normal volunteers, and then in patients with chronic ITP, based on their inappropriately low or low-normal levels of endogenous thrombopoietin.¹⁵ The initial phase I/II and randomized, controlled phase 3 registration trials in chronic ITP were very encouraging, with little toxicity and much higher responses in comparison with placebo²⁰⁻²², which led to its approval by the Food and Drug Administration (FDA) on November 20, 2008 in patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. Eltrombopag is the first oral thrombopoietin (TPO) receptor agonist approved for adult patients with chronic ITP. Recent approval was granted for Hepatitis C associated thrombocytopenia.

Longer follow-up in the EXTEND trial suggests that eltrombopag remains well tolerated (EXTEND Trial, NCT00351468). On December 6, 2011 it was announced that the FDA agreed to modify eltrombopag's Risk Evaluation and Mitigation Services designed to assure safe use of the novel agent, and removed the requirement for healthcare professionals and institutions to enroll in the Promacta Care Program (Promacta[®] Package Insert, 2011). Continued monitoring of adverse events for eltrombopag will be monitored via post-marketing surveillance programs and ongoing clinical trials, rather than a formal prescriber enrollment program.

2.5.1 Eltrombopag for refractory SAA

Reasoning that eltrombopag stimulated primitive hematopoietic stem cells and progenitors and as there was a clear deficit in HSC number and function in SAA, in 2009 we initiated a single-arm dose escalation phase I/II trial for SAA patients with refractory thrombocytopenia³⁸. For protocol entry, all patients had to have severe thrombocytopenia following at least one prior regimen of immunosuppressive therapy for an original diagnosis of SAA. The primary endpoints were safety and clinically significant hematologic response. The study design and response criteria are shown in Supplemental Figure 1 (Appendix B).

At last analysis, 26 patients were enrolled in the protocol, of whom 25 received study drug. As shown in Table 1, this patient population had very prolonged and serious cytopenias before study entry. All were platelet transfusion-dependent, and most also required frequent red blood cell transfusions, and were severely neutropenic and thus susceptible to life-threatening infections. All had failed at least one prior cycle of high dose immunosuppression which was administered more than six months prior to study entry, with the majority failing two and some as many as four prior cycles of immunosuppression. The median time since last immunosuppression was 14 months, with a range of up to 117 months, excluding any chance that responses could be attributed to prior immunosuppressive therapy.

Table 1. Baseline Characteristics of Study Patients.

Age (median)	44
Range	18-77
Race	N (%)
White	12 (46)
African American	7 (27)
Asian	1 (4)
Hispanic	6 (23)
Male sex	14 (54)
Time from last IST (Mo.)	
Median	14
Range	6-117
Transfusion dependent PRBCs	23 (88)

Platelets	25 (100)
Baseline parameters	Median (range)
Platelets (K/uL)	9 (5-15)
Neutrophils (K/uL)	0.8 (0.07-2.8)
Hemoglobin (g/dL)	8.0 (6.0-13.8)

In all but one patient (in whom drug was discontinued at 125 mg/day due to possible cataracts, as described below), drug was escalated to the maximum dose of 150 mg per day, and this dosage was very well tolerated. All severe adverse events (SAE), and all grade 2 and higher adverse events (AE) that were possibly, probably, or definitely attributed to eltrombopag treatment are listed in Table 2. There was one SAE that was possibly related to eltrombopag treatment: a patient with a history of diabetic gastroparesis was hospitalized for recurrent abdominal pain while taking eltrombopag. There were no grade 4 or 5 AEs. One patient developed acute hepatitis B infection with a grade 3 elevation of his hepatic transaminases to greater than 6X the upper limit of normal while on study, so the drug was discontinued; however, the transaminase elevation was almost certainly related to acute infection with hepatitis B. He was taken off study, and with recovery from hepatitis B infection, serum transaminase values returned to baseline.

Table 2. Adverse Events, and Grade 2 or Higher Non-Hematologic Adverse Events

Severe adverse events		N	Dose (mg)	Related to eltrombopag
Allergic	Cephalosporin reaction	1	100	Unlikely
Cardiovascular	Orthostasis	1	Off	Unlikely
Gastrointestinal	Abdominal pain	1	125	Possibly
Hematologic	Gingival bleeding	1	100	Unlikely
Infection	C. difficile colitis	1	150	Unlikely
	Neutropenic fever	6	100, 150 x3, off x 2	Unlikely x 6
		1	Off	Unlikely
	Gastroenteritis	1	150	Unlikely
Non-hematologic AEs	Grade 2	Grade 3	Grade 4	Related to study drug
Constitutional muscle weakness	-	1	-	Possibly
Dermatology/skin Rash	-	1	-	Possibly
Metabolic ALT, AST increased	-	1	-	Possibly
Psychiatric Depression	-	1	-	Possibly

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase;

Prior studies in patients with chronic ITP had raised the concern that TPO mimetics, including eltrombopag, might

increase bone marrow reticulin deposition, although long-term follow up of patients in the ITP EXTEND study has not revealed any significantly increased reticulin in 113 patient marrows performed after up to 4.5 years on drug.²³ We performed bone marrow biopsies with reticulin staining at baseline and after three months of eltrombopag treatment to assess for fibrosis. Patients on the extended access protocol underwent bone marrow biopsies with reticulin staining every six months. Reticulin deposition was graded by a single hematopathologist in a blinded manner on a scale of 0-4 according to standard guidelines. Two patients refused to have the 12-week response assessment bone marrow performed. Among 23 patients assessed, there was no significant increase in reticulin staining either at three months, or on serial biopsies in patients on prolonged eltrombopag treatment (Supplemental Fig. 4 in Appendix B).

There was initial concern that eltrombopag use could be associated with cataracts, based on preclinical animal toxicology studies, and initial trials in patients with ITP incorporated screening for cataract formation, as did our prior study in patients with SAA. We performed ophthalmologic examinations to assess for cataracts at baseline, after three months on eltrombopag, and every six months in patients on the extended access protocol. One patient was found to have possible new grade 2 cataract prompting discontinuation of eltrombopag, but this finding was not confirmed on a second examination performed 2 months later, or on subsequent serial eye examinations. No other patients had new cataract formation or worsening of existing cataracts after treatment with eltrombopag. The concern over cataract formation has been mitigated by lack of evidence for any relationship to eltrombopag in the completed and ongoing eltrombopag randomized controlled trials, and prospective monitoring for cataract formation has not been required by the FDA for subsequent studies.

Among 25 evaluable SAA patients, 11 (44%) achieved protocol-defined hematologic response after 12 weeks of eltrombopag treatment (Supplemental Fig. 2, in Appendix B). Patients reaching a response at 12 weeks and maintaining it to 16 weeks were continued on an extension phase, and continued to receive eltrombopag 150 mg per day. All patients were platelet transfusion-dependent at the time of enrollment, and nine patients achieved platelet transfusion independence after eltrombopag treatment, with patients on the extension phase continuing to show a gradual increase in platelet counts over time (Supplemental Fig. 3A in Appendix B). Two patients achieved a hemoglobin response by 12 weeks, and 4 additional patients had improved hemoglobin levels on the extension phase (Supplemental Fig. 3B in Appendix B), with a median hemoglobin increase of 3.6 g/dL (range 1.5-8.2 g/dL). Four patients who were previously dependent on packed red blood cell transfusions achieved transfusion independence and two were able to be phlebotomized to treat transfusional iron overload. Seven neutropenic patients had increased neutrophil counts after eltrombopag treatment (median increase 590K cells/ μ L), including 4 patients who were severely neutropenic at baseline (Supplemental Fig. 3C in Appendix B). It is notable that patients who reached response criteria and thus were continued on the drug continued to improve over time, suggest that some non-responders potentially could have achieved clinical improvement had the drug been given for more than 12 weeks.

The patient with an unconfirmed finding of cataracts at 12 weeks achieved a platelet response, and he has maintained platelet transfusion independence for 19 months despite discontinuing eltrombopag. This patient also improved his hemoglobin by 3g/dL, which enabled him to undergo therapeutic phlebotomy to treat his transfusional iron overload. The events in this patient suggest that continued eltrombopag might not be required to maintain HSC recovery.

Of the 11 responders, 7 were enrolled in the extension study. With a median follow up of 33 months 4 patients had sufficiently robust counts (platelets $>50,000/\mu$ L, Hb > 10 g/dL in the absence of transfusions, and neutrophils $> 1,000$ for more than 8 weeks) to allow discontinuation of drug. All have maintained their counts off eltrombopag to date, with time off drug between of 4-6 months. Two patients remain on drug at full dose having not attained counts adequate to taper drug. One patient with a partial response became progressively cytopenic after 12 months on drug and went off study after cytogenetic and morphologic evidence of evolution to MDS was seen on bone marrow examination.

Marrow biopsies were performed at study entry, at the 12-week response assessment, and for responders at one year after study entry. Supplemental Figure 4 shows pre-treatment and one-year biopsies. There is striking normalization of cellularity in three of four responders. There was no increase in reticulin on 12-week biopsies in any patient, and no increase in fibrosis in follow-up marrows at 6 or 12 months in responders remaining on the extension phase.

Of the 14 non-responders, two patients died of disease progression, from complications of severe cytopenias. Two nonresponding patients showed morphologic changes and cytogenetic abnormalities (monosomy 7) consistent with progression to myelodysplasia; one patient ultimately died, and the other underwent allogeneic stem cell transplantation. In addition, one non-responder in the unpublished cohort has subsequently developed trisomy 21. One other subject who was a non-responder in an independent ongoing study of eltrombopag for moderate aplastic anemia study (NCT01328587) was then eligible for the SAA study utilizing eltrombopag and ATG/CSA. Three months after study entry she developed monosomy 7 with complex cytogenetics.

In our cohort of over 400 patients with SAA followed long-term, approximately 15% progress to clonal disease including MDS and acute leukemia. Our experience also suggests that patients with long-standing severe refractory disease and who lack of a robust response to initial immunosuppression, as were enrolled on our protocol, are the most likely to progress.

Clonal evolution to myelodysplastic syndrome is a serious complication of aplastic anemia. Rates are in the range of 10-15% over 10 years²⁴. Such patients may go on to develop worsening of their disease or acute myeloid leukemia. There is a theoretical concern that TPO-R agonists, being growth factors, may increase the risk for clonal evolution although across the clinical trials in ITP (n=493) no difference in the incidence of malignancies or haematological malignancies was demonstrated between placebo and eltrombopag treated patients. This is consistent with recent basic research experiments, in which no malignant cell proliferation has been demonstrated upon co-incubation of eltrombopag with MDS cell lines, multiple leukemic cell lines and solid tumor cell lines (colon, prostate, ovary and lung). A randomized, double-blind, controlled trial of romiplostim, an alternative TPO analog, in patients with MDS, was recently stopped early due to DSMB concerns regarding a potentially higher rate of progression from MDS to AML in the romiplostim arm. However, longer-term follow-up and complete analysis of the patients enrolled in this trial was reported at the 2012 American Society of Hematology meeting, and now shown no greater risk of progression to AML in the romiplostim versus the control group. Patients with low, intermediate and high risk MDS and thrombocytopenia are being studied in a number of trials of eltrombopag, and monitoring to date does not indicate concerns regarding an unexpected rate of progression to AML. Refractory AML patients are being treated with eltrombopag as a single agent, and at least one patient with a poor prognosis cytogenetic abnormality (monosomy 7) has gone into a complete remission now for over nine months on treatment with the drug²⁵.

We cannot be certain that eltrombopag treatment does not contribute to progression to clonal marrow disorders, but as detailed above, results to date of treatment of MDS and even AML with eltrombopag or the alternative TPO mimetic romiplostim do not suggest that these drugs increase the risk of progression to more advanced disease even in patients that already have developed MDS or AML.

2.5.2 Rationale for dose selection

Eltrombopag 150 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 our recently-completed non-randomized, off label, phase II study (NCT00922883) of eltrombopag as a single agent in patients with refractory SAA. Twenty-five (25) patients (age range 18-77 years) received 50 mg daily of eltrombopag with dose escalation every two weeks to a maximum dose of 150 mg daily. Patients were successfully escalated to the 150 mg daily dose without dose-limiting toxicities. Hematologic responses were only observed while patients were receiving the 150 mg daily dosing. While it is possible that patients would have responded to lower doses of drug had the dose not been

escalated every two weeks, the lack of toxicity at the 150 mg/day dose in the prior study supports our rationale for utilizing this dose throughout the current trial, unless dose modifications based on toxicity or response criteria are required. A starting dose of 75 mg once daily in East Asian and South East Asian patients will be used. Modified dosing for subjects of East Asian and South East Asian heritage (self-reported) 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 and South East Asian subjects as compared to non-East Asian subjects who were predominantly Caucasian as higher drug exposure in East Asian and South East Asian subjects has been observed.

There is preliminary safety data with doses over 150 mg/day. 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 $\mu\text{g/mL}$ for the 200 mg once daily regimen²⁵. Eltrombopag was well tolerated in healthy subjects at all dose levels. In a recently completed open label study for patients with soft tissue sarcomas (NCT00358540), eltrombopag doses of up to 150 mg have been given in conjunction with chemotherapy, without significant side effects. In several ongoing trials of eltrombopag in myelodysplasia, patients have been escalated to doses of 300 mg/day without dose-limiting adverse events²⁶.

The most extensive data on dosing and long-term side effects has been obtained in patients with chronic ITP. An initial randomized phase 2 trial, followed by two randomized phase 3 trials all showed efficacy for eltrombopag compared to placebo for increasing the platelet count utilizing doses of up to 75 mg per day.²⁰⁻²² In ITP subjects, there was a dose response for eltrombopag 30 mg to 75 mg once daily, with geometric mean $AUC_{(0-\tau)}$ values of 169 $\mu\text{g/mL}$ 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. A recent update of the EXTEND study reported on 302 patients followed up to 5.5 years on eltrombopag therapy for chronic ITP, with a median exposure of 121 weeks and median dosing of 51.4 mg/day. Forty-three (43) patients (14%) discontinued therapy due to adverse events, including headaches, thromboembolic events, or hepatobiliary laboratory abnormalities. An independent central pathology review of bone marrow biopsies did not reveal significant increase in reticulin deposition.

Thrombocytosis is a theoretical risk of eltrombopag treatment when high dosages are administered. However, thrombocytosis has not been observed in the 25 patients with refractory SAA who were treated with 150 mg per day. Thrombocytosis has been observed in healthy volunteers as well as in subjects with ITP, and there was a suggestion of a higher risk of thrombosis in patients on eltrombopag compared to placebo in the phase III randomized trials for chronic ITP.²² However, patients with ITP, in contrast to patients with SAA, have hyper-reactive platelets and an increased endogenous risk of thrombosis. In an extensive analysis of ITP patients treated long-term with romiplostim, an alternative TPO mimetic, there was no evidence for increased thrombotic events in the romiplostim-treated patients compared to controls.²⁷ In a meta-analysis of randomized trials using either eltrombopag or romiplostim, there was a numerically but non-statistically significant trend to increase the occurrence of thromboembolisms compared to controls.²⁸ In the current trial, based on concern regarding thrombosis, dose reductions of eltrombopag will be made if the platelet count reaches 200,000 per μL or greater.

2.5.3 Rationale for pediatric dose selection

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]. In the ongoing open-label phase of PETIT, a phase II pediatric chronic ITP study, subjects between 1 and 5 years received 1.2 – 2.5 mg/kg eltrombopag once daily, while subjects between 6 and 17 years of age received an average daily dose of 58.5 mg daily (NCT00908037). The maximum dose

used in the PETIT trial among all age groups is 75 mg daily dose. Cohort 3 (ages 1 to 5 years) was opened for patient recruitment on 01 June 2011 and the initial group of 5 subjects has been enrolled. Preliminary data have been evaluated for an initial group of 5 subjects aged 1 to <6 years enrolled in Cohort 3 of PETIT. 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. 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.

2.6 Rationale for Permitting Dose Interruption

The effect of dose interruption is unknown in the aplastic anemia population. Thirty-one percent (34 ITP subjects) on the long-term extension study (EXTEND Trial, NCT00351468) had an interruption to eltrombopag dosing at some point in the study. Of the subjects requiring a dose interruption, 7 had a dose interruption lasting 1 to 7 days and 27 had a dose interruption lasting greater than 7 days. Platelet counts decreased back to baseline within 1-2 weeks, although not associated with any bleeding complications. However, the underlying pathophysiology of thrombocytopenia in ITP is very different from SAA, and eltrombopag is being utilized in that disorder to overproduce platelets in the bone marrow to compensate for increased antibody-mediated platelet destruction. In SAA we postulate an effect on HSCs in the bone marrow, and a much more prolonged effect from eltrombopag; therefore, our prediction would be that short or even longer term dose interruptions will not result in any sudden changes in blood counts. We would anticipate some patients on the current trial will be hospitalized for other disease-related issues such as fever and neutropenia and may require suspension of the study drug temporarily.

One patient in our recent trial of eltrombopag for refractory SAA had drug discontinued before the three month time point, despite meeting criteria for response, because of possible cataracts noted on eye examination (later deemed to have been a normal lens examination on repeat testing). His response continued, now for over 18 months, with improvements in all three hematopoietic lineages despite no further eltrombopag treatment, suggesting that the effect of eltrombopag on HSC number or function is long-lasting and prolonged therapy may not be required in SAA. The majority of patients on the extension phase of the prior SAA trial (NCT00922883) have now been tapered completely off eltrombopag, based on meeting blood count criteria for tapering and then discontinuing the drug. None have relapsed and required re-initiation of eltrombopag.

2.7 Rationale for extended access to study medication

Eltrombopag is safe and relatively non-toxic with prolonged use. The EXTEND study (TRA105325), an open label dose modification extension study evaluating the safety and efficacy of extended therapy of eltrombopag in ITP subjects, has been designed to assess long-term effects of eltrombopag therapy. At the most recent analysis, the median average daily dose was 51.4 mg. The median duration of exposure to eltrombopag was 121 weeks (range 0.3-285 weeks), with 253, 217, 176, 59 and 10 subjects treated for at least 6, 12, 24, 48, and 60 months, respectively. Eltrombopag was well-tolerated, and both bleeding and clinically-significant bleeding decreased from baseline at all timepoints. AEs and SAEs occurred in 91% and 29% of patients respectively. The most frequent AEs were headache (27%), nasopharyngitis (24%) and upper respiratory tract infection (22%). 6% had thromboembolic events, including DVT, strokes, or myocardial infarctions. Hepatobiliary abnormalities were seen in 12%, and most resolved while on treatment or after discontinuation. There was no significant increase in marrow reticulin in bone marrow biopsies obtained from 113 patients at time points up to 4.5 years on drug. 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 24 weeks in the current study, in patients responding to the drug until they reach blood count normalization sufficient for tapering as detailed below. Toxicity and efficacy data will continue to be collected during that time to help identify the secondary endpoints of duration of

response, progression to clonal hematopoiesis, and toxicities with extended duration of therapy.

We hypothesize, based on interim results in patients on the extension phase of the prior trial 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.

In the first 25 patients treated, responses only began to be observed at the 3 month time point, and with continued exposure to drug, blood counts of all lineages improved towards the normal range gradually over time periods up to 31 months to date. Bone marrow cellularity began to normalize by 9-15 months. Maximal response was not reached at 6 months, therefore patients in the current trial may continue to improve their blood counts during the extension phase of the study.

2.8 Rationale for tapering eltrombopag during the extension protocol

As discussed above, clonal evolution to myelodysplastic syndrome is a serious complication of aplastic anemia, and given the theoretical concern that chronic stimulation with a growth factor might accelerate progression, the lowest dose and shortest exposure able to improve cytopenias into a safe range would be the goal for eltrombopag therapy.

In our prior study (NCT00922883), we have been able to taper 4 of 7 responding patients on the extension study completely off eltrombopag, and 2 remain on the 150mg dose. None have relapsed during the taper or after drug discontinuation. The only responding patient on the extension trial who lost response was the patient that developed clonal hematopoiesis, a new deletion 13q, and evidence for MDS on his bone marrow biopsy.

In the analysis of the first 25 patients, two non-responding patients who failed to achieve a hematologic response developed clonal evolution to monosomy 7 and MDS after completing three months on the study; one died after progression to acute myeloid leukemia, and a second patient had HSCT. Despite this incidence of progression being within our historical expected rate of evolution to clonal disease in patients with refractory SAA, we feel it is prudent to maintain responding patients in the extension study on the lowest dose of eltrombopag that will maintain stable improved blood counts. It is possible that once the drug expands hematopoietic stem and progenitor cells in vivo, further treatment with eltrombopag will not be required to maintain transfusion independence in these patients. A single patient had drug stopped due to the erroneous diagnosis of a new cataract at week 9 of treatment; this patient continued to show a remarkable multi-lineage response, and maintains a platelet count in a safe range and a hemoglobin level high enough to permit therapeutic phlebotomy to address transfusional iron overload. The schedule for taper of drug is detailed in section 5.2.

2.9 Scientific and clinical justification of the protocol

There is no standard and clearly effective treatment available for SAA patients who are refractory to treatment with standard immunosuppressive therapy and are not eligible for HSCT. Patients with persistent cytopenias are maintained on regular red cell and/or platelet transfusions, and they are at risk of bleeding and serious infections. Eltrombopag may be an alternative approach in these patients as demonstrated by our phase II dose-escalation study³⁸. Eleven of 25 patients (44%) with refractory severe aplastic anemia achieved hematological response in at least one lineage following 12 weeks of dose escalation treatment, with minimal toxicity. Until drug was tapered, the median follow-up was 27 months on drug, with 7 responding patients continuing on drug in the extension phase of the protocol, and had continued normalization of blood counts and marrow cellularity. At most recent follow-up with a median of 27 months, 6 of 7 were transfusion-independent for platelets and red blood cells and had neutrophil counts of greater than 0.75k/ μ L. In these patients serial bone marrow biopsies

demonstrated near normalization of tri-lineage hematopoiesis and marrow cellularity, without increased fibrosis. One responding subject evolved to MDS.

Data from this study has been informative both in determining optimal dosing and the length of treatment required for hematological responses in patients with SAA refractory to ATG/CSA. All patients required and tolerated escalation to the highest planned dose of 150 mg. There was no increase in toxicity associated with higher doses. Several patients began to have detectable changes in transfusion requirements or marrow function by 12 weeks, but they had not achieved the protocol-defined response criteria by that time point and therefore had to discontinue treatment. In retrospect, we hypothesize that additional patients may have attained a response if eltrombopag treatment had been extended for greater than 3 months. Responding patients who remained on drug during the extension study continued to show remarkable progressive improvement in their blood counts, including in lineages other than the platelet lineage. We have tapered responding patients who have reached satisfactory blood count thresholds (platelets greater than 50,000; hemoglobin greater than 10g/dL, and neutrophils greater than 1000/ μ L) off treatment, and of the 4 patients now completely off drug, none have relapsed.

Our hypothesis in the proposed study is that using a fixed dose of 150 mg/day and extending out the response assessment to 6 months will increase the rate of response in this refractory group of patients with few treatment options. All patients would commence eltrombopag at a dose of 150mg per day (75mg in those of East and Southeast Asian ancestry due to lower drug clearance in this population). The primary endpoint would be hematological response rate at 6 months. We believe these modifications would increase response rates appreciably and provide an alternative treatment option in this very difficult to treat patient group.

The improvement in production of not only platelets but also neutrophils and red cells in some responding patients was encouraging and suggests that eltrombopag may be a treatment with significant potential benefit for patients with aplastic anemia. We hypothesize that the drug expanded the number of hematopoietic stem and progenitor cells, replenishing a marrow compartment depleted by prior immune attack, and allowing normalization of hematopoiesis. It suggests that immune attack in patients may not be ongoing, and that poor blood count recovery after immunosuppressive therapy may result from HSC depletion that is not endogenously corrected. The proposed treatment schedule will potentially capture a greater number of responders. The slow cycling of HSCs may require more prolonged treatment with the drug in order to allow sufficient repopulation of the marrow compartment.

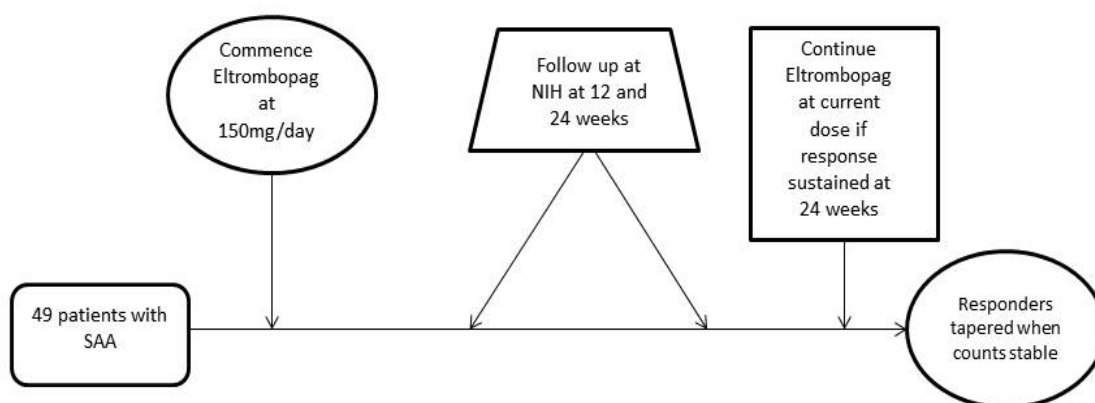
Patients with refractory SAA are at risk of progression to clonal marrow dysfunction, including MDS and leukemia. Continued proliferative stress and inflammatory attack on a very limited number of remaining hematopoietic stem cells may predispose to clonal transformation due to telomere attrition, and oncogenic escape mutations. There is concern that chronic hyperstimulation with hematopoietic cytokines could accelerate or promote clonal transformation. A retrospective Japanese study of 167 children showed that while those with severe aplastic anemia treated with G-CSF and immunosuppression evolved to MDS/AML, there were no evolutions in those children treated with immunosuppression alone²⁹). Another retrospective study by the European Group for Blood and Marrow Transplantation in 2006 showed a significantly higher hazard ratio of developing MDS/AML with the use of G-CSF in patients treated with immunosuppression for severe aplastic anemia³⁰. However large prospective multi-institutional studies did not support this increased risk^{31,32}. Eltrombopag is being investigated as therapy for both MDS and for acute myeloid leukemia, based on experimental laboratory data suggesting that normal HSCs are stimulated relative to leukemic cells by this drug³³. Multiple ongoing trials have shown efficacy in improving platelet counts in some patients with MDS, and to date no evidence for an increased rate of progression to AML. One patient with monosomy 7AML has entered a complete and sustained remission of her leukemia with eltrombopag monotherapy²⁵. In our initial SAA trial, two non-responding patients developed new clonal abnormalities consistent with progression to MDS (monosomy 7) following the 12 weeks of eltrombopag therapy. One additional responding patient aged 68, who had the least robust improvement in counts of those on the extension study, developed new deletion 13 and dysplastic features and came off drug. These observations require longer follow-up and additional patients with SAA to be treated on a clinical protocol with careful follow-

up in order to establish whether there may be increased risk of progression to clonal hematopoiesis in SAA patients treated with eltrombopag.

Eltrombopag is available in 90 countries worldwide, and following the publication of our initial results, many patients apparently are being treated with the drug outside of clinical trials. It is highly desirable to rigorously establish the risk and benefit profile for this promising treatment in a formal research protocol setting.

3 Study Design

The study is designed as a non-randomized, Phase II, dose modification study of the oral TPO-R agonist eltrombopag in refractory severe aplastic anemia. The primary endpoint is measured at 24 weeks (+/- 10 days). Subjects who cannot tolerate the medication or fail to respond by 24 weeks (+/- 10 days) will be taken off study drug. Drug dose during extended access will be at the lowest dosage that maintains a stable platelet count until they meet off study criteria or the study is closed.



4 Eligibility Assessment

4.1 Inclusion criteria

- 4.1.1 Previous diagnosis of refractory severe aplastic anemia and following at least one treatment course of immunosuppression with a regimen containing antithymocyte globulin, alemtuzumab or cyclophosphamide.
- 4.1.2 One or more of the following three clinically-significant cytopenias: platelet count $\leq 30,000/\mu\text{L}$ or platelet-transfusion-dependence (requiring at least 4 platelet transfusions in the 8 weeks prior to study entry); neutrophil count less than $500/\mu\text{L}$; hemoglobin less than 9.0 g/dL or red cell transfusion-dependence (requiring at least 4 units of PRBCs in the eight 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 Infection not adequately responding to appropriate therapy
- 4.2.3 Evidence of a clonal disorder on cytogenetics performed within 12 weeks of study entry.
- 4.2.4 Creatinine > 2.5 mg/dL

- 4.2.5 Direct Bilirubin > 2.0 mg/dL
- 4.2.6 SGOT or SGPT >5 times the upper limit of normal
- 4.2.7 Hypersensitivity to eltrombopag or its components
- 4.2.8 Female subjects who are nursing or pregnant or are unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential
- 4.2.9 Unable to understand the investigational nature of the study or give informed consent
- 4.2.10 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
- 4.2.11 Treatment with ATG, cyclophosphamide or alemtuzumab within 6 months of study entry.

5. 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.

Subjects will initiate eltrombopag at a starting daily dose as detailed below, according to age and ethnicity. Subjects above 12 years of age will receive the adult dose of 150 mg. Those between 6 and 11 will start at 75 mg, and children between 2 and 5 years of age will be started at 2.5 mg/kg (Table 3). To adjust for the higher expected exposure in children of East Asian and South East Asian ancestry, the starting dose for East Asian and South East Asian subjects between 12 and 17 years of age will be 75 mg once daily. For East Asian and South East Asian subjects between 6 and 11 years of age, the starting dose will be 37.5 mg once daily, and for children between 2 and 5, the starting dose will be 1.25 mg/kg (Table 3). Each sachet contains eltrombopag olamine equivalent to 20 mg of eltrombopag per gram of powder. 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.

Table 3: Daily Eltrombopag Dose

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

5.2 Dose adjustments of eltrombopag (See section 2.5)

The daily dose of eltrombopag will be decreased according to the following rules:

Platelet Count	Dose Adjustment or Response
>200,000/ μ L (untransfused) at any time on study	Decrease dosage by 25mg every 2 weeks (+/- 3 days) to lowest dosage that maintains platelet count \geq 50,000/ μ L. In children under 12, the dose will be decreased by 12.5 mg.
>400,000/ μ L (untransfused) at any time on study	Discontinue eltrombopag for one week (+/- 3 days), if platelets fall to <200,000/ μ L; restart at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

5.3 Dose adjustments in extension protocol

Once blood count recovery occurs such that platelets >50,000/ μ L and Hb > 10 g/dL in the absence of transfusions, and neutrophils > 1,000 for more than 8 weeks, eltrombopag will be discontinued. If platelets drop to <30,000/ μ L, Hb to <9g/dL, or ANC to <500/ μ L then eltrombopag can be re-initiated back to the most recent dose.

Additionally, eltrombopag may be stopped if patients continue to meet criteria for response, but fail to show further clinically-significant improvement in their counts over a period of 6 months (stable disease). If after discontinuation of eltrombopag, any lineage falls back below original study inclusion criteria values or if the patient again requires transfusions, eltrombopag will be reinstated at the previously-effective dose.

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

5.4.1 Infection:

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

5.4.2 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 25 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.

5.5 Dose delays, modifications or discontinuation for hematologic side effects

5.5.1 Thrombosis/Embolism:

Subjects who experience a deep venous thrombosis or a pulmonary embolus, transient ischemic attack or stroke, or a myocardial infarction at any time while on eltrombopag will discontinue drug and go off study. Patients with platelet counts of >50,000/ μ L 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/ μ L with discontinuation of eltrombopag. They will be treated for the thrombotic event as otherwise clinically-indicated.

5.5.2 Peripheral blood smear shows new morphological abnormalities:

The presence of persistent morphologic abnormalities (red cell teardrop forms or immature blasts) or the development of significant sudden worsening of cytopenias 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.

5.6 Extended access to study drug

Subjects who demonstrate response by protocol criteria at 24 weeks (+/- 10 days) may be consented for entry into the extended access cohort of the protocol. Per dosing criteria given in section 5.3, patients may remain in the extended access cohort as long as they maintain a response. While on extended access, participants may have drug tapered, stopped, and/or re-started as per section 5.3.

5.7 Permitted supportive care

- Transfusion supportive care (e.g., blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) as clinically indicated. Romiplostim (N-Plate) or IL-11 (Neumega) should not be administered.
- Estrogens or combination oral contraceptives as indicated for uterine bleeding.
- Prophylactic antibiotics and antivirals as clinically indicated.

5.8 Concurrent medications:

Cyclosporine/magnesium: Subjects who are on cyclosporine therapy at enrollment may remain on chronic cyclosporine therapy targeting a stable cyclosporine level as long as eltrombopag is administered 4 hours post any p.o. magnesium given to counteract magnesium-wasting on cyclosporine.

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. Subjects on cyclosporine requiring prophylaxis against PCP should be given inhaled pentamidine instead of TMP/SULF. NIH SAA patients are routinely placed on pentamidine instead of TMP/SULF for PCP prophylaxis to avoid potential marrow-suppressive effects of TMP/SULF anyway. Other CYP inhibitors can be used concomitantly but with careful attention to possible increased eltrombopag activity and toxicity.

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

5.9 Instructions to patients

Timing in relation to food: Subjects 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 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: Vigorous activities are to be avoided, as mild trauma could result in bleeding.

6. Clinical Monitoring

Samples will be ordered and tracked through CRIS. 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.

HRQL surveys will be offered to adult subjects who read English or Spanish. The surveys will be mailed to the subject if they are not required to return to the NIH CC for study evaluations. Any survey time-point that is missed due to the subject's clinical status (e.g. critically ill) will be reported at time of continuing review.

Lab tests not done in NIH will be faxed to the Research Nurse. The PI will review outside test results and they will be filed in as scanned versions in CRIS, and as hard copies in the research charts. Case report forms will be used to record all lab data. All charts will be stored in a secure room.

For the evaluation timeframes that describe the timing of tests/procedures below in the term of month(s), a month is defined as 30 days.

Clinical Studies evaluating iron content: Eltrombopag is structurally similar to clinically available iron-chelators and has been recently shown to reduce intracellular iron levels (Roth et al., July 12, 2012; Blood: 120 (2)). In an in vitro cell culture assay eltrombopag achieved higher iron removal rates (42.9%) when compared to known iron chelators such as Desferrioxamine (22.7%), Deferiprone (19.3%) and Deferasirox (34.9%) (Vlachodimitropoulou Koumoutsea et al. ASH 2024). Aplastic anemia patients require frequent blood transfusions resulting in chronic iron overload. In order to study eltrombopag's iron chelation and excretion capacities in a clinical relevant setting, we will collect urine samples before, during and after eltrombopag treatment in selected patients. For example, we will need to exclude patients currently on chelation therapy from this pilot study. Furthermore, some of our non-local patients may not stay long enough at the clinical center to complete a full 24 hour collection of urine. Serum iron panels (ferritin, transferrin, % saturation) will also be monitored in the selected subjects.

6.1 Pre-study evaluation

Pre-study evaluations to determine eligibility and baseline status may be performed under an IRB approved protocol (for instance the screening protocol 97-H-0041, or studies such as bone marrows performed as follow-up on another NHLBI treatment trial) or as part of this protocol. Patients will be evaluated at the NIH within 12 weeks prior to signing consent. This evaluation includes a bone marrow aspiration and biopsy with cytogenetics to determine eligibility, and these results take up to two weeks to be reported. If a patient is not local, the labs listed below required to be performed within 7 days of signing consent can be performed by their home physician's outside lab, and if they confirm continued eligibility, telephone consent can be obtained. All time frames listed below in section 6.1 are relative to the day consent is signed for this protocol. The source of the pre-study and eligibility data will be appropriately documented. The pre-study evaluations will include the following:

- Medical history and physical examination (at the NIH within 12 weeks prior to consent)
- Concurrent medication review (within 7 days prior to consent)
- Baseline studies
 - Complete blood count with differential (within 7 days prior to consent)
 - Reticulocyte count (within 7 days prior to consent)
 - DAT (direct antiglobulin test) (within 12 weeks prior to consent)

- Acute care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin) (within 7 days prior to consent)
- Total Protein, CK, Uric Acid, LDH, Phosphorus, Magnesium, Albumin, and Calcium (within 12 weeks prior to consent)
- Coagulation and thrombosis screens (PT, PTT) (within 12 weeks prior to consent)
- Viral serologies for hepatitis A, B, C, HIV, HSV, EBV and CMV (positive serologies will have viral nucleic acid testing performed) (within 12 weeks prior to consent).
- Folate level (within 12 weeks prior to consent)
- B12 level (within 12 weeks prior to consent)
- Iron panel (ferritin, transferrin, % saturation) (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
- HLA typing (if not already performed and available)
- Pregnancy test (blood or urine HCG in women of child bearing potential) (within 7-days prior to consent)
- 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 (within 12 weeks prior to consent).
- Bone marrow chromosomal analysis via standard cytogenetic techniques (within 12 weeks prior to consent).
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells (within 12 weeks prior to consent).
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry) (within 12 weeks prior to consent).
- HRQL survey administration
- Research blood as detailed in section 7

6.2 On Study Monitoring (through 6 months +/- 10 days)

Subjects may be followed by their home physician or at the Clinical Center. Progress notes and lab tests not done in 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 Landmark 3-month and 6-month monitoring

Subjects must be evaluated at the NIH Clinical Center at the 3- and 6-month (+/-10 days) time points

- History and physical examination
- Complete blood counts 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
- 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

- 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 dose (3 month time point, unless do not meet requirements per Appendix D, then at 6 month visit) (not required for previously enrolled subjects)
- Blood or urine pregnancy test (woman of childbearing potential only)
- 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
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)
- HRQL survey administration
- Research blood as detailed in section 7

6.4 Off study assessment

Patients with refractory aplastic anemia who fail to respond to eltrombopag after 6 months (+/- 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 unrelated donor or cord blood transplantation, repeated cycles of immunosuppression, or experimental therapies. In our experience on the prior SAA eltrombopag 09-H-0154 study, patients were not willing to return to the NIH for the specified one month or six month evaluation and repeat bone marrow examination, unless they were being offered an additional treatment protocol at the NIH.

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 metabolism and excretion studies

6.5 Long Term Follow Up for Responding Patients Enrolled in Extended Access Cohort

Patients fulfilling response criteria (per section 8.2) at the 6 month 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 month (+/- 7 days). Follow-up on this schedule will continue as long as they remain on eltrombopag on this clinical protocol.

Progress notes and lab tests not done in 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.

6.5.1 Testing required for patients while on extended access eltrombopag:

- Complete blood counts with differential (monthly +/- 7 days)
- ALT, AST, Total Bilirubin or Direct Bilirubin (monthly +/- 7 days)
- Reticulocyte count (monthly +/- 7 days)
- 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 6 months +/- 30 days)
- Bone marrow chromosomal analysis via standard cytogenetic techniques (every 6 months +/- 30 days)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells (every 6 months +/- 30 days)
- HRQL survey administration (every 6 months +/- 30 days)
- Iron panel (ferritin, transferrin, % saturation) at PI's discretion if indicated for iron metabolism and excretion studies (every 6 months +/- 30 days)
- 24 hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 6 months +/- 30 days)

6.5.2 Subjects who have had eltrombopag discontinued during follow-up while in the extended access cohort, due to reaching robust count criteria or responders with stable counts for 6 months as per section 5.3 will be followed in the following manner:

- 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)
- HRQL survey administration (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)
- 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)
- Research blood as detailed in section 7

Patients who discontinue eltrombopag according to the response criteria per section 5.3 who need to have eltrombopag reinitiated due to counts falling will go back onto the monitoring schedule for patients on eltrombopag in the extended access cohort.

6.5.3 Responding patients who discontinue eltrombopag according to the response criteria per Section 5.3 will be followed on study for three years from the time of discontinuation of eltrombopag.

7. Ancillary Laboratory Research Studies

Collection of samples

Intended use: During this study, 60 cc of blood (3 ml/kg not to exceed 60 ml of blood for pediatric subjects) at baseline and at landmark visits at 3, 6 and annually thereafter, and 5 cc of bone marrow aspirate (at the baseline, 3 month, 6 month, and 12 month visits, and then at the annual visits thereafter) will be obtained for the following correlative laboratory research studies. Baseline samples may be obtained under 04-H-0012. Additionally, in selected patients we will collect urine for 24 hours at each of the landmark visits. These studies are not used in assessing the primary endpoint but are undertaken as descriptive or exploratory ancillary studies. Some or all may be performed on each subject, and they may be correlated with response.

- 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.

Storage: Research samples will be stored coded in the secure laboratory of Neal Young, MD.

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 the NIH without IRB notification and an executed MTA.

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.

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. Biostatistical Considerations

8.1 Objectives

The *primary objective* is to assess the efficacy of 6 months of eltrombopag administration in improving bone marrow function in SAA patients with persistent severe cytopenias refractory to treatment with immunosuppressive treatment.

Secondary objectives include assessment of relapse or clonal evolution, pre-treatment characteristics predicting response, and the impact of treatment and treatment response on quality of life.

8.2 Endpoints

The **primary endpoint** will be the proportion of drug responders as defined by changes in the platelet count and/or platelet transfusion requirements, hemoglobin levels, number of red blood cell transfusions, or neutrophil counts as measured by International Working Group criteria and the toxicity profile as measured using the CTCAE criteria. The primary endpoint will be assessed at six months. Platelet treatment response is defined as platelet count increases to 20,000/ μ L above baseline at six months, or stable platelet counts with transfusion independence for a minimum of 8 consecutive weeks prior to response assessment. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by > 1.5 g/dL or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment - compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$ /L as at least a 100% increase in ANC, or an ANC increase $>0.5 \times 10^9$ /L.

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

Secondary endpoints will include hematological responses at 3 months (a) hematological response at 3 and 12 months and yearly thereafter; (b) relapse (c) clonal evolution to PNH, clonal chromosomal population in bone marrow, myelodysplasia by morphology, or acute leukemia; (d) survival and (e) health-related quality of life.

8.3 Sample size

In this cohort study we are omitting the dose escalation and employing instead a fixed dose of 150mg. We are also extending the response assessment to 24 weeks. Based on our preliminary data, we expect the 24-week response rate at this dose level to be at least 44%, and we hypothesize that these modifications will improve outcomes. Thus, for this cohort, we would test the null hypothesis $H_0: p_0=44\%$ versus the alternative $H_1: p_1 \neq 44\%$ at a two-sided significance level of 0.05 and a power of 0.80. Our goal is to detect a $p_1-p_0=20\%$ difference between the response rate. Using a two-sided binomial test ³⁴we need a total sample size of $N=49$ to achieve the required power and significance level. The actual significance level achieved by this test is 0.0429, which requires that the population response rate under the null hypothesis is 44%. Since this is a two-sided test, the null hypothesis H_0 will be rejected if the number of patients who respond to the treatment is ≤ 14 or ≥ 29 . Numerical calculation is calculated using the NCSS/PASS statistical software package (Hintze, J. (2006), NCSS, PASS, and GESS. NCSS. Kaysville, Utah., www.ncss.com).

Subjects who are consented but do not start study drug may be replaced. In order to account for the potential need to replace a subject that signed consent, but did not start drug, up to 60 subjects may be consented.

Subjects who have taken drug, but withdraw or are withdrawn from the study (e.g., toxicity, no response, or other reasons) before the 6-month evaluation will be assessed as intention to treat.

8.4 Statistical methods

The change of quality of life measure from baseline will be examined by the t-test or the Wilcoxon rank-sum test. 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 will be evaluated using Binomial distributions.

The time to responses and the progression-free survival will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate, Kaplan-Meier curves, and Cox regression with age, gender and

ethnicity as covariates, which take consideration of both death without the event of interest as a complete risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. 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. Exploratory subgroup analysis will be use to compare the possible different response rates among male, female and different age categories.

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 24-week study period to be 20% or less. Following Geller et al.³⁵, 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 $(\alpha, \beta) = (1.2, 4.8)$. The parameter are chosen so that the mean $\alpha / (\alpha + \beta) = 0.2$ as the expected proportion of specified TRSAE’s and the sum $\alpha + \beta = 6$ as the “worth” we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is $6/41=15\%$ of the weight we will place on the results of the new study. Hence when we make decisions about stopping the study, the data from the study will dominate over the prior opinion. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 or more subjects have developed a TRSAE. The following table summarizes the threshold numbers for stopping the study.

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE’s reaches:
≤ 5	3
≤ 8	4
≤ 12	5
≤ 16	6
≤ 20	7
≤ 24	8
≤ 28	9
≤ 32	10
≤ 36	11
≤ 41	12
≤ 45	13
≤ 49	14

We investigated the performance of the above stopping rule by simulation. In each run, we generated a study with 49 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	1.6%	24.4%	50.7 %	76.7%	98.1%
Average number of subjects	48.4	41.5	34.2	25.7	13.6
Average number TRSAEs	4.8	8.3	8.5	7.7	5.5

These results suggest that our stopping rule has a low probability of 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

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

Per principal investigator decision: Should any of the following events occur during the study period, or in the extension treatment arm in responders, eltrombopag will be discontinued. The subject will be followed until resolution of the event. For study purposes labs relevant to the adverse event will be monitored for 30 days after discontinuation of eltrombopag. 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 because they met other criteria for coming off study, 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 a patient initiates an alternative disease directed therapy at any 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 by week 24 (+/- 7 days).
- 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
- Evidence of clonal evolution to MDS defined as abnormal cytogenetics identified from bone marrow aspirate
- Subject non-compliance
- Lost to follow-up
- Study completion

Once off study subjects will be referred back to the 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 the best interest of the subject.

9. Data and Safety Monitoring

9.1 Safety monitoring

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

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

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 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 and IND 104,877: An annual progress report, protocol amendments, and any information amendments (e.g., change in the status of the protocol) will be forwarded to FDA Project Manager (designee):

Mara Miller, M.A.
Regulatory Health Project Manager, 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)

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

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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 AEs, including clinically significant abnormal non-hematologic findings on laboratory evaluations, regardless of severity, will be recorded and followed until satisfactory resolution. AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTCAE 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>.

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will 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 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, refractory severe aplastic anemia, 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.

For all subjects enrolled in this protocol, the current laboratory AE collection and reporting procedures are and have been used for all subjects.

Thirty days after the last dose of study drug, adverse event collecting and reporting will be limited to serious adverse 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)	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: <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)	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: <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.

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

9.2.4 Reporting events

All events will be reported to Principal Investigator of this study.

Cynthia E. Dunbar, M.D., HB, NHLBI, NIH, Clinical Center
 10 Center Dr. Building 10, Room CRC 4-5132
 Bethesda, MD 20892-1452
 Tel: 301-496-5093 Fax: 301-594-1290
 E-mail: dunbarc@nhlbi.nih.gov

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 via iRIS using the NIH Problem Report Form.

Reports to the IRB Chair and CD: The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event via iRIS, using the NIH Problem Report Form.

Non-serious Events

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

Deaths

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

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

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

Exclusions to data reporting:

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

- Laboratory values that do not meet the definition of AE listed in Section 9.2.3.
- All grade 1 events listed as expected in the investigator's brochure, package insert.

9.2.4.3 NHLBI DSMB Reporting:

Reports of serious adverse events that are unexpected and thought to be related (possible, probably or definitely attribution) to the experimental drug will also be forwarded no later than seven days in the case of death or life-threatening serious adverse events or within fifteen days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). All SAEs will be included in the DSMB reports for review by the DSMB at the time of scheduled DSMB meetings (DSMB meetings held 2 times a calendar year and DSMB determines the frequency of protocol review per DSMB minutes).

9.2.4.4 Sponsor and FDA Event Reporting

IND #: 104,877

IND Representative: Cynthia E. Dunbar, MD, HB, 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 as either a IND Safety Report or Annual report.

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 the 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 on a MedWatch form (Form FDA 3500A) or in a narrative form to the Project Manager, which currently is:

Mara Miller, MA, Regulatory Health Project Manager
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 (telephone)

9.3 Reporting Serious Adverse Events to 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. Follow-up reports regarding the subject's subsequent course will be submitted until the SAE has resolved or until the subject's condition stabilizes (in the case of persistent impairment) or the subject 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'.

9.4 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.

9.5 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), 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.

Novartis will receive quarterly accrual and toxicity information as detailed in the CTA. 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 subject confidentiality and trial data has occurred, the IRB will be notified.

Data will not be distributed outside NIH without IRB notification and an executed MTA or CTA.

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 Protection (OHSRP).

10 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 cases/million population of aplastic anemia will be diagnosed each year. 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.

This study will be open to all patients who fit the inclusion criteria and provide informed consent to protocol participation. Epidemiologic studies suggest that the gender will be approximately evenly split between male and females, and that 90% of the patients will be Caucasian. However, previous experience at our institution suggests that distribution will be:

by gender: 60% males and 40% females;

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

by age: will range between 2 and 82 (median age of 30) and roughly 20% of patients will be under the age of 18.

For subjects of Asian ethnicity: Plasma eltrombopag area under the curve was approximately 70% higher in East and South East Asian (ethnicity self-reported) subjects as compared to non-Asian subjects who were predominantly Caucasian. Therefore, subjects of Asian heritage will be included but they will be initiated at a lower dose and monitored closely as described in the treatment plan.

For subjects with renal impairment: The pharmacokinetics of eltrombopag has been studied in 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 and given the protocol-defined dosages, but participation will be monitored closely.

For subjects with hepatic impairment: Pharmacokinetics of eltrombopag has been studied in 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. Patients with baseline moderate to severe hepatic impairment will be excluded from the study.

Recruitment efforts: The study will be listed on the clinicaltrials.gov, Clinical Center research studies, the Aplastic Anemia Myelodysplastic Syndrome International Foundation, and the National Heart, Lung and Blood Institute patient recruitment websites. If recruitment goals are not met, a 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.

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

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

10.2 Participation of pediatric patients

Aplastic anemia with severe thrombocytopenia is seen in children, and 15% of all severe aplastic anemia patients referred to the NIH for protocol participation are <18. In the combination immunosuppression with Eltrombopag study (12-H-0150), headed by Dr. Danielle Townsley, pediatric patients are included and we propose enrolling patients 2 and older with an identical dosing schedule.

10.3 Risks and Discomforts:

10.3.1 Related to Promacta[®](eltrombopag)

Boxed warnings related to Promacta[®] (eltrombopag):

Potential Serious Adverse Effects:

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 $200 \times 10^9/L$. The risk of portal venous thrombosis was increased in thrombocytopenic patients with chronic liver disease treated with 75 mg of PROMACTA once daily for 2 weeks in preparation for invasive procedures.

Cataracts

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

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

Clinical Experience:

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

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

Adverse Reactions ($\geq 10\%$) 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.

Severe cutaneous reaction

There is a risk that subject may develop a severe cutaneous reaction that may require hospitalization and discontinuation of eltrombopag.

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 ($\geq 10\%$)

Common: ≥ 1 in 100 and < 1 in 10 ($\geq 1\%$ and $< 10\%$)

Uncommon: ≥ 1 in 1,000 and < 1 in 100 ($\geq 0.1\%$ and $< 1\%$)

Rare: ≥ 1 in 10,000 and < 1 in 1,000 ($\geq 0.01\%$ and $< 0.1\%$)

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

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

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

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

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

Adverse Events considered to be expected for Reporting Purposes in SAA

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

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

Blood and lymphatic system disorders

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

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

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

10.3.2 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.

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. Infections may rarely occur.

10.3.5 Related to HRQL: The only anticipated adverse consequences associated with the HRQL 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 children:

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 probably lethal hematological disease, an alternative to symptomatic therapy.

(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

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 an “*” 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:

- 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 any associated investigators indicated by * on page 1. If not already done, a copy of the consent document will be sent to the potential subject via telefax 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 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 (301-594-1290) 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, Hematology Branch, 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.

If the subject is a minor, the parent who signs the consent for the minor must be a legally authorized 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. 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.

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.

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.

Non-English-speaking research participants: We anticipate enrolling non-English speaking research participants into this study. The IRB approved full consent document will be translated into the subject's native 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 a short form oral consent process as described in MAS Policy M77-2, 45CFR46.117(b)(2) and 21CFR50.27(b)(a). The summary that will be used is the English version of the extant

IRB approved consent document.

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

10.6 Conflict of Interest

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

10.7 FWA Coverage Agreement

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

11 Pharmaceuticals

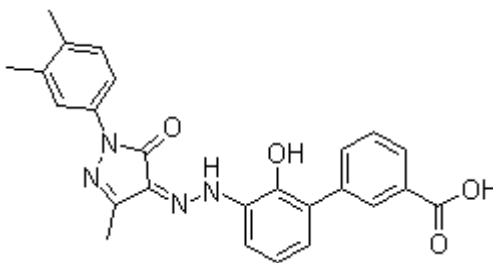
11.1 Eltrombopag (Promacta®):

will be supplied by Novartis

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 provided for a few days of overage in case a patient is delayed returning to clinic.

Stability: Store, both tablets and powder, 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 shipping address for Novartis supplied investigational agent is:

National Institutes of Health
IMDRS, Room 1C230
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196

Shipping Designee Name: ihyun Esther Jeon
Shipping Designee Phone No: (301) 496-4363
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: jihyunesther.jeon@nih.gov

Accountability Procedures: Drug accountability records will be maintained for all clinical supplies. 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.

12 References

1. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood*. Oct 15 2006;108(8):2509-2519.
2. Zoumbos NC, Gascon P, Djeu JY, Trost SR, Young NS. Circulating activated suppressor T lymphocytes in aplastic anemia. *N Engl J Med*. Jan 31 1985;312(5):257-265.
3. Young NS, Leonard E, Platanius L. Lymphocytes and lymphokines in aplastic anemia: pathogenic role and implications for pathogenesis. *Blood Cells*. 1987;13(1-2):87-100.
4. Maciejewski JP, Hibbs JR, Anderson S, Katevas P, Young NS. Bone marrow and peripheral blood lymphocyte phenotype in patients with bone marrow failure. *Exp Hematol*. Oct 1994;22(11):1102-1110.
5. Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. *Br J Haematol*. Sep 1995;91(1):245-252.
6. Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, Young NS. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. *Lancet*. Jul 24-30 2004;364(9431):355-364.
7. Chen J. Animal models for acquired bone marrow failure syndromes. *Clin Med Res*. May 2005;3(2):102-108.
8. Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS. A severe and consistent deficit in marrow and circulating primitive hematopoietic cells (long-term culture-initiating cells) in acquired aplastic anemia. *Blood*. Sep 15 1996;88(6):1983-1991.
9. Maciejewski JP, Kim S, Sloand E, Selleri C, Young NS. Sustained long-term hematologic recovery despite a marked quantitative defect in the stem cell compartment of patients with aplastic anemia after immunosuppressive therapy. *Am J Hematol*. Oct 2000;65(2):123-131.
10. Scheinberg P, Young NS. How I treat acquired aplastic anemia. *Blood*. 2012 Aug 9;120(6):1185-96
11. Zeigler FC, de Sauvage F, Widmer HR, et al. In vitro megakaryocytopoietic and thrombopoietic activity of c-mpl ligand (TPO) on purified murine hematopoietic stem cells. *Blood*. Dec 15 1994;84(12):4045-4052.
12. Qian H, Buza-Vidas N, Hyland CD, et al. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell*. Dec 13 2007;1(6):671-684.
13. Yoshihara H, Arai F, Hosokawa K, et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell*. Dec 13 2007;1(6):685-697.
14. Alexander WS, Roberts AW, Nicola NA, Li R, Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietic receptor c-Mpl. *Blood*. Mar 15 1996;87(6):2162-2170.

15. Emmons RV, Reid DM, Cohen RL, et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. *Blood*. May 15 1996;87(10):4068-4071.
16. Wang W, Matsuo T, Yoshida S, et al. Colony-forming unit-megakaryocyte (CFU-meg) numbers and serum thrombopoietin concentrations in thrombocytopenic disorders: an inverse correlation in myelodysplastic syndromes. *Leukemia*. Oct 2000;14(10):1751-1756.
17. Garnock-Jones KP. Eltrombopag: a review of its use in treatment-refractory chronic primary immune thrombocytopenia. *Drugs*. Jul 9 2011;71(10):1333-1353.
18. Imbach P, Crowther M. Thrombopoietin-receptor agonists for primary immune thrombocytopenia. *The New England journal of medicine*. Aug 25 2011;365(8):734-741.
19. Erickson-Miller CL, Delorme E, Tian SS, et al. Preclinical activity of eltrombopag (SB-497115), an oral, nonpeptide thrombopoietin receptor agonist. *Stem Cells*. Feb 2009;27(2):424-430.
20. Bussel JB, Cheng G, Saleh MN, et al. Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. *N Engl J Med*. Nov 29 2007;357(22):2237-2247.
21. Bussel JB, Provan D, Shamsi T, et al. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet*. Feb 21 2009;373(9664):641-648.
22. Cheng G, Saleh MN, Marcher C, et al. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. *Lancet*. Jan 29 2011;377(9763):393-402.
23. Kuter DJ, Mufti GJ, Bain BJ, Hasserjian RP, Davis W, Rutstein M. Evaluation of bone marrow reticulin formation in chronic immune thrombocytopenia patients treated with romiplostim. *Blood*. Oct 29 2009;114(18):3748-3756.
24. Maciejewski JP, Selleri C. Evolution of clonal cytogenetic abnormalities in aplastic anemia. *Leuk Lymphoma*. 2004 Mar;45(3):433-40
25. Frey N, Hexner E, Porter D et al. A Phase I Clinical Trial Using Eltrombopag in Patients with Acute Myelogenous Leukemia. *ASH Annual Meeting Abstracts*. Nov 2012; 120: 3576
26. Mittelman M, Assouline S, Briasoulis E, et al. Eltrombopag Treatment of Thrombocytopenia in Advanced Myelodysplastic Syndromes and Acute Myeloid Leukemia: Results of the 8-Week Open-Label Part of an Ongoing Study. *ASH Annual Meeting Abstracts*, Nov 2012; 120: 3822.
25. Matthys G, Park JW, McGuire S, et al. Clinical pharmacokinetics, platelet response, and safety of eltrombopag at supratherapeutic doses of up to 200 mg once daily in healthy volunteers. *J Clin Pharmacol*. Mar 2011;51(3):301-308.

27. Gernsheimer TB, George JN, Aledort LM, et al. Evaluation of bleeding and thrombotic events during long-term use of romiplostim in patients with chronic immune thrombocytopenia (ITP). *J Thromb Haemost.* Jun 2010;8(6):1372-1382.
28. Catala-Lopez F, Corrales I, Martin-Serrano G, Tobias A, Calvo G. Risk of thromboembolism treated with thrombopoietin receptor agonists in adult patients with thrombocytopenia: systematic review and meta-analysis of randomized controlled trials. *Med Clin (Barc).* Jan 20 2012.
29. Ohara A, Kojima S, Hamajima N, et al. Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood.* 1997;90(3):1009-1013.
30. Socie G, Mary JY, Schrezenmeier H, et al. Granulocyte-stimulating factor and severe aplastic anemia: a survey by the European Group for Blood and Marrow Transplantation (EBMT). *Blood.* 2007;109(7):2794-2796.
31. Tichelli A, Schrezenmeier H, Socie' G, et al. A randomized controlled study in patients with newly diagnosed severe aplastic anemia receiving antithymocytoglobulin (ATG), cyclosporine, with or without G-CSF: a study of the SAA Working Party of the European Group for Blood and Marrow Transplantation. *Blood.* 2011;117(17):4434-4441.
32. Locasciulli A, Arcese W, Locatelli F, Di Bona E, Bacigalupo A. Treatment of aplastic anaemia with granulocyte-colony stimulating factor and risk of malignancy: Italian Aplastic Anaemia Study Group. *Lancet.* 2001;357(9249):43-44.
33. Will B, Kawahara M, Luciano JP, et al. Effect of the non-peptide thrombopoietin receptor agonist eltrombopag on bone marrow cells from patients with acute myeloid leukemia and myelodysplastic syndrome. *Blood.* 2009;114(18):3899-3908.
34. Chow SC, Shao J, Wang H. *Sample Size Calculations in Clinical Research*, Marcel Dekker, New York 2003.
35. Geller, Nancy L. Dekker, Marcel. *Advances in Clinical Trial Biostatistics 2003*, chapter: Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach.
36. Kantarjian H, Mufti G, Fenaux P et al. Treatment with the Thrombopoietin (TPO)-Receptor Agonist Romiplostim in Thrombocytopenic Patients (Pts) with Low or Intermediate-1 (int-1) Risk Myelodysplastic Syndrome (MDS): Follow-up AML and Survival Results of a Randomized, Double-Blind, Placebo Controlled Study. *Blood ASH Annual Meeting Abstracts*, Nov 2012; 120: 421
37. Bauman JW, Vincent CT, Peng B, Wire MB, Williams DD, Park JW. Effect of hepatic or renal impairment on eltrombopag pharmacokinetics. *J Clin Pharmacol.* May 2011;51(5):739-750.
38. Olnes MJ, Scheinberg P, Calvo KR, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. *N Engl J Med* 2012;367:11-19.

**APPENDIX A NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES
(2/5/2013)**

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	Stem Cell Allograft Transplantation 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

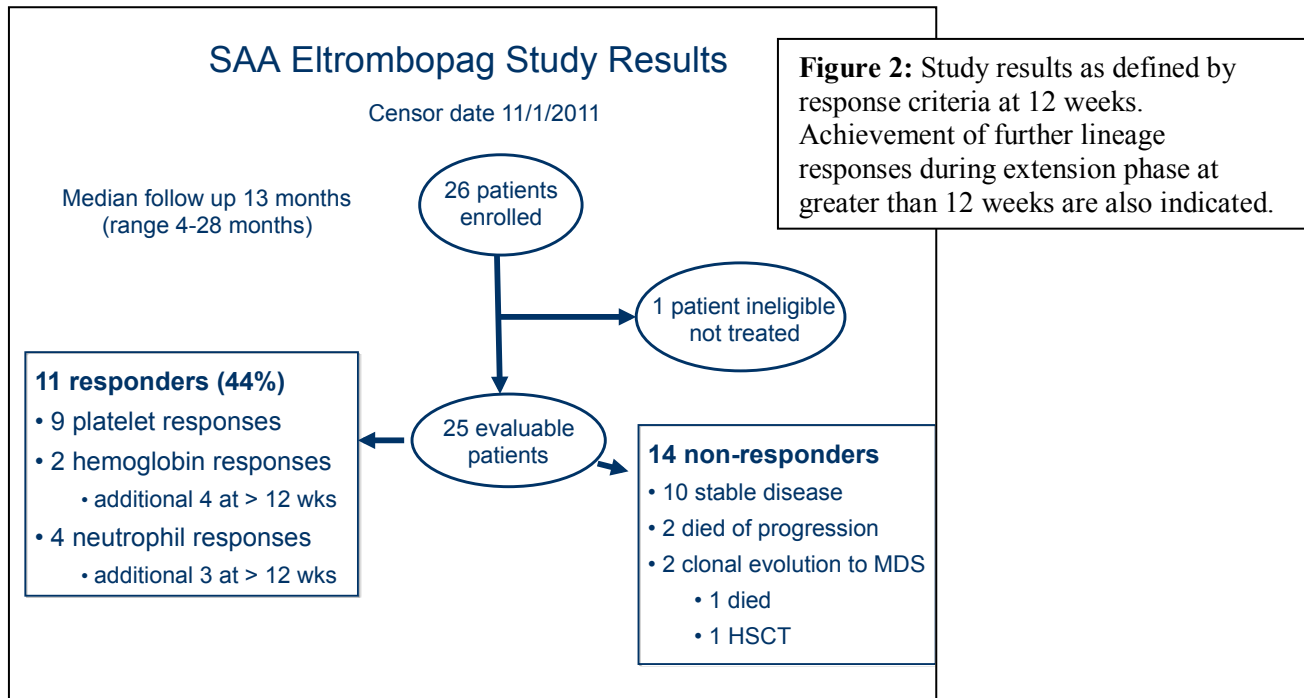
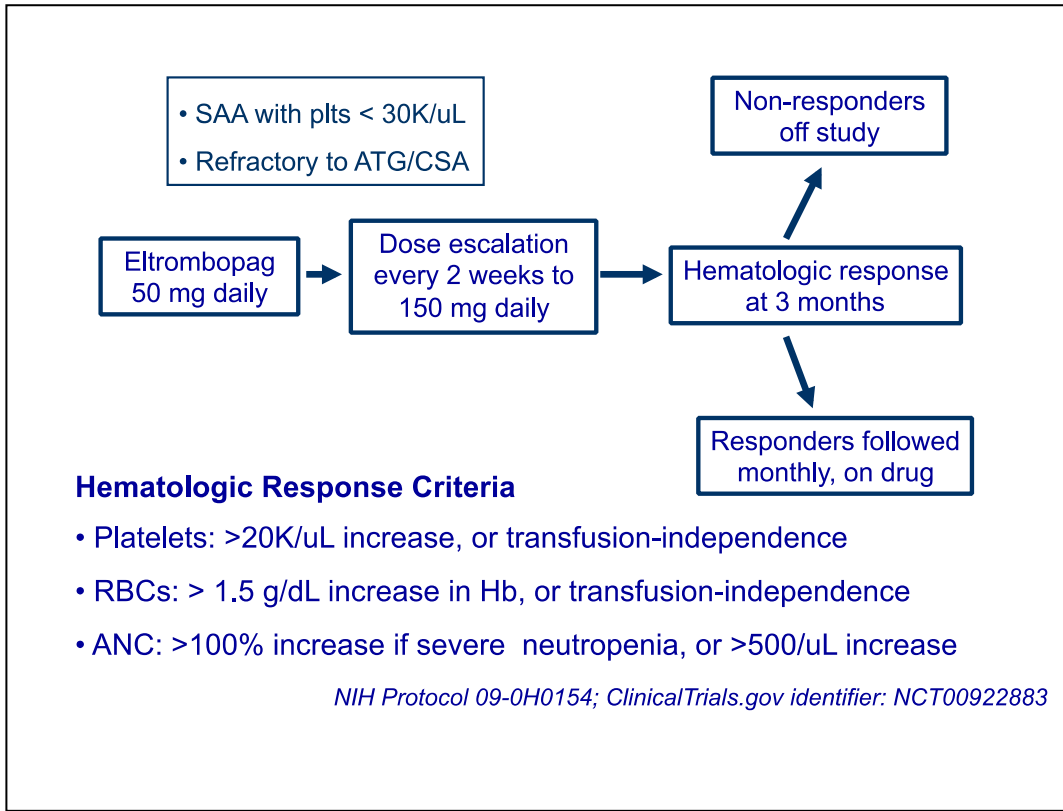
B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
B.5	Testing iron metabolism and excretion of eltrombopag.	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 fluorescence 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, circoviruses, 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 determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and 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 SUPPLEMENTAL FIGURES

Figure 1: Study Design



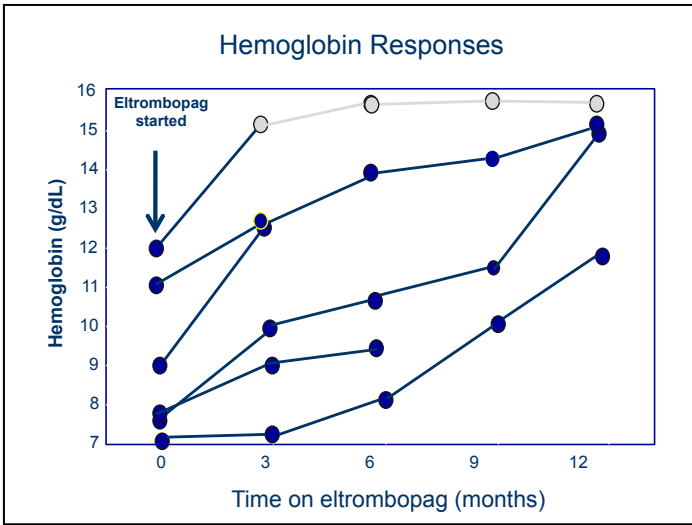
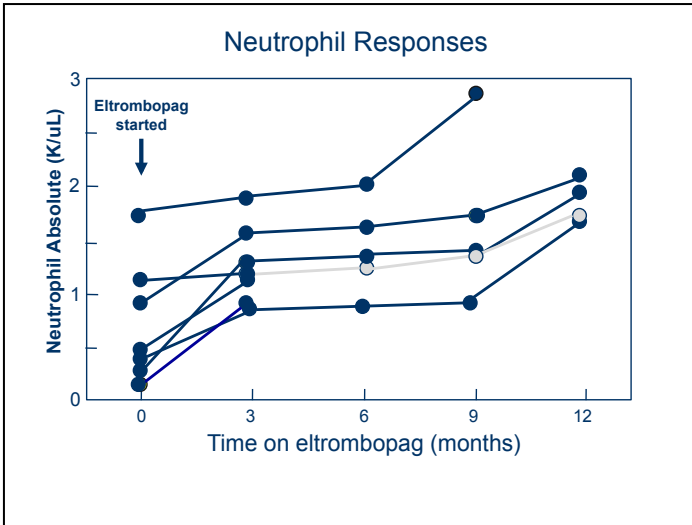
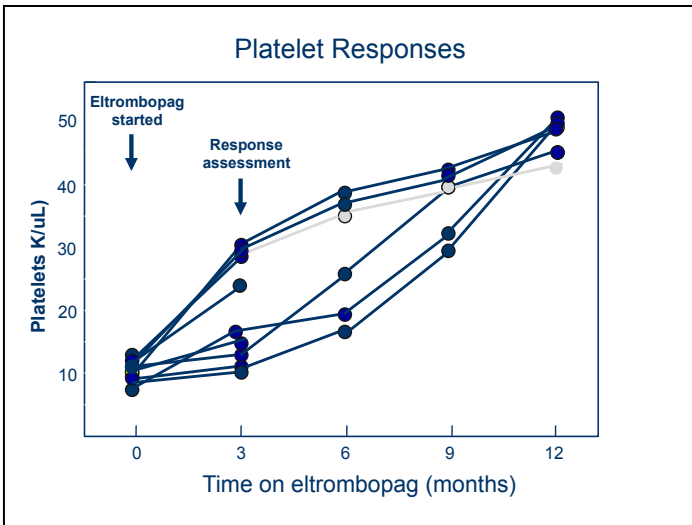


Figure 3: Responses to eltrombopag in responders over time. For each lineage, individual patients reaching response criteria are shown (A. Platelets, B. Hemoglobin, C. Neutrophils). Black lines indicate patients remaining on drug. Gray lines indicate the patient taken off drug at three months due to possible cataract formation.



Bone Marrow Cellularity at 1 Year After Study Entry

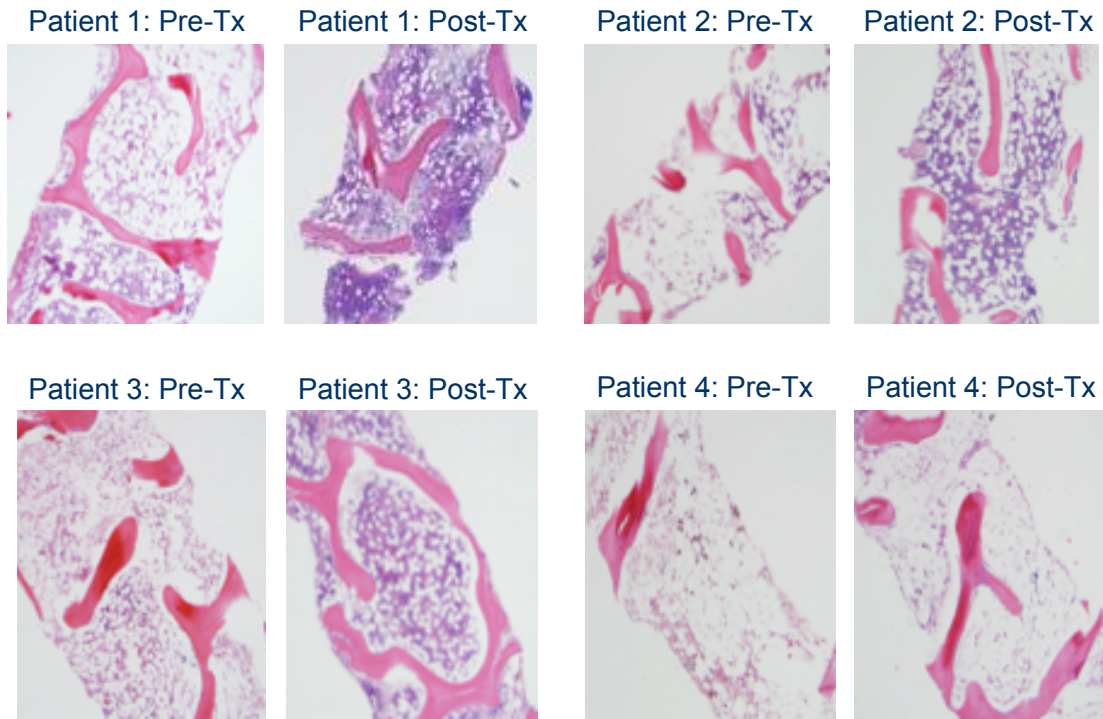


Figure 4: Bone marrow biopsies stained with hemotoxylin and eosin and shown at 100X magnification pre-treatment and 12 months following study entry.

APPENDIX C SCHEDULE OF EVENTS

- Schedule for Initial 6 months
- Schedule for Extended Access phase

Schedule for Initial 6 months

Visit/ Time Point	Consent	Pre-study (w/in 30 days of consent)	Day 1	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Month 3 (+/-10 days)	Wk 14 (+/-5 days)	Wk 16 (+/-5 days)	Wk 18 (+/-5 days)	Wk 20 (+/-5 days)	Wk 22 (+/-5 days)	Month 6 (+/- 10 days)	Month 12-Off study (optional) (+/- 10 days)
Procedure																
Consent	X															
Medical history		X(†)							X						X	X
physical examination		X(†)							X						X	X
Concurrent medication review		X(\$)														
Complete blood count with differential		X(\$)		X	X	X	X	X	X	X	X	X	X	X	X	X
Reticulocyte count		X(\$)							X						X	X
DAT (direct antiglobulin test)		X(†)														
Acute care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)		X(\$)							X						X	X
Mineral (Phosphorus, Magnesium, Albumin, and Calcium)		X(†)							X						X	X

Visit/ Time Point	Consent	Pre-study (w/in 30 days of consent)	Day 1	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Month 3 (+/-10 days)	Wk 14 (+/-5 days)	Wk 16 (+/-5 days)	Wk 18 (+/-5 days)	Wk 20 (+/-5 days)	Wk 22 (+/-5 days)	Month 6 (+/- 10 days)	Month 12- Off study (optional) (+/- 10 days)
Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)		X(\$)													X	X
Hepatic (ALT, AST, Total Bilirubin, OR Direct Bilirubin)				X	X	X	X	X	X	X	X	X	X	X		
Total Protein		X(†)							X						X	X
CK		X(†)							X						X	X
Uric Acid		X(†)							X						X	X
LDH		X(†)							X						X	X
Coagulation and thrombosis screens (PT, PTT)		X(†)														
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(†)														
Iron panel (ferritin, transferrin, % saturation)		X(†)							X(##)						X(##)	X(##)

Visit/ Time Point	Consent	Pre-study (w/in 30 days of consent)	Day 1	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Month 3 (+/-10 days)	Wk 14 (+/-5 days)	Wk 16 (+/-5 days)	Wk 18 (+/-5 days)	Wk 20 (+/-5 days)	Wk 22 (+/-5 days)	Month 6 (+/- 10 days)	Month 12- Off study (optional) (+/- 10 days)
24 hour urine collection to determine the total iron		X(##)							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	
Research Bloods		X(†)							X						X	X
Bone marrow aspiration and core biopsy,*		X(†)							X						X	X
Bone marrow chromosomal analysis via standard cytogenetic techniques		X(†)							X						X	X
Flow cytometry of the peripheral blood to quantitate GPI-negative cells		X(†)							X						X	X

Visit/ Time Point	Consent	Pre-study (w/in 30 days of consent)	Day 1	Wk 2 (+/-5 days)	Wk 4 (+/-5 days)	Wk 6 (+/-5 days)	Wk 8 (+/-5 days)	Wk 10 (+/-5 days)	Month 3 (+/-10 days)	Wk 14 (+/-5 days)	Wk 16 (+/-5 days)	Wk 18 (+/-5 days)	Wk 20 (+/-5 days)	Wk 22 (+/-5 days)	Month 6 (+/- 10 days)	Month 12- Off study (optional) (+/- 10 days)
Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)		X(†)							X						X	X
HRQL survey administration		X (‡)							X(‡)						X(‡)	
PK									X (‡)							
First Dose of Medication			X													
Document Drug Accountability									X						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

(‡) Per Appendix D, if subjects do not meet requirements for PK at month 3, then can be performed at month 6, performed at PI's discretion

(##) at PI's discretion if indicated for iron metabolism and excretion studies

Schedule of Events Extended Access Phase

Visit/ Time Point	Monthly (+/- 7 days)	Every 6 Months (+/- 30 days)	Every 12 months	Off study (optional) (+/- 30 days)
Procedure				
Complete blood count with differential	X	X		X
Reticulocyte count	X(‡‡)	X(‡‡)		X
Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)	X	X		X
Research Blood			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
Bone marrow chromosomal analysis via standard cytogenetic techniques		X(%)		X
Flow cytometry of the peripheral blood to quantitate GPI-negative cells		X(%)		X
Lymphocyte peripheral blood phenotyping(analysis of T, B, and NK subsets via flow cytometry)		X(%)		X
HRQL survey administration		X(%)		
Document Drug Accountability		X		
Iron panel		X(##)	X(##)(%)	
24 hour urine collection to determine the total iron		X(##)	X(##)(%)	

% Per section 6.5.2, only every 12 months +/- 60 days for robust responders that discontinue eltrombopag

(‡‡) per section 6.5.2, not required for robust responders

(##) at PI's discretion if indicated for iron metabolism and excretion studies

APPENDIX D PHARMACOKINETIC STUDIES

Collection of samples for PK Assessments

Subjects will have PK assessments at the landmark 3-month study visit. Subject must have received once daily eltrombopag for at least 7 days prior to this visit (i.e., be at PK steady-state with no recent dose interruptions). If a subject is not currently receiving eltrombopag at the time of this visit (because of a dose interruption) or eltrombopag has been reinitiated after a dose interruption within the 7 days prior to this visit, PK assessments will be deferred until the landmark 6-month study visit. The eltrombopag dosing history for the 2 weeks prior to the PK visit will be recorded (any dose interruptions, actual dose administered).

Blood samples (2 mL) for PK analysis will be collected in K2EDTA-containing tubes. One sample will be collected at each of the following times: within 30 min prior to eltrombopag dosing (pre-dose sample), and at 2, 4, 6, and 8 h after eltrombopag dosing. An optional sample will be collected 24 h post-dose, prior to administration of eltrombopag the next day.

Record the date, time, and amount (in mg) of the dose administered after the pre-dose PK sample. Collect each whole blood PK sample as close as possible to the planned time relative to dosing. Record the actual date and time that each sample was collected.

If a cannula is used, the cannula will be inserted into an arm vein within sufficient time prior to dosing, will be kept patent with normal saline and will be removed after the last blood sample is collected or earlier if the subject requests. In order to avoid artificial dilution of the PK sample by the saline, 0.5-1 mL of whole blood will be collected and discarded before each PK sample is collected.

7.7.1 PK Sample Processing and Storage

Each PK samples will be gently mixed by inversion 8 to 10 times (do not shake). Place the samples on ice immediately after collection. Within 1 hour of sample collection, the samples will be centrifuged in a refrigerated (2°C to 8°C) centrifuge at 1500 RPM for 10 minutes. The resulting plasma will be transferred into a properly-labeled polypropylene tube. Immediately, place the plasma samples upright in a -20°C freezer and retain the samples in the freezer until they are shipped for analysis.

7.7.2 Shipping Instructions

Samples should be shipped **only on Monday, Tuesday, or Wednesday**, not less often than every 2 months. Samples must be shipped on dry ice via overnight courier to:

LiMajor Pittman
PPD
2246 Dabney Road
Richmond VA, 23230, USA
Tel: (804) 977-8017
[e-mail: limajor.pittman@ppdi.com](mailto:limajor.pittman@ppdi.com)