CLINICAL RESEARCH PROJECT

Protocol # 12-H-0150
Drug Name: eltrombopag (Promacta®)
IND: 104877
IND holder: Cynthia E. Dunbar, M.D.

Date: October 22, 2015

To: Richard Cannon, MD, Chair, NHLBI IRB

Title: Eltrombopag added to standard immunosuppression in treatment-naïve severe aplastic anemia

Other Identifying Words: Immunosuppression, T-cells, hematopoiesis, autoimmunity, thrombocytopenia, neutropenia, pancytopenia.

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Subjects of Study:	Number	Sex	Age-range
Cohort 1	31	either	≥ 2 years and weight>12 kg
Cohort 2	33	either	≥ 2 years and weight >12 kg
Cohort 3	31	either	≥ 2 years and weight >12 kg
Extension Cohort	26	either	> 2 years and weight >12 kg

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

Tech Transfer: CRADA, MTA Yes

PRECIS

Severe aplastic anemia (SAA) is a life-threatening bone marrow failure disorder characterized by pancytopenia and a hypocellular bone marrow. Allogeneic bone marrow transplantation offers the opportunity for cure in younger patients, but most are not suitable candidates for transplantation due to advanced age or lack of a histocompatible donor. Comparable long-term survival in SAA is attainable with immunosuppressive treatment with horse anti-thymocyte globulin (h-ATG) and cyclosporine (CsA). However, of those patients treated with h-ATG/CsA, one quarter to one third will not respond, and 30-40% of responders relapse. The majority of the hematologic responses observed following initial h-ATG/CsA are partial, with only a few patients achieving normal blood counts. Furthermore, analysis of our own extensive clinical data suggests that poor blood count responses to a single course of ATG (non-robust responders), even when transfusion-independence is achieved, predicts a worse prognosis than when robust hematologic improvement is achieved (protocol 90-H-0146). The explanation for partial recovery and relapse are not fully understood, but incomplete elimination of auto-reactive T cells and insufficient stem cell reserve are both possible. Furthermore, 10-15% of SAA patients treated with standard immunosuppression will develop an abnormal karyotype in follow-up, with monosomy 7 being most common, which portends progression to myelodysplasia and leukemia. In contrast, malignant clonal evolution is rare in complete responders to immunosuppression. Although horse ATG/CsA represented a major advance in the treatment of SAA, refractoriness, incomplete responses, relapse, and clonal evolution limit the success of this modality. Thus, newer regimens are needed to address these limitations, and provide a better alternative to stem cell transplantation.

One approach to augment the quality of hematologic responses is to improve underlying stem cell function. Previous attempts to improve responses in SAA with hematopoietic cytokines including erythropoietin, G-CSF, and stem cell factor, have failed. Thrombopoietin (TPO) is the principal endogenous regulator of platelet production. In addition, TPO also has stimulatory effects on more primitive multilineage progenitors and stem cells *in vitro* and in animal models. Eltrombopag (Promacta®), an oral 2nd generation small molecule TPO-agonist, is currently approved for treatment of chronic immune thrombocytopenic purpura (ITP), chronic hepatitis C-associated thrombocytopenia, and severe aplastic anemia who have had an insufficient response to immunosuppressive therapy. Eltrombopag increases platelets in healthy subjects and in thrombocytopenic patients with chronic ITP and hepatitis C virus (HCV) infection. Our Branch recently completed a pilot study of eltrombopag in refractory SAA. We saw encouraging clinical results in a cohort of patients who have failed on average two prior immunosuppressive regimens (Olnes et al. ASH Annual Meeting Abstracts, San Diego, CA, 2011, oral presentation and N Engl J Med 2012;367:11-9.[1]). Of the twenty-five SAA patients treated with eltrombopag by mouth for three months, eleven (44%) patients met protocol criteria of clinically meaningful hematologic responses, without significant toxicity. Nine patients demonstrated an improvement in thrombocytopenia (>20k/μL increase or transfusion independence), hemoglobin improved in two patients (>1.5g/dL or achieved transfusion independence, and four patients had a significant response in their neutrophil count. When responders continued the drug beyond three months, the hematologic response to eltrombopag increased; a trilineage response was observed in four patients, and a bilineage response occurred in another four, with median follow-up of 13 months. These results suggest that stem cell depletion, a major component of the pathophysiology of SAA, might be directly addressed by eltrombopag administration. The aim of the current study would be to improve the hematologic response rate and its quality, as well as prevent late complications such as relapse and clonal progression, by addition of eltrombopag to standard immunosuppressive therapy.

[1]Olnes MJ et al. Eltrombopag and Improved Hematopoiesis in Refractory Aplastic Anemia. N Engl J Med 2012;367:11-9.

This trial will evaluate the safety and efficacy of combining eltrombopag with standard hATG/CSA as first line therapy in patients with SAA. The primary endpoint will be the rate of complete hematologic response at six months. Secondary endpoints are relapse, robust hematologic blood count recovery at 3, 6, and 12 months, survival, clonal evolution to myelodysplasia and leukemia, marrow stem cell content, and hematological response of relapse patients that re-start treatment.

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1. OBJECTIVES

To determine the safety and efficacy of h-ATG/CsA + eltrombopag in untreated subjects with severe aplastic anemia (SAA).

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 Treatment of Aplastic Anemia

2.3.1 Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic bone marrow transplantation from a histocompatible matched sibling is curative therapy in the majority of aplastic anemia patients who undergo the procedure.[10] Survival rates with allogeneic

hematopoietic stem cell transplantation from a histocompatible sibling have been reported to be as high as 90% from single institution studies, and approximately 70% in composite registry data, which more likely reflects the general experience.[11,12] The frequency and severity of graft-versus-host disease correlates with patient age and continues to be the major limiting factor in terms of both morbidity and mortality as well as long-term quality of life.[13] In general, adults have a lower survival rate compared to children, and most experts do not suggest allogeneic stem cell transplantation as first-line therapy, even from a fully matched sibling donor, for older SAA patients. Allogeneic bone marrow transplantation is available to only a minority of patients, since 70% will lack a suitable matched sibling donor. Alternative donor transplantation using a matched unrelated source is almost never the initial treatment, given the extended time period required to identify and recruit an appropriate matched unrelated donor, and the reported lower survival and higher rates of graft-versus-host disease compared to sibling donor transplants. Cord blood transplants are even less frequently utilized in SAA, due to higher rate of delayed hematopoietic reconstitution and persistent immune dysfunction in SAA patients and resulting poor survival in published trials.

2.3.2 Immunosuppressive Regimens

Horse Anti-thymocyte Globulin (ATGAM®; h-ATG) is currently approved for the treatment of aplastic anemia by the United States Food and Drug Administration. h-ATG as a single agent resulted in response rates of 30-50% in SAA in several large studies carried out in the 1980s.[14-16]The mechanism by which h-ATG improves bone marrow failure in aplastic anemia is not fully understood. h-ATG preparations contain a variety of antibodies recognizing human T-cell epitopes, many directed against activated T-cells or activation antigens.[17,18] After treatment with h-ATG, circulating levels of lymphocytes drop to 10% of pretreatment level, through a variety of mechanisms; Fc receptor complement-dependent lysis, opsonization and phagocytosis by macrophages, and immunomodulation leading to long-term depletion via antibody dependent cell-mediated cytotoxicity and activation-induced apoptosis. Although the decline in circulating levels of lymphocytes is transient, the number of activated T -cells is decreased for more prolonged periods of time; this effect is also reflected in decreased lFN-γ and possibly TNF production after h-ATG.[19,20] The response to h-ATG may be mediated by circulating factors produced in this immunologically activated state, although response to ATG has not correlated with severity or presence of clinical serum sickness.

Tissue culture preparations of peripheral blood lymphocytes treated with ATG produce hematopoietic colony stimulating factors, suggesting a possible additional activity of ATG in stimulating hematopoiesis *in vivo*. h-ATG binds to numerous other cell types in addition to lymphocytes, including cells of the bone marrow.[21-23]

Cyclosporine (CsA) is a major immunosuppressive drug and probably secondary only to corticosteroids in worldwide utilization in this role. In addition to its longstanding use in bone marrow and solid organ transplant recipients; CsA has been widely employed as an immunosuppressive drug in many autoimmune diseases. In contrast to ATG, CsA has a selective inhibitory effect on T lymphocytes, suppressing early cellular response to antigenic and regulatory stimuli. By blocking expression of nuclear regulatory proteins, it leads to reduced T cell proliferation and activation with diminished release of cytokines such as interleukin-2 and interferon-γ. CsA binds to intracellular receptors termed immunophilins, inhibiting in turn the activity of calcineurin, which results in the blocking of interleukin-2 production and T cell activation and proliferation. *In vivo*, CsA inhibits the release of IL-2 from activated T-cells and consequently decreases T cell proliferation.[24,25]

h-ATG/CsA is the current standard immunosuppressive regimen in SAA.[1]The addition of CsA to ATG improved response rates to 60-70% and the 5-year survival in responding patients to 80-90%.[26-28]With

this regimen, relapses occur in 1/3 of responders and clonal evolution to myelodysplasia and leukemia in 10-15% of cases overall.[1] Although this non-transplant therapy represents a therapeutic success, the significant minority of unresponsive patients, frequent relapse, and progression to late clonal disease remain problematic and result in significant morbidity and mortality. Robust and rapid hematologic recovery has been associated with better survival outcomes,[27] and in our experience, complete hematologic responders are unlikely to evolve to myelodysplasia or leukemia (unpublished data).

Alternative immunosuppressive agents have been used with limited success. At Johns Hopkins, high dose cyclophosphamide without stem cell rescue produced hematologic response rates similar to those seen with ATG combined with CsA in an initial small study, with no relapse or evolution to paroxysmal nocturnal hemoglobinuria (PNH) or myelodysplasia observed.[29] However, a prospective randomized trial conducted at the NHLBI, which compared ATG and cyclosporine to cyclophosphamide and cyclosporine, was terminated prematurely due to excessive toxicity, severe fungal infections and deaths in the group that received cyclophosphamide.[30] In contrast to the Hopkins' experience, some of our patients relapsed or developed cytogenetic abnormalities.[31] The explanation for the increased toxicity seen in the cyclophosphamide-treated patients is the serious immunosuppression and resulting neutropenia. With the cyclophosphamide regimen (at 200 mg/kg) the rate of hematologic complete response (40-50%) appears higher than that of h-ATG regimen (10-15%). In an attempt to circumvent the immediate toxicity with cyclophosphamide, we have investigated a lower dose (120 mg/kg) in treatment-naïve SAA patients in the past 15 months. Thus far complete responses have been observed in accordance to our and the Hopkins experience, but there is continued concern with toxicities despite lower dose cyclophosphamide.

As the addition of CsA clearly improved outcomes compared to the use of ATG alone, other immunosuppressive drugs were predicted, based on their mode of action, animal studies, and experience in other human diseases and with organ transplant, to increase response rates or decrease relapse. The addition of mycophenolate mofetil (MMF) to ATG and CsA in an NIH trial of 104 patients (protocol 00-H-0032) did not change hematologic response (about 62%), relapse (37%), or evolution rates.[32] In a follow up study sirolimus was added as a third agent to standard h-ATG/CsA. Again, when tested in a randomized protocol, results were not superior to standard h-ATG/CsA (protocol 03-H-0193). More lymphocytotoxic agents that are active in the relapsed and refractory settings were studies as first line therapy in the context of a large randomized study (protocol 06-H-0034). Results were disappointing. Both rabbit ATG and alemtuzumab yielded inferior response rates when compared to horse ATG.[33] Thus, h-ATG plus CsA remains the standard immunosuppressive regimen in SAA.

2.2.3 Hematopoietic Cytokines

The isolation and cloning of hematopoietic growth factors was rapidly followed by testing of their clinical activity in aplastic anemia. The rationale for use of cytokines that act on committed hematopoietic progenitors in specific differentiated lineages, such as erythropoietin and G-CSF, has been suspect. First, their receptors are not present on primitive stem cells, at best might be expected to shorten time to recovery of blood counts following therapy, once immunosuppression or transplantation allows some recovery of stem cell numbers. Second, endogenous erythropoietin levels are extremely elevated in SAA, calling into question the rationale for exogenous administration. Clinical trials have examined the addition of GM-CSF, G-CSF, and IL-3,all considered myeloid cell cytokines, alone or added to standard immunosuppressive therapy. In this setting, G-CSF has been widely studied in combination with horse ATG plus CsA. This regimen has not been shown to improve outcomes in several randomized trials.[34-36]. The lack of benefit may be secondary to the activity of G-CSF on more committed myeloid progenitors, suggesting that a growth factor that acts on less differentiated progenitor cells might have activity. A recent meta-analysis of 19 individual trials concluded that there was no impact on survival or response rate with the addition of these cytokines to standard immunosuppressive regimens.[37]

The relationship between extended treatment with G-CSF and clonal evolution of SAA to myelodysplasia and leukemia is unclear. A Japanese study in pediatric patients with SAA found a relationship between the cumulative number of days of G-CSF and eventual progression to MDS/AML, however, the study was not randomized or controlled regarding whether G-CSF was given or not.[38] A smaller case series in adults also suggested a relationship.[39] However, patients with SAA clearly have an increased risk of late clonal disease, and those who are non-responders or less robust responders are more likely to progress[38](our unpublished data). Non-responding patients are more likely to receive G-CSF for prolonged periods of time, despite lack of efficacy, as some patients may show an increase in neutrophil counts with G-CSF, despite lack of overall survival or response benefit. At present, there is no clear role for G-CSF in the treatment of patients with SAA, and the relationship of G-CSF to late clonal disease is remains uncertain.

2.4 Thrombopoietin and Hematopoiesis

Thrombopoietin (TPO) was purified, identified and cloned by independent research groups in academia and industryin 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.[40] 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.[41,42]*In vitro* expansion of functional and phenotypic HSCs can be stimulated by TPO, either alone or in combination with other cytokines.[43]

The control of TPO levels and TPO production in 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.[44]More recent studies also confirm TPO levels to be high in SAA and moderately elevated in myelodysplastic syndromes compared to normal controls.[45]

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.[46]Studies conducted *in vitro* have shown that eltrombopag is an effective agonist binding to *mpl*, the thrombopoietin receptor (TPO-R), to stimulate thrombopoiesis. It binds *mpl* at a position distinct from the ligand binding site, within the juxtamembrane domain of the receptor, and thus does not compete with TPO for binding to its receptor.[47] 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 HSCs to thrombopoietin.[48]

In vivo, eltrombopag increased platelet number in the chimpanzee (the only nonclinical species which is pharmacologically responsive to eltrombopag).[48] 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.[44] The initial phase 1/ 2 and randomized, controlled phase 3 registration trials in chronic ITP were very encouraging, with little toxicity and much higher responses by comparison with placebo[49-51], 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. A second TPO-agonist, romiplostim (Nplate) was developed by Amgen, is also available for the treatment of chronic ITP and is given subcutaneously weekly.

Longer follow-up in EXTEND suggests that eltrombopag remains well tolerated (EXTEND Trial, NCT00351468). On December 6, 2011 the FDA agreed to modify eltrombopag's Risk Evaluation and Mitigation Strategies to assure safety use 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 by post-marketing surveillance programs and ongoing clinical trials.

2.6 Eltrombopag for Refractory Severe Aplastic Anemia

Reasoning that eltrombopag stimulated primitive HSCs and progenitors and as there was a clear deficit in HSC numbers and function in SAA, in 2009 we initiated a single-arm dose escalation phase 1/2 trial for patients with refractory SAA. For protocol entry, all patients had to have severe thrombocytopenia in addition to fulfilling criteria for SAA, and they must have failed at least one prior regimen of standard ATG-containing immunosuppressive therapy. The primary endpoints were safety and clinically significant hematologic response. The study design and response criteria are shown in Supplemental Figure 1.

At termination, 26 patients were enrolled in the protocol and 25 had received study drug. As shown in Table 1, this patient population had very prolonged and serious cytopenias. 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 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.

Number of patients	**26**
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/μL)	9 (5-15)
Neutrophils (K/μL)	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 maximum 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 hepatitis B. He was taken off study, and with recovery from hepatitis B infection, serum transaminase values returned to baseline.

In March 2014 data from this clinical trial was also published (Desmond, Townsley, et al. Blood 2014) reporting safety and efficacy data on a further 18 patients and long-term follow-up on the entire cohort of 43 patients. The overall response rate was 17 of 43 patients (40%) at 3 to 4 months, including trilineage and bilineage responses. Most patients (14/17) continued to show trilineage improvement and 5 patients had drug discontinued for near normalization of blood counts without relapse. Eight patients developed new cytogenetic abnormalities on eltrombopag, including 5 with chromosome 7 loss or partial deletion, but none evolved to leukemia. Eltrombopag was immediately discontinued when cytogenetic abnormalities were observed.

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

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

Category	Event	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 2Off x 6	Unlikely
		1	150	Unlikely
	Gastroenteritis	1		Unlikely
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; SAE, severe adverse event.

Prior studies in patients with chronic ITP have raised the concern that TPO mimetics, including eltrombopag, might increase bone marrow reticulin deposition.[52]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).

Eltrombopag has an FDA warning for cataract formation based on a prior study in ITP patients. 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 a new lens opacification 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.

Among 25 evaluable patients 11 (44%) achieved protocol-defined hematologic response after 12 weeks of eltrombopag treatment (Supplemental Fig. 2). 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). Two patients achieved a hemoglobin response by 12 weeks, and 4 additional patients had improved hemoglobin levels on the extension phase (Supplemental Fig. 3B), 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/μL), including 4 patients who were severely neutropenic at baseline (Supplemental Fig. 3C). 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 since 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. The remaining responders on the extension study will have their dose of eltrombopag tapered in order to determine whether responses more generally can be maintained off of drug. There have been no other suggestions of new cataracts in this study.

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 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. Progression to monosomy 7/MDS is concerning, given the controversy regarding whether chronic G-CSF increases progression to MDS in SAA as summarized above, and the warning regarding progression of MDS to AML for patients being treated with an alternative TPO-mimetic, romiplostim. Of note, romiplostim directly binds to the thrombopoietin receptor (c-mpl) and competes with endogenous thrombopoietin; while eltrombopag binds to a transmembrane domain of c-mpl leading to signal transduction. The distinct mechanism of action between the 2 thrombopoietin mimetics may account for differences in efficacy and safety profiles between them. In our cohort of over 400 patients with SAA followed long-term, approximately 15% progress to clonal disease including myelodysplasia and leukemia, and therefore two patients out of 25 enrolled is not unexpected. 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.

2.7 **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, pilot phase II study (NCT00922883) of eltrombopag as a single agent in patients with refractory SAA. 25 patients (age range 18-77 years) received 50mg daily of eltrombopag with dose escalation every two weeks to a maximum dose of 150mg daily. Patients were successfully escalated to the 150mg daily

dose without observing any dose-limiting toxicities. Hematologic responses were only observed while receiving the 150mg daily dosing; it is possible that patients would have responded to lower doses of drug had the dose not been escalated every two weeks. Therefore, the duration of therapy in this setting has not been well defined.

There is preliminary safety data with doses up to 300 mg per day in a number of different patient populations. 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-τ) values of 302 μg/mL for the 200 mg once daily regimen. [53] 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. 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.[49-51] 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 μg/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 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.

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 and 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.

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. It is possible although unlikely that patients with previously untreated SAA, who may have better residual hematopoietic stem cell function, could develop very high platelet counts on eltrombopag. 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 3 randomized trials for chronic ITP.[51] However, patients with ITP, in contrast to patients with SAA, have hyper-reactive platelets and an increased endogenous risk of thrombosisIn an extensive analysis of ITP patients treated long-term with romiplostim, an alternative thrombopoietin mimetic, there was no evidence for increased thrombotic events in the romiplostim-treated patients compared to controls.[54]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.[55]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.

2.8 Rationale for Permitting Dose Interruption

The effect of dose interruption is unknown in the aplastic anemia population. 31% (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 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 drug on HSC number or function is long-lasting and prolonged therapy may not be required in SAA.

2.9 Scientific and Clinical Justification of the Protocol

The combination of ATG, which lyses lymphocytes, and CsA, which blocks T-cell function, is responsible for survival rates comparable to those observed with transplant recipients.[27,56] Hematology Branch efforts for the past 10-15 years have been focused on developing immunosuppressive regimens in SAA that would circumvent the limitations presently observed with h-ATG/CsA. The failure of more intensive immunosuppressive regimens to improve outcomes suggests that even if immune attack on the bone marrow can be interrupted, deficits in the number or expansion ability of HSCs may limit hematopoiesis in these patients. Clinical and laboratory data suggest that greatly diminished stem cell numbers limit the effectiveness of immunosuppression and contribute to relapse and evolution.[57] It is reasonable therefore to conclude that the addition of a hematopoietic growth factor capable of expanding primitive HSCs and progenitors would be useful.

One such hematopoietic growth factor, TPO, is a potent endogenous cytokine and the principal regulator of platelet production, as summarized above. A 2nd generation TPO-agonist, the nonpeptide mimetic eltrombopag, is administered orally, well tolerated and does not induce auto-antibodies, in contrast to first-generation TPO-R agonists such as megakaryocyte growth and development factor (MDGF).

Eltrombopag has been shown to increase platelets in healthy subjects, patients with chronic immune thrombocytopenic purpura (ITP), and more recently shown to increase blood counts in patients with hepatitis C associated thrombocytopenia[58,59] and now in SAA as single agent, in our recently completed trial which has been presented in abstract form and now published (N Engl J Med 2012;367:11-9).[60]These surprising results suggested that eltrombopag, like thrombopoietin itself, might act on the hematopoietic stem cell and offer real clinical benefit in SAA by increasing stem cell number.

Since outcome is strongly related to the presence of early recovery and to the quality of the blood cell count response at 3 months after receiving antithymocyte globulin, an agent like eltrombopag in enhancing the quality and speed of hematologic recovery could both shorten time at risk for infections, bleeding and transfusions as well as potentially reduce the rates of late events linked to a deficient stem cell compartment, and thus improve survival. In a cohort of 122 patients with SAA at our institution treated with H-ATG and CsA, survival was associated with early attainment of hematologic recovery (86% vs. 40% at 5 years, P<.001), and degree of blood count recovery at 3 months (90% vs. 42% for patients with less robust recovery).[27]Therefore, the addition of eltrombopag to standard immunosuppressive therapy is likely to have an impact on survival if the quality of hematologic recovery can be improved during the first 3 - 6 months of treatment.

The objectives of this trial are therefore to assess the effectiveness of eltrombopag alongside standard immunosuppressive therapy with h-ATG/CsA for treatment naïve severe aplastic anemia and to define the additive toxicity of eltrombopag with h-ATG/CsA. A secondary objective for this study will be to describe the common aspects of health-related quality of life (HRQL) in the adult subjects pre- and post-study treatment which is recommended by the FDA[61] and the IOM[62] as indicators of treatment effects.

2.9.1 Justification for adding Cohort 2 (August 2013)

In this study we are observing more rapid hematologic improvement than observed with immunosuppression alone. The overall response rate of 84% (11/13) at 6 months, with 100% of these responses observed by 3 months, is also higher than in historical data. As subjects currently receive eltrombopag for 6 months but blood counts have improved by 3 months, it is possible that administration of eltrombopag may only be necessary for the shorter duration, lowering risk, cost, and inconvenience. In particular, limiting exposure to eltrombopag may reduce the risk of cytogenetic evolution. Further, in the event of relapse, expected with discontinuation of treatment, we can now not distinguish whether cyclosporine or eltrombopag is responsible, as both are administered concurrently. Therefore, we propose to treat another cohort (cohort 2) of 31 subjects, with no change to this protocol other than a reduction in eltrombopag administration from 6 to 3 months. The study objectives, primary and secondary endpoints, statistics – sample size, stopping rules, eligibility, monitoring and ancillary studies will remain unchanged. Responses at 3 months can be assessed for the two cohorts, providing a confirmation cohort for our early results, whereas new information will be provided by comparison of long-term outcomes between the two cohorts. These data can be used to inform design of a future randomized, multicenter phase III trial. Note amendment 17 was submitted on October 23, 2014 to increase Cohort 2 sample size to 33 subjects in order to allow subjects to be enrolled and treated while amendment 16 to add Cohort 3 was being reviewed. Note, only 31 subjects were enrolled into Cohort 2 because the Cohort 3 amendment was approved prior to needing to enroll the 2 additional subjects into Cohort 2.

2.9.2 Justification for adding Cohort 3 (September 2014)

As of September 15, 2014, 57 subjects have been enrolled on the protocol (31/31 in cohort 1, and 26/31 in cohort 2) and the combination of cyclosporine and eltrombopag has been safe and well tolerated. Higher overall response rates with eltrombopag compared to immunosuppression alone are a consistent observation from both the first and second cohort. Most subjects respond within 2-3 weeks of the first dose of eltrombopag alleviating the transfusion burden sooner than our historical cohort. In cohort 2,

where eltrombopag is discontinued at 3 months in all subjects, all subjects have either maintained or normalized their blood counts between 3 and 6 months. In cohort 1 and cohort 2 eltrombopag was implemented to begin on Day 14 to avoid any potential hepatotoxicity from the combination of cyclosporine and h-ATG. However, now that we have ample data indicating the combination of cyclosporine and eltrombopag is safe, the earlier initiation of eltrombopag on Day 1 in combination with hATG should be tested in a third cohort. Therefore we propose to treat another cohort (cohort 3) of 31 subjects, where eltrombopag will be administered without delay, starting Day 1, with the intention of accelerating hematologic recovery. Eltrombopag will be discontinued at the 6-month landmark visit.

2.9.3 Justification for adding Extension Cohort

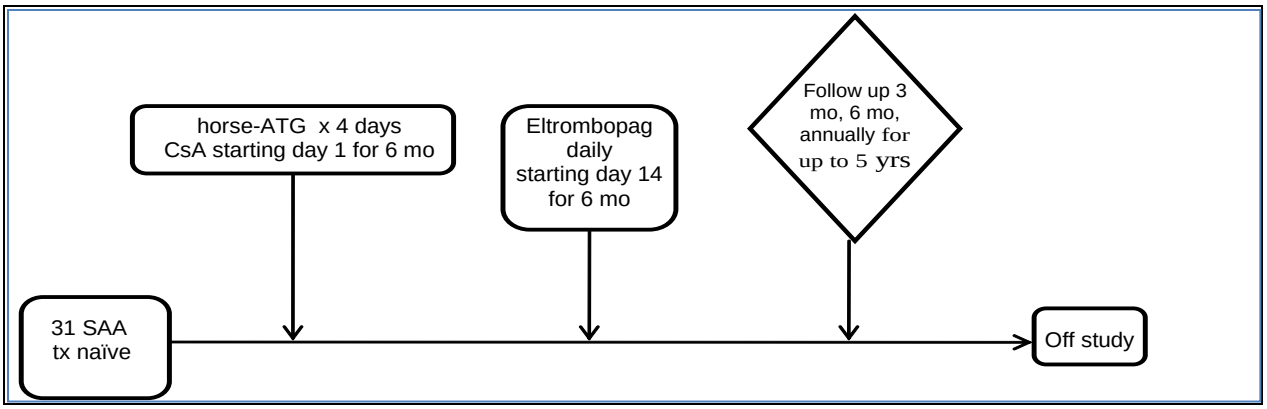
Amendment 22 (V) is being submitted to add an Extension Cohort of 26 subjects to the protocol once enrollment is completed in cohort 3 in order to allow additional subjects to be enrolled and treated while a new SAA treatment naïve protocol is developed and approved. We anticipate enrollment will be completed before we have a new SAA treatment naïve protocol developed and approved. The time frame to receive approval from Novartis will be prolonged due to the recent acquisition of GSK Oncology by Novartis.

3 STUDY DESIGN

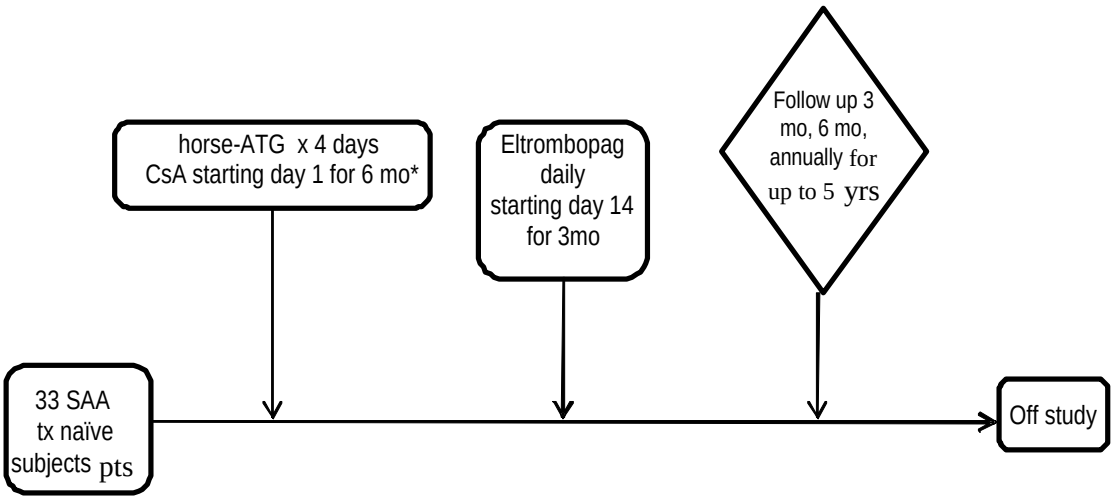
This study is designed as a non-randomized, pilot phase I/II study in which 121 subjects with severe aplastic anemia who have not received prior definitive immunosuppressive therapy will be treated with a standard regimen of h-ATG/CsA, combined with eltrombopag as experimental therapy. The first 31 subjects will be enrolled to cohort 1. Then up to 33 subjects will be enrolled to cohort 2. Then up to 31 subjects will be enrolled in cohort 3. Subjects enrolled to cohort 1 will receive eltrombopag for 6 months whereas subjects enrolled to cohort 2 will receive eltrombopag for 3 months. Subjects enrolled in cohort 3 will initiate h-ATG, CsA and eltrombopag on Day 1. The primary clinical endpoint of this study is the quality of hematologic response at 6 months. In cohorts 1 and 2 eltrombopag will be initiated on day 14 to avoid overlap with the known transient hepatotoxicities associated with ATG and cyclosporine. Cohort 3 will initiate h-ATG, CsA, and eltrombopag on Day 1 if there are no significant hepatotoxicities seen in cohorts 1 and 2. Patient-reported outcome questionnaires (Appendix E) will be collected initially when the subject enrolls on-study (pre-ATG/CSA), pre-eltrombopag, at 3 and 6 months, and annually for 5 years.

Up to an additional 26 subjects may be enrolled into the Extension Cohort in order to allow subjects to be enrolled and treated while a new SAA treatment naïve protocol is developed and approved. The Extension Cohort's treatment schedule will be the same as per Cohort 3.

Protocol Design for Cohort 1

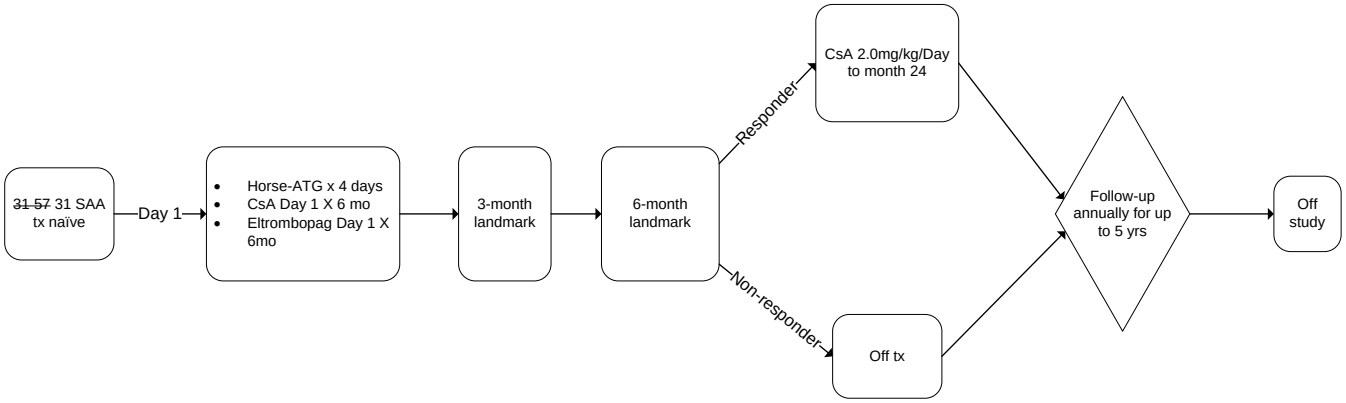


Protocol Design for Cohort 2 added August 2013

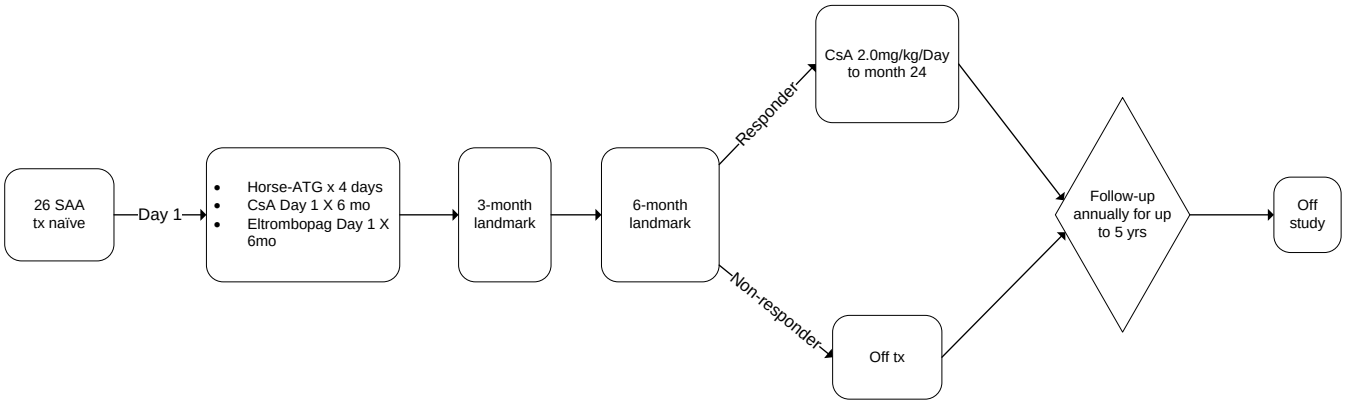


*Cohort 2 will continue CsA administration month 6 to month 24 at 2.0mg/kg per amendment number 15

Protocol Design for Cohort 3 added September 2014



Protocol Design for Extension Cohort



4 ELIGIBILITY ASSESSMENT

All subjects age 2 and older with SAA who have not received prior ATG-based immunosuppressive therapy and lack a suitable matched sibling marrow donor, or are not allogeneic transplantation candidates due to patient choice, advanced age, or infeasibility of transplantation will be considered for enrollment.

Eligibility will be determined on another screening Hematology Branch protocol (97-H-0041) or any another active Hematology branch protocol. The time between determination of eligibility and signing consent to participate on this protocol and initiate treatment on this protocol will not exceed 90 days. Due to the nature of SAA, counts may fluctuate depending on transfusions. Because of this, the lowest clinical laboratory result (ANC, platelet, and/or absolute reticulocyte count) obtained within 30-days prior to treatment initiation will be used for eligibility determination.

4.1 Inclusion Criteria

4.1.1 Severe aplastic anemia characterized by
Bone marrow cellularity <30% (excluding lymphocytes) *

AND

At least two of the following:

- Absolute neutrophil count< 500/ μL
- Platelet count< 20,000/ μL
- Absolute reticulocyte count<60,000/ μL

4.1.2 Age ≥ 2 years old
4.1.3 Weight> 12 kg

* If a range is provided instead of the overall bone marrow cellularity the median value of that range will be used for this inclusion criterion.

4.2 Exclusion Criteria

4.2.1 Known diagnosis of Fanconi anemia

4.2.2 Evidence of a clonal disorder on cytogenetics performed within 12 weeks of study entry. Patients with super severe neutropenia (ANC < 200 /μL) will not be excluded initially if cytogenetics are not available or pending. If evidence of a clonal disorder consistent with myelodysplasia is later identified, the patient will go off study – see section 9.6.

4.2.3 Prior immunosuppressive therapy with any ATG, alemtuzumab, or high dose cyclophosphamide

4.2.4 SGOT or SGPT >5 times the upper limit of normal

4.2.5 Subjects with known liver cirrhosis in severity that would preclude tolerability of cyclosporine and eltrombopag as evidenced by albumin < 35g/L

4.2.6 Hypersensitivity to eltrombopag or its components

4.2.7 Infection not adequately responding to appropriate therapy

4.2.8 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

4.2.9 Potential subjects with cancer who are on active chemotherapeutic treatment or who take drugs with hematological effects will not be eligible

4.2.10 Current pregnancy, or unwillingness to take oral contraceptives or use a barrier method of birth control or practice abstinence to refrain from pregnancy if of childbearing potential during the course of this study

4.2.11 Inability to understand the investigational nature of the study or to give informed consent or does not have a legally authorized representative or surrogate that can provide informed consent per section 11.6.

5 TREATMENT PLAN

5.1 Horse Anti-thymocyte Globulin (h-ATG) Administration

A single treatment course of h-ATG will be administered at a dose of 40 mg/kg/day for 4 consecutive days. Dose will be calculated based on actual body weight. H-ATG will be infused intravenously for approximately 4 hours. The infusion time may vary based on the patient and circumstances. Infusion times may be extended up to 24 hours to improve tolerance of infusional side effects such as fever, chills and hypotension if necessary. Serum sickness prophylaxis with oral prednisone at 1 mg/kg/d will begin prior to the first dose of h-ATG, and will be continued for 10 days total and then tapered over the subsequent 7-14 days. Those subjects who develop serum sickness may require a longer tapering schedule and will be dosed individually as clinically indicated.

5.2 Cyclosporine (CsA)

Day 1 to Month 6 dosing:
For subjects ≥ 12 years of age, cyclosporine will be started on day 1 at 3 mg/kg/dose by mouth administered every 12 hours (total daily dose of 6 mg/kg/day). Dosing will be based on actual body weight except in obese subjects. For obese subjects (defined as a body mass index > 35 in adult subjects [> 20 years of age] and > 95th percentile in subjects 12 to 20 years of age), cyclosporine dosage will be based on an adjusted body weight that is calculated as the midpoint between the ideal body weight and actual body weight (see below for definition of ideal body weight).

For subjects < 12 years of age, cyclosporine will be started on day 1 at 6 mg/kg/dose by mouth administered every 12 hours (total daily dose of 12 mg/kg/day). Dosing will be based on actual body weight except in obese subjects. For obese subjects (defined as a body mass index > 95th percentile in subjects 12 to 20 years of age), cyclosporine dosage will be based on an adjusted body weight that is calculated as the midpoint between the ideal body weight and actual body weight (see below for definition of ideal body weight).

In subjects who had a therapeutic CsA level established prior to protocol enrollment, the same CsA dose will be initiated with the h-ATG and adjusted accordingly. Cyclosporine dose may be interrupted when clinically indicated at the discretion of the investigator. Interruptions will not be reported as deviations; however, when the interruption is the consequence of a serious adverse event, the interruption will be included in the report.

Cyclosporine dosing will be adjusted to obtain a goal therapeutic trough level between 200 and 400 mcg/L.

Ideal Body Weight Definitions:
Ideal body weight (adult male, age > 20): 50 kg + 2.3 kg per inch over 5 feet
Ideal body weight (adult female, age > 20): 45.5 kg + 2.3 kg per inch over 5 feet
Ideal body weight (pediatrics, ages 2–20): 50th percentile weight based on CDC growth curves.

Month 6 to Month 24 cyclosporine dosing:
On review of the data as of September 17, 2014 where a trend towards a higher than expected number of relapses after the 6 month landmark visit were observed and salvaged by resuming cyclosporine, the protocol was amended to prolong administration of cyclosporine beyond 6 months per the following:

At the 6-month landmark visit, responders will have the CsA dose reduced to 2.0mg/kg/day administered orally at a fixed dose through the 24 month timepoint. Previous anecdotal experience suggested that a gradual taper of CsA may avoid relapse, but when we adopted this strategy prospectively in our clinical trials (03-H-0193 and 06-H-0034) the rate of relapse was unchanged (Scheinberg, Rios, et al. AJH 2014[67]). However, time to relapse was delayed by 1 year with the longer course of CsA and may be dose-dependent. The CsA dose at the time of relapse during the taper phase was at a median of 1.32 (range: 0.52-4.18) mg/kg/day and a mean of 1.8 mg/kg/day. This suggests that a CsA dose level above these levels might preclude relapse and improve tolerability of chronic maintenance. For remaining subjects due for the 6 month timepoint on cohort 2 we will prolong administration of cyclosporine beyond 6 months. CsA will continue to be administered from Day 1 to 6 months, but from 6 months to 2 years CsA will be administered at a lower fixed daily dose (2 mg/kg/day) in an attempt to improve relapse, a secondary endpoint, among responders in the study.

5.3 Eltrombopag

Subjects will initiate eltrombopag at a starting daily dose as detailed in Table 3, according to age and ethnicity. Subjects between 12 and 17 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). Eltrombopag will be administered orally as tablets or an oral suspension from sachet of eltrombopag powder. 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. If an adult receives the oral suspension administration of eltrombopag, the required number of sachets will be used to administer the below adult doses.

Table 3. Dosing according to age and ethnicity

Age groups	Daily dose
Non-Asian	
12-85	150 mg
6-11	75 mg
2-5	2.5 mg/kg
East Asian, South East Asian	
12-85	75 mg
6-11	37.5 mg
2-5	1.25 mg/kg

Eltrombopag may be taken on an empty stomach (1 hour before or 2 hours after a meal) or with food containing little (<50 mg) or preferably no calcium or dairy products. Allow at least a 4-hour interval between eltrombopag and other medications or products containing polyvalent cations (e.g. calcium, magnesium, aluminum, zinc, selenium or iron) such as antacids, dairy products, and mineral supplements

to avoid significant (70-75%) reduction in eltrombopag absorption due to chelation.

Dose delay or dosing interruptions:
Cohort 1:
Eltrombopag dose may be interrupted when clinically indicated at the discretion of the investigator. Interruptions will not be reported as deviations; however when the interruption is consequence to a serious adverse event, the interruption will be included in the report.

Interruptions will not be reported as deviations to the IRB, but will be recorded in the medical record. However, when the interruption is consequence to a serious adverse event, the interruption will be included in the SAE report.

Cohort 2, 3, and Extension Cohort:
The first dose of eltrombopag may be delayed if it is clinically indicated or other unforeseen events affect the start of eltrombopag (for example weather related) at the discretion of the investigator.

Eltrombopag dose may be interrupted when clinically indicated at the discretion of the investigator (for example if the patient is in the intensive care unit and unable to take PO medications). For subjects on cohort 2. if eltrombopag dosing is delayed or interrupted for more than 1 week (7 days) and the interruption is not the result of a severe adverse event related to eltrombopag, then the subject will receive additional eltrombopag in order to receive a total of 10 weeks as originally planned for cohort 2.

Interruptions will not be reported as deviations to the IRB, but will be recorded in the medical record. However, when the interruption is consequence to a serious adverse event, the interruption will be included in the SAE report.

5.4 Dose Adjustments of Eltrombopag

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

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

5.5 Dose Delays, Modifications or Discontinuation of Eltrombopag for Non-Hematologic Side Effects

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

5.5.2 Liver function abnormalities:
Cohorts 1 and 2:
In the event of an increase in the ALT level to > 6 times the ULN, subjects will return to clinic or

have blood tests drawn by their home physician every 3-4 days. If the ALT remains > 6 times the ULN on a second blood test, eltrombopag will be discontinued until ALT is < 5 times the ULN. Eltrombopag will be restarted at a dose level 25 mg/day lower than the prior dose. If liver test abnormalities return to an ALT of > 6 times ULN on this reduced dose, the eltrombopag dose with be reduced by 25 mg/day until there is reduction of ULN to < 5 ULN.

Cohort 3 and Extension Cohort:
Transient hepatotoxicity is an expected and common side effect of h-ATG that occurs during the first 14 days following the start of h-ATG administration. In the event of an increase in the ALT level to > 6 times the ULN during Days 1 – Days 14, eltrombopag will be held until ALT is < 5 times the ULN and then resumed at the same dose. If the ALT rises to > 6 times ULN after resuming eltrombopag (and is not attributable to other inciting factors such as serum sickness, sepsis, or azole antifungal agents) then the ALT will be monitored at least every 3-4 days. If the ALT remains > 6 times the ULN on repeat blood tests, eltrombopag will be stopped until the ALT is < 5 times the ULN. Then eltrombopag will be restarted at a dose level that is 25 mg/day lower than the prior dose. If liver test abnormalities return to an ALT of > 6 times ULN on this reduced dose, the eltrombopag dose with be reduced by 25 mg/day until there is reduction of ULN to < 5 ULN.

5.6 Dose Delays, Modifications or Discontinuation of Eltrombopag for Hematologic Side Effects

5.6.1Thrombosis/Embolism: Subjects who experience a deep venous thrombosis (other than a line-related upper extremity thrombosis) or a pulmonary embolus, a TIA or stroke, or a myocardial infarction at any time while on eltrombopag will discontinue eltrombopag but remain on CsA and hATG. Subjects with platelet counts of > 50,000/ μL at the time of thrombosis will be treated with enoxaparin or another appropriate anticoagulant as clinically indicated until the platelet count drops below 20,000/ μL or they complete a standard 3-6 month course of anticoagulation.

5.7 Extended Access to Study Drug

Responders:
Cohorts 1 and 2: Study drug (eltrombopag) administration will be discontinued at 6 months for subjects in cohort 1 and at 3 months* for subjects in cohort 2; cyclosporine will be discontinued at 6 months for both cohorts.

Cohorts 1 and 2 Subjects that Relapse:
In cohort 2, if a subject who received eltrombopag for 3 months* has evidence of relapse as defined in Section 7, eltrombopag and/or cyclosporine can be restarted at the clinical investigator's discretion. Such cohort 2 subjects, who require treatment prior to month 6, will be denoted as non-responders at 6 months for statistical purposes.

Subjects in cohort 1 or 2 that relapse (defined in section 7) after the end of 6-month treatment period while in follow-up may have eltrombopag and/or cyclosporine restarted at the clinical investigator's discretion. Subjects in cohort 2 that received the reduced CsA dose (2mg/kg/day) starting after the 6-month landmark visit who relapse despite the prolonged reduced dose of CsA may have the CsA dose increased to achieve therapeutic levels (trough levels 200-400) at the clinical investigator's discretion. Subjects can remain on the drugs under this protocol until the end of the 5 year follow-up period.
*Study drug (eltrombopag) administration will be discontinued at 3 months for subjects in cohort 2, except for subjects experiencing dose delays/interruptions as outlined in Section 5.3.

Cohort 3 and Extension Cohort Subjects that Relapse:
Subjects that relapse (defined in section 7) after the end of 6-month treatment period while in follow-up may have eltrombopag restarted at the clinical investigator's discretion. In addition, the cyclosporine dose may be increased to achieve a therapeutic level. Subjects can remain on the drugs under this protocol until the end of the 5-year follow-up period.

5.8 Pre-medications and Management of Infusion Reactions

Subjects will receive pre-medication approximately 30 minutes prior to infusion of ATG as follows:

- oral diphenhydramine 1 - 1.5 mg/kg/dose (NTE 50 mg) administered orally or intravenously, and
- oral acetaminophen 10-15 mg/kg/dose (NTE 650 mg)

Oral prednisone at 1 mg/kg/d will begin prior to the first dose of h-ATG for serum sickness prophylaxis, and will be continued for 10 days total and then tapered over the subsequent 7-14 days. Infusion reactions will be treated symptomatically (e.g., antiemetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators, meperidine). Prednisone dose will be calculated based on actual body weight.

In case of moderate or severe reactions hydrocortisone will be given and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided. If a subject has a persistent severe infusion reaction that does not respond to measures to ameliorate the signs/symptoms associated with the infusion, the h-ATG infusion will be discontinued (see section off study criteria) and subjects will go off study.

5.9 Permitted Supportive Care

- Transfusion support (blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) if deemed necessary by the investigator or treating physician. Romiplostim (N-Plate) or IL-11 (Neumega) should not be administered.
- Estrogens or combination OCP's as indicated for uterine bleeding

5.10 Concurrent Mediations:

Cyclosporine/magnesium: Subjects will be on chronic CsA therapy targeting a stable drug level as long as eltrombopag is administered 4 hours after oral magnesium given to counteract magnesium-wasting on CsA. Magnesium supplementation will not be given concurrently with eltrombopag as it may interfere with eltrombopag's absorption. The drug-drug interaction potential between eltrombopag and CsA is unknown. Both CsA and eltrombopag are inhibitors of OATP and BCRP drug transporters, and eltrombopag is a substrate of BCRP in vitro. It is not known if the combination will result in any PK changes to either drug. Subjects will be monitored for signs of CsA toxicity during the study, and therapeutic drug monitoring can be instituted as required. In the event of liver function abnormalities as a consequence of a drug-drug interaction between eltrombopag and CsA, eltrombopag will be dose-reduced according to Section 5.5.2

Rosuvastatin: In vitro studies demonstrated that eltrombopag is not a substrate for the organic aniontransporter 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. All subjects on cyclosporine require prophylaxis against PCP and will 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. Subjects aged 5 years and over will receive pentamidine for PCP prophylaxis but children under 5 years of age (approximate) are often not able to complete the inhalation treatment with pentamidine and will receive dapsone or another prophylactic regimen. 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, with the exception of N-Plate and Neumega (see 5.9).

5.11 Infection Prophylaxis and Monitoring

***Pneumocystis jiroveci* prophylaxis:** Aerosolized pentamidine will be used as prophylaxis against *Pneumocystis jiroveci,* 300 mg approximately every 4 weeks by inhalation beginning the first month of therapy and to continue for 6 months totalfor subjects 5 years of age and older for all cohorts. Dapsone or another prophylactic regimen against *Pneumocystis jiroveci* may be substituted at the discretion of the PI. Bactrim (TMP/SULF) will be avoided because trimethoprim is a moderate to strong inhibitor of CYPs that may theoretically result in enhanced activity of eltrombopag. Children under 5 years of age (approximate) are often not able to complete the inhalation treatment with pentamidine and will receive dapsone or another prophylactic regimen at the discretion of the PI.

Antiviral prophylaxis: Valacyclovir, 500 mg once daily, will be administered for at least 1 month in all subjects regardless of HSV serology status. Pediatric subjects less than 40 kg, will receive acyclovir (or equivalent) at 20mg/kg PO q12h to a maximum dose of 800mg q12h. Prophylaxis may be extended at the discretion of the PI.

Antibacterial and antifungal prophylaxis will not be included systematically with the immunosuppressive regimen, but may be administered at the discretion of the PI or treating physician on a case-by-case basis.

5.12 Management of Fever in Neutropenic Subjects(All subjects)

Subjects with a single temperature of 38.5 °C or two readings of 38.0 °C or greater will be evaluated for infection including cultures of blood and urine and any other suspicious sites prior to beginning empiric therapy. Antibiotics will be initiated following current infectious disease guidelines.

5.13 Instructions to Subjects

Special Instructions regarding CsA:

Regarding concomitant medications: Certain other medications can change the level of cyclosporine in the blood. Some of these medications are erythromycin, ketoconazole, diltiazem, rifampin, phenytoin and phenobarbital. We will ask subjects to inform us of any medication taking concomitantly while on the study.

Regarding prohibited foods: Grapefruit and grapefruit juice may increase the effects of CsA by increasing the amount of this medicine in the body. Subjects will be advised not to eat grapefruit or drink grapefruit juice while taking this medicine.

Regarding Immunizations: While taking CsA and for at least three months following discontinuation, immunizations should be avoided, and any planned immunization should be discussed with study investigators. There is almost no possibility that a vaccination given during this time period will be effective in stimulating immunity. Any live or attenuated vaccine may result in an infection, due to compromised immunity on CsA and h-ATG. Subjects should also avoid close household contact with individuals receiving the live oral polio vaccine for at least 72 hours following administration.

Special Instructions regarding eltrombopag:

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

5.14 HRQL Questionnaires

The relevant dimensions of HRQL being assessed in this study include (1) PROMIS Global Health, Sleep Disturbance, Applied Cognition-Abilities, Anxiety and Depression and (2) FACT- Anemia, Thrombocytopenia and Neutropenia.

Patient-Reported Outcomes Measurement Information System (PROMIS®), is an initiative based on an NIH grant to establish and provide the public a free, reliable and validated commonly used measures of patient-reported outcomes.[63]

The FACT instruments[64] are a health assessment instrument designed to measure multi-dimensional quality of life in chronic illness and its associated therapy. The different subscales selected for this study are specific for patients with diseases or treatments with hematological effects. All measures will be offered to adult subjects who read English or Spanish. Any survey time-point that is missed due to the subject's clinical status (e.g. critically ill) will be reported as a protocol deviation at time of continuing review.

6 CLINICAL MONITORING

Samples will be ordered and tracked through the CRIS 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. HRQL surveys will be mailed to the subject if they are not required to return to the NIH CC for study evaluations.

6.1 Pre-study Evaluation - PI may accept results from studies done outside of NIH.

Baseline studies will be conducted as follows:

- Medical history and physical examination
- Concurrent medication review
- HRQL survey administration
- Baseline clinical studies
  - Complete blood count with differential
  - Reticulocyte count
  - DAT (direct antiglobulin test)
  - Type and screen
  - 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 LD panel)
  - Coagulation and thrombosis screens (PT, PTT, D-dimer)
  - Thyroid function tests
  - Viral serologies for hepatitis A, B, C, HIV, HSV, EBV and CMV
  - EBV and CMV PCR
  - PPD (at risk subjects only, based on history or geography)
  - Folate level
  - B12 level
  - Iron panel (ferritin, transferrin, % saturation)
  - HLA typing (if not already available)
  - Pregnancy test (urine or blood HCG in women of child bearing potential)
  - 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
  - Telomere length of leukocytes
  - 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)
  - Chest X-ray- this does not need to be performed if a clinically indicated chest CT is performed to reduce the radiation burden
  - EKG
  - Placement of a central line if subject does not have a pre-existing indwelling central venous catheter

6.2 On Study Monitoring, Day 1 of h-ATG Through Hospital Discharge

On treatment monitoring will consist of:

- Pregnancy test (urine or blood HCG in women of child bearing potential) does not need to be repeated if done within two weeks (Day 0 to Day -14).
- CBC with differential (daily)

- Acute care, Mineral, Hepatic and Other panel (every other day)
- Reticulocyte count (weekly +/- 3 days)
- Vital signs (daily)
- CsA blood level will be monitored every week (+/- 3 days) while inpatient in the hospital and continued per section 6.3. The blood level will be monitored to ensure therapeutic range of 200 – 400 ng/ml is achieved. CsA dosage will be adjusted to target this range. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity.

6.3 On Study Monitoring (hospital discharge through 6 months)

After completing h-ATG administration, subjects will remain hospitalized until clinically stable (7-14 days total). Post-discharge, subjects may be followed by their home physician or at the Clinical Center. Progress notes and laboratory results from home physicians will be faxed to the research nurse, Olga Rios, RN (301) 496-4462. Standard of care tests will be done as needed and may include the tests listed below.

- Complete blood counts with differential every 1-2 weeks +/- 3 days
- Chemistry panel (NIH Acute care, Mineral, Hepatic and Other panel, home laboratory chemistry panel must include electrolytes, hepatic transaminases, urea nitrogen (BUN), serum creatinine, and total bilirubin)(weekly +/- 3 days)
- CsA blood level will be monitored every week (+/- 3 days) for the first month and then every other week (+/- 3 days) for the remainder of the treatment period once levels are stabilized in the therapeutic range of 200 – 400 ng/ml. CsA dosage will be adjusted to target this range. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity. CsA monitoring will be discontinued at 6 months for all cohorts.
- HRQL survey administration (pre-eltrombopag; within 2 days of day 14 post ATG on cohorts 1 and 2)

Landmark 3-month and 6-month monitoring

Subjects must be evaluated at the NIH Clinical Center at the 3- and 6-month (+/-7 days) time points

- History and physical examination
- Complete blood counts with differential
- Acute care, Mineral, Hepatic and Other-panel
- Reticulocyte count
- Urine pregnancy test (woman of childbearing age 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)
- Peripheral blood for pharmacokinetic sampling (3-month only) (cohort 1 only)
- HRQL survey administration
- EKG (required at month 3 visit, may be performed at other time points)

6.4 Long Term Follow Up (12 months to 5 years)

After the 6 month visit, subjects must be evaluated at the Clinical Center at 12 months (+/- 30 days) and then yearly thereafter to 5 years (+/- 60 days). Subjects will be seen by their home physician as clinically indicated and the Hematology Branch investigators and home physicians will remain in communication. Home physicians will monitor blood counts and other clinical parameters as clinically indicated. The following tests will be performed at each Clinical Center visit. At the clinical investigator's discretion, participants may be evaluated more frequently if medically indicated based on disease status.

- Complete blood counts with differential
- Acute care, Mineral, Hepatic and Other panel
- Reticulocyte count
- 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
- HRQL survey administration

6.5 Extended Access for Relapse

Indicated below are the procedures that can be performed on subjects that re-start drug due to relapse. At the clinical investigator's discretion, participants may be evaluated as medically indicated based on disease status. The procedures may be performed by the subjects' home physician or at the Clinical Center. If testing is done by home physician, progress notes and laboratory results from home physician will be sent to the research team. Subjects will be seen at the Clinical Center every 3 to 6 months (+/- 30 days) while on extended access for relapse. Below is the list of procedures that will be performed as medically indicated.

Procedures that may be performed when drugs are re-started

- History and physical examination
- Pregnancy test (urine or blood HCG in women of child bearing potential)
- Complete blood counts with differential
- Chemistry panel (NIH Acute care, Mineral, Hepatic and Other panel, home laboratory chemistry panel must include electrolytes, hepatic transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin, and reticulocyte count)
- CsA blood level will be monitored until levels are stabilized in the therapeutic range of 200 – 400 ng/ml. CsA dosage will be adjusted to target this range. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity.
- 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

7 CRITERIA FOR RESPONSE

Response is defined as blood counts no longer meeting the standard ("Camitta") criteria for severe pancytopenia in SAA (see section 4.1), equivalent to 2 of the following values obtained on 2 serial blood count measurements at least one week apart at landmark time points (3, and 6 months)

- Absolute neutrophil count > 500/ μL
- Platelet count > 20,000/ μL
- Reticulocyte count > 60,000/ μL

A complete response (CR) will be defined as (all 3 must be met):

- Absolute neutrophil count >1,000/ μL
- Platelet count >100,000/ μL
- Hgb>10 g/dL

A partial response will be defined as blood counts that do not meet criteria for severe pancytopenia but are not sufficient for a CR.

Improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling response criteria.

The presence of evolution to PNH will be defined by flow cytometric detection of > 1% GPI-deficient neutrophils at baseline and landmark time points through 5 years. Evolution to myelodysplasia and/or acute leukemia will be assessed at landmark time points, or as clinically indicated between landmarks by examination of peripheral blood and bone marrow and diagnosis and classification according to the WHO criteria. Evolution to clonal hematopoiesis will be defined by detection of new bone marrow cytogenetic abnormalities at landmark time points.

Relapse: Clinical definition determined by observation of a decline in blood counts not explained by another clinical process (e.g. acute infection) that is either (a) a substantial decline in one or more blood counts, or (b) a progressive decline in one or more blood counts on at least two consecutive blood draws.

8 EXPLORATORY LABORATORY RESEARCH STUDIES

8.1 Collecting, tracking and disposition of samples

Intended use: During the course of participating on 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, 12 months and annually thereafter, and 5 cc of bone marrow aspirate (baseline, 3 months, 6 months, 12 months, and annually thereafter) will be obtained for the following correlative laboratory research studies. Baseline samples may be obtained on another protocol, such as 04-H-0012. 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.

- Thrombopoietin level
- CD34 cell number in whole blood and bone marrow aspirate by flow cytometry
- 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 in Cohort 1 only (Appendix D)
- 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 B.

Storage: Research samples will be stored coded in the secure laboratory of the Dr. Young.

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). Bone marrow samples obtained for cytogenetic studies will be submitted to Quest Laboratories under a fee-for-service contract. 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 breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

9 BIOSATISTICAL CONSIDERATIONS

9.1 Sample sizes

The statistical section of this protocol has been amended to first (**amendment B**) increase the accrual

ceiling to 31 subjects from 25 subjects to increase the likelihood to have at least 25 evaluable subjects at the 6-month time point for analysis. Next, with **amendment (8), submitted on August 22, 2013,** to add a second cohort with a reduced eltrombopag schedule while keeping the primary endpoints unchanged. Next, with **amendment (16), submitted on October 2, 2014,** to add a third cohort revising the administration schedule to allow for h-ATG, CsA, and eltrombopag to all be administered starting Day 1 while keeping the primary endpoints unchanged. Eltrombopag will be administered for 6 months and the primary endpoint will remain the same, response at month 6.

Our past experience with h-ATG/CsA suggests that the CR probability at 6 months for previously untreated patients given this regimen is approximately 10%~12%. Our data supports that among those in which a CR is observed, evolution to monosomy 7 and leukemia did not occur. Therefore CR will be used as a surrogate for late events for the purpose of study design. We hypothesize that the actual CR probability using this treatment would reach 30% or more and a CR probability of 10% or less would warrant terminating the treatment on this patient population. Let p be the CR probability at 6 months for this patient population.

In the **original protocol, approved on June 13, 2012**, our sample size was determined by testing the null hypothesis, H0: $p \leq 10\%$, versus the alternative, H1: $p \geq 30\%$, at 0.05 significance level and 0.80 of the power. We intended to test this treatment using a small number of patients, and terminate the study if early evidence suggests that the CR probability fell below the benchmark rate of 10%. We determined the sample size using the Two-Stage Minimax Design of Simon (1989), since it required a smaller total number of subjects (n=25) compared to the Two-Stage Optimal Design (n=29). At the first stage, 15 subjects would be accrued and the null hypothesis would be accepted (i.e., the treatment would be terminated) if no more than 1 subject had a CR to the treatment within 6 months. If 2 or more subjects had a CR to the treatment within 6 months at the first stage, 10 additional subjects would be accrued. The null hypothesis of $p \leq 10\%$ would be accepted if the total number of patients having CR within 6 months was 5 or less.

With amendment B, approved on December 3, 2012, we increased the accrual ceiling to 31 subjects from 25 subjects to increase the likelihood to have at least 25 evaluable subjects at the 6-month time point for analysis of secondary endpoints while maintaining the statistical power for the primary endpoint of the study. This modified the statistical design as follows: Let p be the CR probability at 6 months for this patient population. Our sample size is determined by testing the null hypothesis, H0: $p \leq 10\%$, versus the alternative, H1: $p \geq 30\%$, at 0.05 significance level and 0.865 for power. We would like to test this treatment using a small number of patients, and terminate the study if early evidence suggests that the CR probability falls below the benchmark rate of 10%. We determine the sample size using the Two-Stage Minimax Design of Simon (1989), since it requires a smaller total number of subjects (n=31) compared to the Two-Stage Optimal Design (n=34). At the first stage, 24 subjects will be accrued and the null hypothesis will be accepted (i.e., the treatment will be terminated) if no more than 2 subjects demonstrates a CR to the treatment within 6 months. If 3 or more subjects have a CR to the treatment within 6 months at the first stage, an additional 7 subjects will be accrued. The null hypothesis of $p \leq 10\%$ will be accepted if the total number of subjects having a CR within 6 months is 6 or less. The "ph2simon" function in the "Clinfun" package "Clinical Trial Design and Data Analysis Function, Version 1.0.5" of the statistical software R was used to compute the numerical results of the Two-Stage Design.

The current pilot study design has a cohort of 31 subjects receiving eltrombopag starting day 14 and ending at the 6 month time point (cohort 1). With **amendment (8), submitted on August 22, 2013**, once accrual to cohort 1 has been completed without reaching any study stopping rules we propose to treat another cohort (cohort 2) of 33 subjects whereby they would be treated with the same regimen except that they would receive a reduction in exposure to eltrombopag from 6 to 3 months. The primary and secondary endpoints, objectives, eligibility, statistics, including sample size and stopping rules,

monitoring and ancillary studies apply the same way for cohort 2 as for cohort 1. With amendment 16, submitted on October 2, 2014, once accrual to cohort 2 has been completed without reaching any stopping rules we propose to treat another cohort (cohort 3) of 31 subjects whereby they would be treated with the same regimen except that they would start h-ATG, CsA, and eltrombopag on Day 1. Eltrombopag will be administered up to the 6-month landmark visit. The primary and secondary endpoints, objectives, eligibility, statistics, including sample size and stopping rules, monitoring and ancillary studies apply the same way for cohort 3 as for cohorts 1 and 2 with the exception of one additional secondary objective (relapse rate between month 6 to month 24 when receiving low CsA during this time period).

	Sample size (n)	Eltrombopag	Primary Efficacy Endpoint	Statistical Design
Cohort 1	31	D 14 – 6 months	CR rate at 6 months	Simon Two-Stage
Cohort 2	33	D14 – 3 months	CR rate at 6 months	Simon Two-Stage
Cohort 3	31	D1 – 6 months	CR rate at 6 months	Simon Two-Stage

Amendment 22 (V) is being submitted to add an Extension Cohort of 26 subjects to the protocol in order to allow subjects to be enrolled and treated while a new SAA treatment naïve protocol is developed and approved. Data from subjects enrolled into the Extension Cohort would improve precision of exploratory analysis of secondary endpoints. However, this data will not be used for the primary analysis of cohort 3.

9.2 Statistical Methods

For each cohort, the planned analyses will include descriptive statistics on the proportions of responses (% subjects with partial or complete response), cumulative incidence of response with death considered to be a competing risk and the time to complete response. The response probabilities, including complete response probability and partial response probability, will be estimated using the sample proportions, and their inferences, including confidence intervals and hypotheses testing, will be evaluated using Binomial distributions. Survival analysis will include methods based on Kaplan-Meier estimates and Cox regression. Although we have a small sample size, if it is appropriate, we will consider additional analyses for the primary and secondary endpoints using the analysis of variance, multiple regression and logistic regression models. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions and Kaplan-Meier curves) and their corresponding 95% confidence intervals.

Results for each cohort will be compared separately to the historical data from the large numbers of patients treated at the NIH previously with either a single or repeat course of immunosuppression from prior NHLBI studies: the h-ATG/CsA protocol (90-H-0146); the h-ATG/CsA vs. Cytoxan/CsA protocol (97-H-0117); the h-ATG/CsA/MMF protocol (00-H-0032); the h-ATG/CsA/Rapamune protocol (03-H-0193); the r-ATG vs. alemtuzumab protocol (03-H-0249); and h-ATG/CsA vs. r-ATG/CsA protocol (06-H-0034). More specifically, results from the current trial will be compared to our large historical experience with a horse ATG based regimen in nearly 400 patients (from the aforementioned studies) of a hematologic response rate of 60-70%, a complete response rate of 10-15%, a relapse rate of 30-40%, a clonal evolution rate (any clonal abnormality) of 15-20%, and a high risk evolution rate (to monosomy 7, high risk MDS, leukemia, or complex cytogenetics) of 10-15%.

9.3 Primary Endpoints

The primary objective of this phase I/II study is to evaluate the safety and activity profile of h-ATG/CsA/eltrombopag in treatment naïve SAA.

- The primary safety endpoint will be toxicity profile in the 6 months following h-ATG/CsA/eltrombopag.
- The primary efficacy endpoint is CR rate at 6 months (see section 7 for CR definition).

Subjects who drop out, who have failed to respond and opt for alternative therapy (e.g. bone marrow transplant), or who require eltrombopag to be restarted (cohort 2 only) before the 6-month evaluation will be counted as non-responders.

9.4 Secondary Endpoints

Secondary endpoints will also be evaluated for the study to include: (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; (e) health-related quality of life; (f) hematological response of relapse subjects that re-start treatment; and (g) affects of a 2.0mg/kg/day CsA dose starting month 6 for 18 months until month 24 on the rate of relapse of subjects deemed responders at month 6.

Subjects will be followed up to 60 months so that long-term disease-free and overall survival can be estimated. Response and toxicity comparisons will also be made with the results obtained from prior NHLBI studies: the h-ATG/CsA protocol (90-H-0146); the h-ATG/CsA vs. Cytoxan/CsA protocol (97-H-0117); the h-ATG/CsA/MMF protocol (00-H-0032); the h-ATG/CsA/Rapamune protocol (03-H-0193); the 3-arm randomized study (06-H-0034) and the r-ATG vs. alemtuzumab (03-H-0249) trials.

9.5 Stopping Rules

The study will be monitored to ensure that the occurrence of a specified set of treatment related serious adverse events (TRSAEs) within 6 months of treatment in the trial does not substantially exceed an anticipated rate. The following TRSAEs will be monitored for early stopping of the study:

- Death considered to be probably or definitely related to eltrombopag.
- Any grade IV toxicity considered to be probably or definitely related to eltrombopag.
- Grade IV thrombosis/embolism

The study will be monitored using the stopping rules as outlined below for early stopping if the number of subjects in the study who have developed one or more of the above specified TRSAEs is over a pre-specified threshold value. TRSAEs are those attributed as definitely or probably related to eltrombopag. Dr. John Tisdale will serve as the independent monitor who reviews the attribution of TRSAEs.

From our experience using this agent in other clinical settings, we anticipate the rate of developing at least one of the above specified TRSAEs for this patient population to be 20% or less. Following Geller et al. 2004, (Nancy L. Geller, Dean Follmann, Eric S. Leifer, and Shelly L. Carter "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach" Chapter 2, pp 41-52, Advances in Clinical Trial Biostatistics, 2004, Marcel Dekker: New York), our stopping rule is determined by a Bayesian approach. The stopping boundary for the study is reached if the Bayesian posterior probability that the true probability of developing one or more of the above specified TRSAEs exceeds this benchmark rate of 20% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 3, i.e. the parameters of the beta prior distribution are 0.60 and 2.40. 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 the study when 3 or more subjects have developed specified TRSAEs. The following tables summarize the threshold numbers for stopping the

study:

Number of Tx naïve SAA subjects enrolled	Stop if the number of Tx naïve SAA subjects who develop any of the specified TRSAEs reaches:
≤ 6	3
≤ 9	4
≤ 13	5
≤ 17	6
≤ 21	7
≤ 25	8
≤ 29	9
≤ 31	10

For the stopping rule we generated a study with 25 independent Bernoulli trials, each had a probability p for having the above TRSAE and q=1-p for not having such 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 "Monitored" TRSAE= p	5%	10%	15%	20%	25%	40%
Proportion of stopped studies	0.27%	2.45%	9.19%	22.57%	41.44%	89.81%
Average number of Tx naive SAA subjects	24.95	24.58	23.59	21.78	19.30	11.02
Average number of Tx naive SAA with a specified TRSAE	1.25	2.47	3.53	4.35	4.83	4.41

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

Evolution to clonal hematopoiesis will be monitored and documented in this study. Currently, there are no standards to determine if evolution to clonal hematopoiesis with respect to this study is a serious adverse event or treatment related serious adverse event. Therefore, evolution to clonal hematopoiesis will not be included in stopping rules. Currently, none of the active SAA NHLBI Hematology Branch treatment protocols includes evolution to clonal hematopoiesis as a stopping rule, including all protocols administering eltrombopag.

9.6 Off Study Criteria

Subject choice: Subjects may withdraw from the study at any time. The risks of withdrawing will be discussed, as will alternative treatment options. The subject will be informed that his/her condition poses a high mortality rate. The risks of not receiving further therapy include a high mortality rate with supportive care only. Those subjects who choose to withdraw will be strongly encouraged to continue to have blood counts monitored until initiation of alternative SAA therapy or through the 6 month off study medication to assess for late occurring adverse events.

Principal investigator decision: Should any of the following events occur, study drug administration will be discontinued. The subject will be followed until resolution of the event and laboratory values monitored through 6 months or until initiation of alternative SAA therapy:

- Intolerance of eltrombopag not resolved by dose reduction
- Thrombosis/embolism (DVT, PE, stroke or TIA, myocardial infarction) other than central line thrombosis.
- Persistent hepatotoxicity as defined in section 5.3.2
- Infusion-related h-ATG reactions refractory to all appropriate supportive measures
- Life threatening acute hypersensitivity reactions
- Pregnancy or unwillingness to refrain from pregnancy
- Initiation of additional immunosuppressive therapy other than steroids or cyclosporine
- Evidence of a clonal disorder identified in subjects with super severe baseline neutropenia (ANC < 200 /μL) who were not initially excluded because cytogenetics was not available or pending.

Completion of protocol participation: After 5 years, protocol participation will be deemed complete. 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) or evaluated for eligibility for another Branch protocol, depending on the wishes of the subject and availability of appropriate additional clinical trials at the NIH.

9.7 Data Management

The Principal Investigator 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, Hematology Branch fellows, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from home physician. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system. Data will not be sent outside of the NIH without IRB approval and an executed agreement.

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., unique code, or minimum PII required for subject identification. Study data will be housed in the Hematology Branch P Drive, a secure limited access drive.

Novartis will receive quarterly accrual and toxicity information as detailed in the CRADA. In order to

maintain subject 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 breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Publication policy: Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

10 DATA AND SAFETY MONITORING

10.1 Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Danielle Townsley, M.D.

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

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

FDA: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to the FDA Project Manager or designee:

Mara Miller, Regulatory Project Manager
CDER Therapeutic Biological Products Document Room
Center for Drug Evaluation and Research, Food and Drug Administration
5901 B Ammendale Road, Beltsville, MD 20705-1266

(301) 796-0683

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

Kelly Haines
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Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080
USA
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Mobile +1 201 452 8479
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10.2 Event Characterization and Reporting

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance. The characterization (e.g., seriousness, expectedness, etc…) of the event will determine the reporting requirements.

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.

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

10.2.1 Unanticipated Problem (UP) Characterization

Any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected: (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied,
2. Related or possibly related: to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research),

and

3. Suggests that the research places 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.

10.2.2 Protocol Deviation (PD) Characterization

Any change, divergence, or departure from the IRB approved research protocol. The impact of a PD is characterized by designation as serious or not serious. The serious adverse event definition (see below)

will be used to determine if the PD is serious in nature.

It is anticipated that approximately 50% of the clinical monitoring laboratory testing performed during the first 6 months will not be performed within +/- 3 days of the required time points, with the exception of the month 3 and 6 landmark visits. If the clinical laboratory monitoring occurs outside the +/- 3 day window, these events will not be reported to the IRB as non-serious protocol deviations on the NIH problem form within 14 days of the occurrence unless the event occurs at greater than 50% in a single subject or greater than 50% cumulatively for all subjects. The events will be recorded in the database and reported at time of continuing review. If the number of events per subject or per cumulative enrolled subjects exceeds 50%, this will be reported within 14 days to the IRB as a serious protocol deviation. Please note, subjects and providers are reminded of the importance of the timely completion of the clinical laboratory testing.

Questionnaires that are not completed at the required time point due to clinical status (e.g. critically ill), will be recorded in the database and reported as protocol deviations at the time of continuing review.

Interruptions in eltrombopag dosing that are clinically indicated per section 5.3, will not be reported as deviations to the IRB, but will be recorded in the medical record. However, when the interruption is a consequence of a serious adverse event, the interruption will only be included in the SAE report.

10.2.3 Adverse Events (AEs) Characterization

Adverse events are defined as any untoward 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 subject's participation in the research.

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.

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

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.

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

Duration of adverse event collecting and reporting: The collection and recording (in the database) of AEs (and SAEs) will begin on the first day of initiation of the study drug and will continue for 30 days after the 6 month landmark visit or for 30 days after treatment is discontinued if before the 6 month landmark visit, after which the events will be captured in the medical record but not abstracted (recorded in the database) to toxicity tables. Serious adverse event recording and reporting will continue as long as the subject remains on study. AEs will be followed until satisfactory resolution as long as the subject remains on study.

10.2.4 Serious Adverse Events Characterization

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death
- in-patient hospitalization or prolongation of existing hospitalization
- persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- congenital anomaly/birth defect
- life threatening adverse event
- other important medical events that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above may be considered serious based upon appropriate medical judgment

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 (clear not related to the research).

TRSAEs are those attributed as definitely or probably. As detailed in section 9.5 stopping rules, TRSAE that will be monitored and considered for early stopping the study according to statistically determined criteria include Death and any grade IV toxicity considered to be probably or definitely related to study medication.

Hospitalizations for administrative issues (e.g., to receive a transfusion after hours) or movement to the ICU for routine monitoring per administrative requirements or nosocomial isolation will not be reported as an SAE.

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

10.2.5 Non-compliance Characterization

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

10.2.6 Event Reporting

All events listed in section 10.2 will be reported to Danielle Townsley M.D., Principal Investigator of this study:

Danielle Townsley, M.D.
Bldg 10, Room CRC 3E-5132
Phone: (301) 402-3477
Pager: 102-10433
E-mail: townsleydm@NHLBI.NIH.GOV

10.2.6.1 Reporting Timeframes to IRB Chair, Clinical Director, and IRB

- Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event.

- Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event.

- Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

- SAEs that do not meet the criteria of UP must be reported to the IRB Chair and Clinical Director within 14 days of learning of the event using the SAE form in PTMS.

- Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

10.2.6.1.1 Reports at the time of continuing IRB review:

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

- All UPs
- All PDs (except for those granted a waiver of reporting)
- 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:

- In view of the underlying illness, 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 abnormalities as adverse events.

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

- Because CsA, h-ATG (ATGAM®) and eltrombopag (Promacta®) are FDA approved drugs with known toxicity profiles, any observed or volunteered adverse events that are listed on the package insert will not be reported unless (1) the adverse event is previously unknown (not on the package insert); (2) the adverse event is more severe than on the package insert; or (3) meets the criteria for a serious adverse event. The collection of AEs information will begin on the first day of initiation of therapy.
- Cohorts 1 and 2: Because grade 3 and 4 laboratory abnormalities which do not result in any clinical action frequently occur during h-ATG administration, only those events that result in a clinical action (i.e., dose reduction/discontinuation, prolongation of hospitalization, etc) will be recorded in the database and reported to the IRB during the first week of h-ATG treatment.
- Cohort 3: Because grade 3 and 4 adverse events which do not result in any clinical action frequently occur during h-ATG administration, those events that result in a clinical action (i.e., dose reduction/discontinuation, prolongation of hospitalization, etc) will be recorded in the database and reported to the IRB from the start of h-ATG to 14 days post-treatment. The exception to this will be if the event, which did not result in a clinical action, can be associated with both eltrombopag and h-ATG, and the event attribution cannot be assigned with a high level of certainty to only h-ATG, then these events will be recorded in the database and reported to the IRB at the time of continuing review.
- Cohort 3: Grade 3 and 4 LFTs will be recorded in the medical record, and only recorded in the database and reported as adverse events to the IRB from the start of h-ATG to 14 days post treatment if the increased LFT persists at a grade 3 or 4 for greater than 5 days.
- All grade 1 events listed as expected in the protocol, consent forms, or other applicable protocol documentation.

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

10.2.6.3 Sponsor and FDA Reporting
IND: 104877
IND Sponsor: Cynthia E. Dunbar, MD

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.

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 in a narrative format or on FDA Form 3500A.

FDA contact: Mara Miller, Regulatory Project Manager
CDER Therapeutic Biological Products Document Room
Center for Drug Evaluation and Research, Food and Drug Administration
5901 B Ammendale Road, Beltsville, MD 20705-1266
(301) 796-0683

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

10.2.6.4 Reporting Serious Adverse Events to CRADA Sponsor
Novartis: All unexpected and possibly, probably or definitely related SAEs occurring during the study or within 30 days of the last administration of eltrombopag will be reported to Novartiswithin 24 hours of the research team learning of the event. A copy of the SAE report (NHLBI SAE report form,) 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 the study drug the SAE report (in the appropriate format, e.g., NHLBI SAE report form, narrative, and/or Medwatch form, Appendix A) will be forwarded to Novartis and FDA within 24 hours of learning of event. 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 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.'

Reports will be forwarded to Novartis

10.3 Reporting of Pregnancy

Subjects who become pregnant during the study should discontinue the study drugs (CsA and eltrombopag) immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her designee, will submit pregnancy information to the NHLBI Clinical Director, NHLBI IRB and Novartis within two weeks of learning of a subject's pregnancy. Information on the status of the mother and child will be forwarded to 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.

10.4 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.5 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

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

11 HUMAN SUBJECT PROTECTION

11.1 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.

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 AUC0-∞ of eltrombopag was increased by 41% in subjects with mild hepatic impairment and by 80% to 90% in subjects with moderate or severe hepatic impairment compared with healthy volunteers. Therefore patients with minimally impaired hepatic function will be included but participation will be monitored closely. 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, and may be listed on The Aplastic Anemia 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 NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: $0. The study participants will not be reimbursed for their time and inconvenience.

Travel (air/train/bus): Travel from home for the first NIH visit will not be reimbursable. If the patient consents to the research protocol, travel home following the first visit will be reimbursable. Subjects will be reimbursed 100% of government rate for travel to required landmark visits or additional protocol-related visits as deemed clinically-indicated by the PI once the subject has been determined eligible to participate and signs consent.

Local Travel (< 50 miles) (car/taxi/shuttle): Subjects will be reimbursed for local train/bus and/or shuttle costs. Reimbursement for car mileage less than 50 miles from home is not provided. Taxi will be paid

only when medically necessary and authorized by the PI

Long distance travel (> 50 miles): Car mileage will be reimbursed $0.41/mile when the distance from home is greater than 50 miles. Subjects will not be reimbursed for rental car cost beyond the car mileage rate. Taxi will be paid only when medically necessary and authorized by the PI.

Meals: Subjects will not be reimbursed for meals.

Lodging: Subjects will be reimbursed for hotel/motel lodging at a rate of $60/night for a maximum of 7 days after which the reimbursement is $30/night. If space is available, the children's inn ($64/night) or the family lodge ($65/night) will be paid directly.

Guardian coverage: Subjects will be reimbursed for guardian travel (100% of government rate) and lodging ($15.00/night) provided the services of a guardian are medically indicated and pre-approved.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population. Protocol 10-H-0176 for treatment naïve SAA will end enrollment soon and the H-ATG/CsA/eltrombopag protocol is being developed to replace 10-H-0176.

11.2 Participation of Children

In principle, age is not a consideration. But in practice, we are limiting the protocol to subjects who are age 2 years and older because our clinic does not have the expertise to care for infants. In addition, per Clinical Center guidelines, we are limiting participation to children who weigh >12 kg.

11.3 Exclusion of Pregnant Women and Nursing Mothers

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

11.4 Risks and Discomforts

11.4.1 Related to Horse ATG (ATGAM)

Potential Serious Adverse Effects:

Anaphylaxis (less than 1% of patients): Rarely, patients may develop potentially fatal anaphylaxis. Production by the patient of antibodies to horse proteins leads to the formation of immune complexes and the clinical development of serum sickness, characterized by fever, a characteristic rash, arthralgia, myalgia and non-specific gastrointestinal and neurologic symptoms. Onset is typically at day ten to eleven, and the course is self-limited; symptoms may be improved by corticosteroids. Transient reduction in peripheral granulocyte and platelet counts and in the hemoglobin may occur during the period of administration of ATG and may lead to a temporary increase in transfusion requirements.

Severe lung injury (less than 1% of patients): Several cases of a severe lung injury related to Atgam treatment have been reported. Although this side effect appears extremely rare, it is serious and can be fatal. There is no information about the mechanism or specific treatment for this condition. A few patients recovered after intensive medical support including use of a breathing machine.

Cardiac Failure and Pulmonary Edema (less than 5% of patients): h-ATG is associated with pulmonary edema or congestive heart failure

Potential side effects related to h-ATG (ATGAM) include:

*Very common side effects (occurring in 10% or more of patients):*fever (51%), chills (16%), thrombocytopenia (30%), leukopenia (14%), skin rash (27%)

Common side effects (occurring in 5 to 10% of patients): serum sickness like symptoms, dyspnea/apnea, arthralgia, headache, chest, back or flank pain, diarrhea and nausea and/or vomiting,

Events reported with frequency of less than 5% of patients in a pre-marketing clinical trial in the treatment of aplastic anemia include: diaphoresis, joint stiffness, peri-orbital edema, aches, edema, muscle ache, vomiting, agitation, lethargy, listlessness, light-headedness, seizures, diarrhea, bradycardia, myocarditis, cardiac irregularity, hepatosplenomegaly, post viral encephalopathy, hypotension, congestive heart failure, hypertension, burning soles/palms, foot sole pain, lymphadenopathy, post cervical lymphadenopathy, tender lymph nodes, pleural effusion, respiratory distress, and proteinuria.

Related to pregnancy and/or nursing mothers: h-ATG has not been evaluated in either pregnant or lactating women therefore administration of h-ATG to pregnant women is not recommended and should be considered only under exceptional circumstances.

Postmarketing Experience:
During approximately 5 years of post approval marketing experience, the frequency of adverse reactions in voluntarily reported cases is as follows: fever 51%; chills 16%; thrombocytopenia 30%; leukopenia 14%; rashes 27%; systemic infection 13%. Events reported in 5% to 10% of reported cases include abnormal renal function tests; serum sickness-like symptoms; dyspnea/apnea; arthralgia; chest, back, or flank pain; diarrhea and nausea and/or vomiting. Events reported with a frequency of less than 5% include: hypertension, Herpes Simplex infection, pain, swelling or redness at infusion site, eosinophilia, headache, myalgias, or leg pains, hypotension, anaphylaxis, tachycardia, edema, localized infection, malaise, seizures, GI bleeding or perforation, deep vein thrombosis, sore mouth/throat, hyperglycemia, acute renal failure, abnormal liver function tests, confusion or disorientation, cough, neutropenia or granulocytopenia, anemia, thrombophlebitis, dizziness, epigastric or stomach pain, lymphadenopathy, pulmonary edema or congestive heart failure, abdominal pain, nosebleed, vasculitis, aplasia or pancytopenia, abnormal involuntary movement or tremor, rigidity, sweating, laryngospasm/edema, hemolysis or hemolytic anemia, viral hepatitis, faintness, enlarged or ruptured kidney, paresthesias, and renal artery thrombosis.

11.4.2 Related to CsA:

Potential Serious Side Effects Include:

Infection related: Because of low white blood cell counts, patients with aplastic anemia are susceptible to infections. By further blocking the immune system, CsA further increases this risk.
Cancer related: When used at high doses in transplant patients, CsA may be associated with an increased risk of cancer, especially lymphoma (4 of every 10,000 patients who receive the medication). Transplant patients receive higher doses than you will be given and are treated for longer periods than the duration of this study. However, because of the way that CsA acts on the body, there is a chance that it may cause effects that may not occur until years after the medicine is used
Blindness: In very rare instances (less that .01%), CsA has been reported to cause blindness

Potential side effects:

Although it is metabolized primarily in the liver, CsA major toxicity is renal. CsA causes a decrease in creatinine clearance, which almost always returns to normal range on cessation of the drug or lowering of the dose. Rare development of a hemolytic-uremic syndrome has been reported in patients with CsA after allogeneic bone marrow transplant. In our patients with SAA, frequent creatinine measurements have allowed prompt adjustment of dose and serious renal complications are infrequent.

Evidence of hepatotoxicity is common, usually as transient increases in bilirubin and transaminases. These levels often normalize with continued administration of the drug; reduction of the dose is uniformly associated with a return to normal levels.

Additional complications include hypertrichosis, gingival hypertrophy (possibly related to pre-existing poor dental hygiene), hyperesthesia, hirsutism, tremors, headaches, nausea and nonspecific gastrointestinal complaints. Hypertension may occur, and be high enough to require treatment.

Neurologic complications include insomnia, dizziness, anxiety, confusion, and vertigo. We have observed seizures in patients receiving CsA, when drug levels were within the therapeutic range. Posterior Reversible Encephalopathy Syndrome (PRES) is an increasingly recognized neurologic disorder seen in 1% of patients on cyclosporine following solid organ transplantation which manifest with acute to subacute hypertension and/or seizures.[77] In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.[71]

Hypomagnesemia and hyperkalemia may occur but are asymptomatic. Increases in uric acid may occur and attacks of gout have been rarely reported. Cyclosporine therapy may be associated with a modest increase of serum triglycerides or cholesterol.

Less frequent adverse events include:

Autonomic Nervous System: dry mouth, increased sweating
Systemic: allergy, asthenia, hot flushes, malaise, weight decrease, weight increase
Cardiovascular: abnormal heart sounds, cardiac failure, myocardial infarction, peripheral ischemia
Central and Peripheral Nervous System: hypoesthesia, neuropathy, vertigo
Endocrine: goiter
Gastrointestinal: constipation, dysphagia, enanthema, eructation, esophagitis, gastric ulcer, gastritis, gastroenteritis, gingival bleeding, glossitis, peptic ulcer, salivary gland enlargement, tongue disorder, tooth disorder
Infection: abscess, bacterial infection, cellulitis, folliculitis, fungal infection, herpes simplex, herpes zoster, renal abscess, moniliasis, tonsillitis, viral infection
Hematologic: anemia, epistaxis, leukopenia, lymphadenopathy
Liver and Biliary System: bilirubinemia

Metabolic and Nutritional: diabetes mellitus, hyperkalemia, hyperuricemia, hypoglycemia
Musculoskeletal System: arthralgia, bone fracture, bursitis, joint dislocation, myalgia, stiffness, synovial cyst, tendon disorder
Neoplasms: breast fibroadenosis, carcinoma
Psychiatric: anxiety, confusion, decreased libido, emotional lability, impaired concentration, increased libido, nervousness, paranoia, somnolence
Reproductive (Female): breast pain, uterine hemorrhage
Respiratory System: bronchospasm
Skin and Appendages: abnormal pigmentation, angioedema, dermatitis, dry skin, eczema, nail disorder, pruritus, skin disorder, urticaria
Special Senses: abnormal vision, cataract, conjunctivitis, deafness, eye pain, taste perversion, tinnitus, vestibular disorder, blindness
Urinary System: abnormal urine, hematuria, increased BUN, micturition urgency, nocturia, polyuria, pyelonephritis, urinary incontinence

Prolonged low dose administration of CsA:
Patients who undergo solid organ transplantation, such as kidney transplantation, are given cyclosporine for life at a high dose (usually 5-10 mg/kg/day) whereas we are proposing to continue at low-dose, 2mg/kg/day. Cyclosporine is considered a weak immunosuppressant and few infectious complications are observed. There is ample data on the use of cyclosporine from long-term studies where cyclosporine is used for solid organ transplantation. Nephrotoxicity is the main toxicity, usually reversible, but is typically only observed when troughs over 200ng/mL are targeted. Nephrotoxicity is rarely observed with low-dose cyclosporine and monitoring of levels is unnecessary. The risk of relapse of severe aplastic anemia however, can be fatal, and this risk far exceeds the risks long term cyclosporine poses and is why many centers have adopted this approach. We also did not adopt this approach at the start of the protocol because we were uncertain whether eltrombopag may actually reduce the risk of relapse and warrant this approach unnecessary. However now we see from our maturing data that this is unlikely to be the case.[68,69]

11.4.3 Related to Promacta®(eltrombopag)

The following information is excerpted from Promacta product label (dated 08/2014) and the Investigator Brochure (updated version 10 dated 4/23/2013)

Potential Serious Adverse Effects:

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

Precautions with eltrombopag:
In clinical studies, hemorrhage was the most common serious adverse reaction and most hemorrhagic reactions followed discontinuation of eltrombopag. Other events of concern include:

Increased Liver Chemistries
Eltrombopag administration may cause hepatotoxicity. In the ITP controlled clinical studies, one patient experienced Grade 4 (NCI Common Terminology Criteria for Adverse Events [NCI CTCAE] toxicity

scale) elevations in serum liver test values during therapy with Eltrombopag, worsening of underlying cardiopulmonary disease, and death. One patient in the placebo group experienced Grade 4 liver test abnormalities. In controlled studies, elevations of ALT and indirect bilirubin were observed more frequently on the eltrombopag arm than placebo. Overall, serum liver test abnormalities (predominantly Grade 2 or less in severity) were reported in 11% and 7% of the eltrombopag and placebo groups, respectively. In the controlled studies, four patients (1%) treated with eltrombopag and three patients in the placebo group (2%) discontinued treatment due to hepatobiliary laboratory abnormalities. Eighteen of the patients treated with eltrombopag in the controlled studies with hepatobiliary laboratory abnormalities were re-exposed to eltrombopag in the ITP extension study. Seven of these patients again experienced liver test abnormalities (predominantly Grade 1) resulting in discontinuation of eltrombopag in one patient. In the ITP extension study, one additional patient had eltrombopag discontinued due to liver test abnormalities (all ≤Grade 3).

Serum ALT, AST, and bilirubin should be measured prior to initiation of eltrombopag, every 2 weeks during the dose adjustment phase and monthly following establishment of a stable dose. 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 the abnormality(ies) resolve, stabilize, or return to baseline levels. Permanently discontinue eltrombopag if ALT levels increase to ≥6X the upper limit of normal (ULN) and are:

- progressive, or
- persistent for ≥4 weeks, or
- accompanied by increased direct bilirubin, or
- accompanied by clinical symptoms of liver injury or evidence for hepatic decompensation.

Exercise caution when administering eltrombopag to patients with hepatic disease. Use a lower starting dose of eltrombopag in patients with hepatic impairment i.e. cirrhosis and monitor closely.

Bone Marrow Reticulin Formation and Risk for Bone Marrow Fibrosis (Note this risk has been since removed from the eltrombopag package insert approved revisions on 2/2014.)

Eltrombopag is a thrombopoietin (TPO) receptor agonist and TPO-receptor agonists may increase the risk for the development or progression of reticulin fiber deposition within the bone marrow.

In the Promacta®extension study, patients had bone marrow biopsies evaluated for increased reticulin and collagen fiber deposition. Bone marrow biopsies taken after 1 year of therapy showed predominantly myelofibrosis (MF) Grade 1 or less in 140/151 (93%) of patients. There were 11/151 (7%) of patients with MF Grade 2. Four patients had collagen deposition reported. One patient with a pre-existing MF Grade 1 developed a MF Grade 2 and subsequently discontinued treatment with eltrombopag. Clinical studies have not excluded a risk of bone marrow fibrosis with clinical consequences.

Prior to initiation of eltrombopag, the peripheral blood smear should be examined closely to establish a baseline level of cellular morphologic abnormalities. Following identification of a stable dose of eltrombopag, peripheral blood smears and CBCs should be examined monthly for new or worsening morphological abnormalities (e.g., teardrop and nucleated red blood cells, immature white blood cells) or cytopenia(s). If the patient develops new or worsening morphological abnormalities or cytopenia(s), treatment with eltrombopag should be discontinued and a bone marrow biopsy, including staining for fibrosis considered.

Thrombotic/thromboembolic Complications

Eltrombopag may increase the risk of thrombotic/thromboembolic events. In the controlled ITP clinical studies, four thrombotic/thromboembolic complications were reported within the groups that received eltrombopag and none within the placebo groups. Thrombotic/thromboembolic complications have also been reported in the ITP extension study.

In a placebo-controlled double-blind study (ELEVATE) of 292 patients with chronic liver disease who were undergoing an elective surgical procedure, the risk of thrombotic events was increased in patients treated with 75mg eltrombopag. Six thrombotic complications were reported within the group that received eltrombopag and two within the placebo group. All of the thrombotic complications reported within the eltrombopag group were of the portal venous system. Malignancy is known to increase the risk for developing a thrombotic event. Four of the 6 subjects receiving eltrombopag either had a diagnosis or suspicion of malignancy (2 hepatocellular carcinoma; 1 possible lymphoma and 1 brain tumor). The ELEVATE study was terminated in November 2009 and a Dear Health Care Professional Letter (DHCPL) was sent to all physicians enrolled in *Promacta* Cares in May 2010. Eltrombopag is not indicated for the treatment of thrombocytopenia in patients with chronic liver disease. Caution should be used when administering eltrombopag to patients with known risk factors for thromboembolism (e.g., Factor V Leiden, ATIII deficiency, antiphospholipid syndrome and portal hypertension).

Malignancies and Progression of Malignancies

Stimulation of the TPO receptor on the surface of hematopoietic cells by eltrombopag may increase the risk for hematologic malignancies. Across the ITP clinical program, hematologic malignancies were reported in one eltrombopag patient and one in a patient receiving placebo. Please note the most recent updated version of the package insert (11/2012) lists “hematologic malignancies” as being removed from the Warning and Precautions section.

Cataracts

Cataracts were observed in toxicology studies of eltrombopag in rodents (see Non-clinical Information). To date, there is however, no evidence that eltrombopag increases the incidence nor progression of cataracts in patients who have received eltrombopag. In the three placebo-controlled ITP studies, 7% of patients in both the placebo and eltrombopag treatment groups had a report of cataract. In the extension trial, cataracts developed or worsened in 4% of patients who underwent ocular examination prior to therapy with PROMACTA. A significant proportion of patients in the ITP clinical studies were also exposed to chronic corticosteroid administration. Routine monitoring of patients for cataracts is recommended. Patients treated with eltrombopag who experience visual difficulties should have an appropriate ophthalmologic evaluation. Despite the concern for cataracts in pre-clinical studies, the Clinical Events Committee overseeing eltrombopag studies have not observed signals to date of ocular toxicities in clinical trials (Investigator’s Brochure, Promacta). In recent longer follow-up report, occurrences of cataracts have not differed significantly among patients who received eltrombopag or placebo in clinical trials (Cooper at al. ASH Annual Meeting Abstracts, San Diego, CA, 2011).

Adverse reactions:

As detailed on the product label for ITP: The data described below reflects the experience of exposure to 446 patients with chronic ITP aged 18 to 85, of whom 65% were female across the ITP clinical development program including 3 placebo-controlled studies. Eltrombopag was administered to 277 patients for at least 6 months and 202 patients for at least 1 year.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in

the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Table 4 presents the most common adverse drug reactions (experienced by more than 3% of patients receiving eltrombopag) from the 3 placebo-controlled studies, with a higher incidence in eltrombopag versus placebo.

Table 4: Adverse Reactions (≥3%) from Three Placebo-Controlled Studies

Preferred Term	PROMACTA 50mg n = 241 (%)	Placebo n = 128 (5)
Nausea	9	3
Diarrhea	9	7
Upper respiratory tract infection	7	6
Vomiting	6	<1
Increased ALT	5	3
Myalgia	5	2
Urinary tract infection	5	3
Oropharyngeal pain	4	3
Increased AST	4	2
Pharyngitis	4	2
Back pain	3	2
Influenza	3	2
Paresthesia	3	2
Rash	3	2

As detailed on the product label for Severe Aplastic Anemia (refractory): 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.

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

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

Ecchymosis	12
Muscle spasms	12
Transaminases increased	12
Arthralgia	12
Rhinorrhea	12

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.

As detailed on the product label for Chronic Hepatitis C-associated Thrombocytopenia: In the 2 placebo-controlled trials, 955 patients with chronic hepatitis C-associated thrombocytopenia received PROMACTA. The below Table presents the most common adverse drug reactions (experienced by greater than or equal to 10% of patients receiving PROMACTA compared with placebo).

Table. Adverse Reactions (≥10% and Greater than Placebo) from Two Placebo controlled Trials in Adults with Chronic Hepatitis C

Adverse Reaction	**PROMACTA +Peginterferon/Ribavirin n = 955 (%)**	**Placebo +Peginterferon/Ribavirin n = 484 (%)**
Anemia	40	35
Pyrexia	30	24
Fatigue	28	23
Headache	21	20
Nausea	19	14
Diarrhea	19	11
Decreased appetite	18	14
Influenza-like illness	18	16
Asthenia	16	13
Insomnia	16	15
Cough	15	12
Pruritus	15	13
Chills	14	9
Myalgia	12	10
Alopecia	10	6
Peripheral edema	10	5

In the 2 controlled clinical trials in patients with chronic hepatitis C, hyperbilirubinemia was reported in 8% of patients receiving PROMACTA compared with 3% for placebo. Total bilirubin greater than or equal to 1.5 X ULN was reported in 76% and 50% of patients receiving PROMACTA and placebo, respectively. ALT or AST greater than or equal to 3X ULN was reported in 34% and 38% of patients for PROMACTA and placebo, respectively.

As detailed in published literature: The safety and efficacy of eltrombopag has been demonstrated in two randomized, double blind placebo controlled studies in adults with previously treated ITP. Most undesirable reactions associated with eltrombopag were mild to moderate in severity, early in onset and rarely treatment limited. Adverse events among the 311 patients who received either eltrombopag or placebo included: (very common ≥ 10%; common ≥ 1% but less than 10%)

- Infections and infestations: pharyngitis (common), Urinary tract infection (common)
- Gastrointestinal: nausea (very common), diarrhea (very common), dry mouth(common), vomiting (common)
- Hepatobiliary disorders: increased AST (common), ALT (common)
- Skin and subcutaneous tissue disorders: alopecia (common), rash (common)
- Musculoskeletal and connective tissue: Back pain, musculoskeletal chest pain, musculoskeletal (pain myalgia)

The safety of eltrombopag has also been studied in healthy volunteers who received the drug for 5 days at doses as high as 200mg daily with safety profiles similar to placebo.[53]

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

Nonclinical Pharmacology

Studies conducted in vitro have shown that eltrombopag is an effective agonist binding to *mpl*, the thrombopoietin receptor (TPO-R), to stimulate thrombopoiesis. It binds *mpl* at a position distinct from the ligand binding site, and thus does not compete with TPO for binding to its receptor.[47] In vivo, eltrombopag increases platelet number in the chimpanzee (the only nonclinical species which is pharmacologically responsive to eltrombopag). These findings, coupled with supporting clinical efficacy data, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous thrombopoietin (TPO). Additionally, in vitro electrophysiology studies have been performed and in vivo safety pharmacology studies assessed the effects of eltrombopag on cardiovascular, respiratory and central nervous systems.

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

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

polypeptide (OATP1B1), although it was an inhibitor of this transporter with the potential for such an interaction confirmed clinically.

Nonclinical toxicology

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

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

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

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

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

Genotoxicity: The toxic potential of eltrombopag was also assessed in a battery of in vitro and in vivo genetic toxicology studies and the weight of evidence provided by these assessments suggests that

eltrombopag does not pose a genotoxic risk in humans.

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

Teratogenicity: Eltrombopag was not teratogenic in rats or rabbits and did not affect fertility in male rats or fertility, early embryonic development, embryofetal development, maternal reproductive function, or development of offspring in female rats at non-maternally toxic doses. No effect on embryofetal development was observed in rabbits. At a maternally toxic dose in rats, treatment with eltrombopag was associated with embryolethality, a low incidence of cervical ribs (a non-teratogenic fetal variation) and reduced fetal body weight. In definitive juvenile toxicity studies in rats, eltrombopag was not associated with adverse effects. In vitro, eltrombopag was toxic in the presence of ultraviolet-A (UV-A) radiation, indicating a phototoxic response. However, there was no evidence of cutaneous phototoxicity in hairless mice or ocular phototoxicity in pigmented or albino mice or rats. Eltrombopag also showed evidence of photoclastogenicity in vitro that was associated with cytotoxic drug concentrations (15 to 29 μg/mL) and high intensity UV exposure [30 minimal erythematous dose (MED)]. However, no evidence of photoclastogenicity was observed at a 2-fold higher concentration (58.4 μg/mL) and UV exposure of ~15 MED. Eltrombopag did not adversely affect immune function in an immunotoxicity study in rats.

Clinical Pharmacology (based on studies done in healthy subjects and subjects with hepatic impairment or renal impairment)[53,65]

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

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

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

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

Race: Based on both non-compartment analysis and population pharmacokinetic analysis, plasma

eltrombopag exposure was approximately 70% higher in some Asian subjects (East and South East Asian) with ITP as compared to non-Asian subjects who were predominantly Caucasian in these trials. In addition, the pharmacodynamic (PD) response to eltrombopag was qualitatively similar in the Asian subjects, but the absolute PD response was somewhat greater. An approximately 40% higher systemic eltrombopag exposure in healthy African- American subjects was noted in at least one clinical pharmacology study. The effect of African-American ethnicity on exposure and related safety and efficacy of eltrombopag has not been established.

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

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

Safety Findings from Completed and Ongoing Studies in Patients with Thrombocytopenia

A comprehensive clinical program was designed to assess the clinical utility of eltrombopag in the treatment of chronic idiopathic thrombocytopenia purpura (ITP). On Nov 20, 2008, the United States Food and Drug Administration (FDA) granted accelerated approval for eltrombopag (Promacta®) for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The approved indication is based on data from three pivotal studies in the short-term treatment (TRA100773A and B, and TRA102537), and one ongoing long-term treatment study of patients with chronic ITP (EXTEND). Safety data from 462 eltrombopag-treated subjects in 8 completed or ongoing GSK sponsored clinical efficacy studies are as follows:

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

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

- No dose-dependent pattern of adverse events (AEs) was observed across the eltrombopag 30 mg, 50 mg, and 75 mg treatment groups.
- No clinically meaningful differences in incidence or severity of the most common ($\geq$5%) AEs were observed between subjects treated with eltrombopag 50 mg compared to placebo.
- Similar incidences of serious adverse events (SAEs) (12% and 11%) and discontinuations due to AEs (7% and 5%) were observed in the placebo and eltrombopag 50 mg treatment groups, respectively.
- Increases in hepatobiliary values (ALT, AST, bilirubin, alkphos) were seen in 16/164 subjects (9.7%) in the eltrombopag group (all doses), compared with 5/67 (7.5%) in the

placebo group. These elevations in liver aminotransferase were generally asymptomatic and returned to baseline after discontinuation of therapy.

- One case of thromboembolism was observed (platelet count 108,000/uL) in the eltrombopag 50 mg treatment group in a subject who died from sepsis of pulmonary origin.
- Preclinical findings that indicated potential for phototoxicity, cataracts and renal tubular toxicity did not appear to translate to clinical consequences during short-term use.
- Transient decreases in platelet counts to levels below baseline were observed in both treatment groups after eltrombopag treatment ended. However, the decreases in platelet count were not accompanied by a clinically meaningful increase in bleeding symptoms.

TRA102537(RAISE).In RAISE, the primary efficacy endpoint was the odds of achieving a platelet count >50,000/μL and <400,000/μL, during the 6 month treatment period, for subjects receiving eltrombopag relative to placebo. One hundred and ninety seven subjects were randomized 2:1, eltrombopag (n=135) to placebo (n=62), and were stratified based upon splenectomy status, use of ITP medication at baseline and baseline platelet count. Subjects received study medication for up to 6 months, during which time the dose of eltrombopag could be adjusted based on individual platelet counts. In addition, subjects could have tapered off concomitant ITP medications and received rescue treatments as dictated by local standard of care.

- The odds of achieving a platelet count between 50,000/μL and 400,000/μL during the 6-month treatment period were 8 times higher for eltrombopag treated subjects than for placebo-treated subjects (Odds Ratio: 8.2 [99 % Cl: 3.59, 18.73] p = < 0.001). Median platelet counts were maintained above 50,000/μL at all on-therapy visits starting at Day 15 in the eltrombopag group; in contrast, median platelet counts in the placebo group remained below 30,000/μL throughout the study.

- At baseline, 77 % of subjects in the placebo group and 73 % of subjects in the eltrombopag group reported any bleeding (WHO Grades 1-4); clinically significant bleeding (WHO Grades 2-4) at baseline was reported in 28 % and 22 % of subjects in the placebo and eltrombopag groups, respectively. The proportion of subjects with any bleeding (Grades 1-4) and clinically significant bleeding (Grades 2-4) was reduced from baseline by approximately 50% throughout the 6 month treatment period in eltrombopag-treated subjects. When compared to the placebo group, the odds of any bleeding (Grades 1-4) and the odds of clinically significant bleeding (Grades 2-4) were 76 % and 65 % lower in the eltrombopag-treated subjects compared to the placebo-treated subjects (p < 0.001).

- Eltrombopag therapy allowed significantly more subjects to reduce or discontinue baseline ITP therapies compared to placebo (59 % vs. 32 %; p < 0.016).Significantly fewer eltrombopag-treated subjects required rescue treatment compared to placebo-treated subjects [19 % vs. 40 %; p = 0.001].Four placebo and 14 eltrombopag subjects had at least 1 hemostatic challenge (defined as an invasive diagnostic or surgical procedure) during the study. Fewer eltrombopag- treated subjects (29 %) required rescue treatment to manage their hemostatic challenge, compared to placebo-treated subjects (50 %).

- In terms of improvements in health related quality of life, statistically significant improvements from baseline were observed in the eltrombopag group in fatigue, including severity and impact on thrombocytopenia-impacted daily activities and

concerns [as measured by the vitality subscale of the SF36, the motivation and energy inventory, and the 6-item extract from the thrombocytopenia subscale of the FACIT-Th]. Comparing the eltrombopag group to the placebo group, statistically significant improvements were observed with thrombocytopenia impacted activities and concerns specifically regarding motivation, energy and fatigue, as well as physical and emotional role and overall mental health. The odds of meaningful improvement in health related quality of life while on therapy was significantly greater among patients treated with eltrombopag than placebo.

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

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

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

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

The primary analysis of this endpoint was performed on a dataset that classified subjects as either responders or non-responders (primary dataset). For this primary analysis of response, only on-therapy platelet counts were included. Responders either achieved a platelet count of ≥50 K/uL (from a baseline platelet count of <30 K/uL) at the Day 43 Visit, or achieved a platelet count >200 GK/uL and discontinued study medication prior to Day 43; and non-responders either did not achieve a platelet count ≥50 K/uL at Day 43 or discontinued treatment prior to Day 43 for any reason other than a platelet count

>200 K/uL.

Supportive data analyses were performed using a dataset of all platelet counts during the treatment and follow-up periods, whether or not the subject discontinued treatment prematurely (observed dataset).

The odds of responding were significantly greater for the eltrombopag 50 mg treatment groups compared to placebo in both TRA100773A and TRA100773B (Table 5-). The primary method of analysis was a logistic regression model adjusted for ITP medication use at randomization, splenectomy status and baseline platelet count ≤15 K/uL. Results using observed Data were similar.

Table 5. Primary Endpoint in Studies TRA100773A and TRA100773B

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

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

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

Figure 1

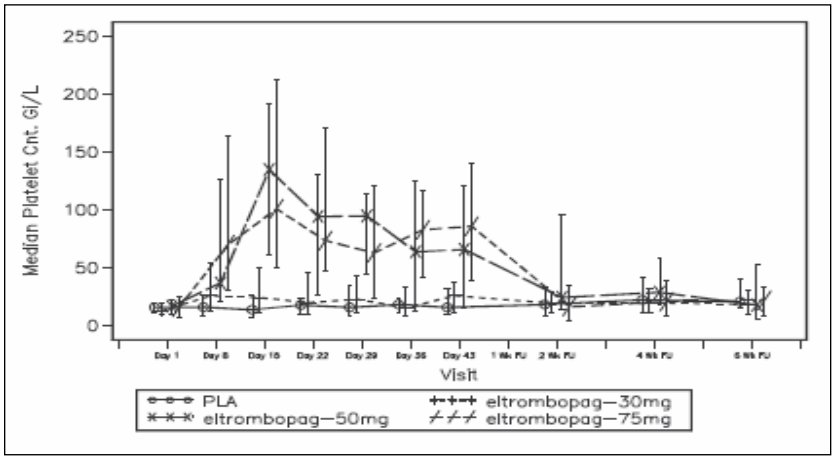
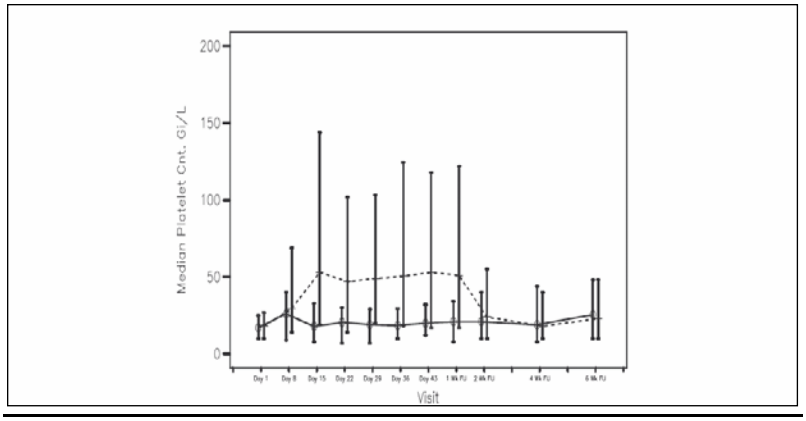


Figure 2



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

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

Analysis of Bleeding: Results of bleeding signs and symptoms reported via the World Health Organization (WHO) Bleeding Scale during the TRA100773A and TRA100773B are presented. The WHO Bleeding Scale has 5 grades: Grade 0 - no bleeding; Grade 1 – petechiae; Grade 2 - mild blood loss; Grade 3 - gross blood loss; and Grade 4 - debilitating blood loss. To analyze the data, subjects' assessments were summarized into categories: no bleeding (Grade 0), any bleeding (Grade 1 to Grade 4) and clinically significant bleeding (Grade 2 to Grade 4) (Table 6).

Table 6 **WHO Bleeding Scale Assessment**

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

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

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

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

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

TRA112940/Longitudinal Bone Marrow Study: TRA112940 is an ongoing study designed to ascertain the baseline levels of bone marrow reticulin and/or collagen fibers in previously treated adults with

chronic ITP and to evaluate any potential long-term effect of eltrombopag on bone marrow reticulin and/or collagen fibers. The study is closed to enrollment and at time of study enrollment closure, 167 subjects were enrolled. As of the cut-off date of 31 May 2012, an interim analysis was performed to assess for bone marrow fibrosis (reticulin and/or collagen) at baseline (before treatment with eltrombopag) and 1 year of treatment (n =101) subjects (Brynes, 2012). Specimens underwent central independent pathology review of reticulin grade and presence of collagen (European Consensus scale-MF; Thiele, 2005).

At baseline, 91 subjects had reticulin grade MF-0, 10 MF-1, and 0 MF≥2. At 1 year, 59 subjects had MF-0, 38 MF-1, 3 MF-2, and 1 MF-3. Compared with baseline, there was no change at 1 year in MF grade in 61 subjects, a decrease by 1 grade in 3, an increase by 1 grade in 35, and an increase in 2 or 3 grades in 1 subject each. Three subjects had collagen at 1 year (1 subject each with MF-1, MF-2, and MF-3). None of the 4 subjects with MF≥2 had adverse events or hematologic abnormalities considered related to impaired bone marrow function, and none withdrew due to bone marrow findings. Among the 8 subjects with prior TPO-RA treatment, all had baseline reticulin of MF-0 and none had collagen; at 1 year, 6 remained MF-0, 1 was MF-1, and 1 MF-3 (collagen demonstrated).

10% of subjects had MF-1 at baseline. After 1 year of treatment, no increase or a mild increase in reticulin was observed in 63% and 35% of subjects. No subject with MF≥2 (n=4) had clinical signs or symptoms indicative of bone marrow dysfunction and none withdrew from the study.

TRA108062/PETIT: TRA108062/PETIT was designed to investigate the efficacy, safety, tolerability and pharmacokinetics of eltrombopag in previously treated pediatric patients with chronic ITP.
Enrollment: As of 01 March 2013, a total of 82 subjects were enrolled: 29 subjects in Cohort 1 (12-17 years), 33 subjects in Cohort 2 (6-11 years), and 20 in Cohort 3 (1-5 years). In total, 80 subjects received at least one dose of eltrombopag. Subjects in Part 1 received open-label eltrombopag. Subjects in Part 2 received blinded study medication (eltrombopag or placebo), and in Part 3 all subjects received open-label eltrombopag. As of 11 January 2012, in the open label phase of this study, 15 patients were enrolled into the 3 age-determined cohorts (5 subjects per cohort). Patients were between 2 and 17 years of age, with the median age of 11 years. Cohorts 1, 2 and 3 had a median age of 14 years (13-17), 11 years (9-11), and 5 years (2-5), respectively.

Adverse Events: All subjects in Part 1 reported an AE. Grade 1 and 2 AEs were reported in 88% of patients. There was one Grade 4 AE of neutropenia reported. The most commonly reported AEs were headache (53%), vomiting (40%), and diarrhea (33%).

Summary of On-therapy Adverse Events Reported by Subjects in Part 1 TRA108062

Adverse Events, n (%)	Cohort 1 n=5	Cohort 2 n=5	Cohort 2 n=5	Total n=15
Headache	4 (80)	2 (40)	2 (40)	8 (53)
Vomiting	2 (40)	1 (20)	3 (60)	6 (40)
Diarrhea	2 (40)	1 (20)	2 (40)	5 (33)
URT Infection[a]	2 (40)	2 (40)	0	4 (27)
Abdominal pain	1 (20)	1 (20)	0	2 (13)
Cough	1 (20)	2 (40)	0	3 (20)
Epistaxis	0	1 (20)	1 (20)	2 (13)

Serious Adverse Events: As of 01 March 2013, 19 SAEs had been reported in 12 subjects. Four SAEs were assessed by the investigator as related to study medication: febrile neutropenia, lenticular opacity, vitreous opacaties, and pyrexia. Fifteen SAEs were assessed as unrelated to study medication by the

investigator: neutropenia (2); epistaxis (3); anaemia; rectal hemorrhage (2); post procedural hemorrhage; thyroid cancer; varicella, impetigo and conjunctivitis in the same subject; and urinary tract infection and systemic lupus erythematosus in the same subject.

Deaths: No deaths have occurred in PETIT as of the clinical cut-off date.

TRA115450/PETIT2: TRA115450/PETIT2 was designed to investigate the efficacy, safety, and tolerability of eltrombopag in previously treated pediatric patients with chronic ITP. At the time of the clinical cut-off date (March 1, 2013), 92 subjects were enrolled; 33 subjects in Cohort 1, 39 subjects in Cohort 2, and 20 in Cohort 3.

Serious Adverse Events: As of 01 March 2013, 27 SAEs had been reported in 17 subjects. Four SAEs reported by 3 subjects were assessed by the investigator as related to study medication: abnormal alanine aminotransferase and abnormal aspartate aminotransferase (same subject), haemolytic anaemia, and thrombocytosis. Twenty-three SAEs were assessed as unrelated to study medication by the investigator: pneumonia (3), epistaxis (3), thrombocytopenia (2), haemorrhage (2), hypertensive crisis, pleural effusion, fungal pneumonia (2 events in 1 patient), influenza, aseptic meningitis, petechiae, gingivitis, hematoma infection, eye injury, rhinitis, upper respitory tract infection, and dengue fever.

Deaths: No deaths have occurred in PETIT2 as of the clinical cut-off date.

11.4.4 Related to Corticosteroids

Corticosteroids can make the body retain water and salt, cause diabetes and acne, and worsen high blood pressure. In addition, they will probably increase your appetite and may cause insomnia or mood changes. Steroids can also cause stomach ulcers and soften bones, leading to osteoporosis. The more dangerous problems, like bone thinning, only occur with long-term use; the other side effects will stop when the medication is discontinued. A small number of cases of "aseptic necrosis" have been observed when steroids have been used in high doses for durations comparable to those we will use in this study. Aseptic necrosis is a thinning of the bone near the joints, which can lead to chronic pain, sometimes requiring joint replacement. Steroids also suppress the immune system and raise your susceptibility to infection; taking CsA and steroids together increases this risk still further. We will ask that you plan to stay in the hospital for the first two weeks of your therapy so that infections, if they occur, can be treated promptly.

11.4.5 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.

11.4.6 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, vasovagal reactions or infections may rarely occur.

11.4.7 Related to Cardiac Monitoring

EKG: An electrocardiogram (EKG) is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or “wave”) travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put patches (electrodes) on the chest, arms and legs. The electrodes are soft and don’t cause any discomfort when they’re put on or taken off by the technician. The machine only records the EKG. It doesn’t send electricity into the body. There’s no pain or risk associated with having an electrocardiogram.

11.4.8 Related to Central Line Placement:

A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the placement procedure. Only trained experienced staff will place the line in order to minimize these procedure related risks.

The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Very rarely (less than 1% of the time), the line placement may nick a vein causing one lung to collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, patients will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will remain in place until drug administration is complete.

11.4.9 Related to Concomitant Medications

- ***Pentamidine:*** cough (31-47%), bronchospasm (10-23%), decreased appetite (53-72%), fatigue, metallic taste, shortness of breath, decreased appetite, dizziness, rash, nausea, pharyngitis, chest pain/congestion, night sweats, chills, vomiting.

- ***Valacyclovir***: Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who received valacyclovir at greater than the recommended dose.

11.5 Risks in Relation to Benefit

For adult subjects:
The benefits to the subjects could be reduction or even abolition of transfusion requirements and/or improvement of cytopenia, 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.

The benefits of this study and the acquisition of bone marrow and blood samples important for the understanding of the pathophysiology of immune-mediated bone marrow failure states have been described in the previous paragraphs.

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

For pediatric subjects:

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

(a) the risk is justified by the anticipated benefit to the subjects: We are offering pediatric subjects with a 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).

11.6 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 Principal Investigator, Dr. Townsley, or the following Associate Investigators on this protocol (Drs. Dunbar, Dumitriu, Winkler, or Young or Janet Valdez, MPAS, PA-C) from the Hematology Branch 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 the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. 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. Is she does not agree she will be advised not to sign the assent.
If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

When a pediatric subject reaches age 18, continued participation will require re-consenting with the adult consent document. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, an attempt will be made to contact the subject for re-consent in accordance with NIH HRPP SOP 14D 7.4.

Informed Consent of Non-English Speaking Research Participants.

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2.If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the Principal Investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2) and 21 CFR50.27 (b) (a). The summary that will be used is the English version of the extant IRB approved consent document. We request IRB approval for the use of the short form for up to 5 research participants in a given language and will notify the IRB at the time of the Continuing Review of the frequency of the use of the Short Form. Should we reach a threshold of 5 we will notify the IRB and have the consent document translated into the given inherent language. IRB approval will be obtained for each individual use since this study is greater than minimal risk and involves pediatric

participants.

Informed Consent for adult research participants unable to provide consent:
If there is an unexpected enrollment of a research participant unable to provide informed consent, the following justification and procedures per NIH HRPP SOP 14E will be used to enrolled participants in the this protocol.

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

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

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

Procedures for obtaining consent for legally authorized representative (LAR) (Risk Level B per SOP 14E): The following procedures will be followed starting with (1) in order to determining the LAR.
(1) For adults who cannot consent and have a court appointed guardian from a jurisdiction that allows it or a Durable Power of Attorney (DPA) for health care and/or research participation, the PI/designee or ACAT confirms appropriateness of surrogate to consent to research, including that:
(a)The surrogate understands that the protocol involves research;
(b)The surrogate understands the risks, potential benefits, (if any), and alternatives to the study;
(c)The surrogate has sufficient reason to believe participation in the study is consistent with the subject's preferences and values.

(2) Adults who cannot consent and who do not have a DPA or court-appointed guardian, but who are capable of understanding the DPA process and can assign a DPA, then ACAT confirms appropriateness of surrogate to consent to research, which includes assessing criteria (a)-(c) above.
(3) Adults who cannot consent, who do not have a DPA or court-appointed guardian, and who are not able to understand the DPA process to appoint a DPA, then a person at the highest level of the following list may serve as surrogate and authorize subject's participation if ACAT confirms surrogate appropriateness (which includes assessing criteria (a)-(c) above):
1. spouse or domestic partner;
2. adult child;
3. parent;
4. sibling;
5. other close relative

If at any time there is a question about the authority of the LAR to provide consent based on the jurisdiction appointing the durable power of attorney or other legal question regarding the LAR to provide consent, the Office of the General Counsel will be consulted.

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

11.7 Conflict of interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

This protocol has an associated CRADA - with Novartis.

12 PHARMACEUTICALS

12.1 ELTROMBOPAG (PROMACTA®)

Supply: The drug Novartis is providing for this study is investigational material and is available in tablets (white to off-white 10.3mm standard bi-convex tablets) and as a powder for oral suspension (Eltrombopag PfOS, a reddish-brown to yellow powder contained inside an elongated sachet). Each sachet contains eltrombopag olamine equivalent to 20 mg of eltrombopag per gram of powder. The tablets are available as 12.5, 25, 50, 75 and 100 mg tablets.

Preparation: There is no parental dose for eltrombopag.

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

Administration: Eltrombopag may only be given orally.

Toxicities: see section 11.3.3Risks related to eltrombopag.

Eltrombopag Powder for Oral Suspension:
Eltrombopag powder for oral suspension (Eltrombopag PfOS) is a reddish-brown to yellow powder contained inside an elongated sachet. Each sachet will contain eltrombopag olamine equivalent to 20 mg of eltrombopag per gram of powder. PfOS sachets will be packaged in a carton. Each carton pack will hold 35 sachets along with a plastic reconstitution container and a syringe-adapt cap. The pack will also contain an extra syringe-adapt cap as a spare. The clinical site will provide a 10cc syringe with each carton. The sachet should not be opened until ready to use. Add 9.5 mL of water drawn using a 10cc syringe into the provided plastic container. Cut open the sachet and add the entire content of the sachet into the container with water. The container is capped and shaken for 10-20 seconds. The resulting suspension contains 2 mg/mL of eltrombopag dose. The prescribed volume (dose) is drawn through the syringe port on the cap with a syringe. Upon dosing, the rest of the remaining suspension in the container is discarded. The container and the syringe are rinsed with water and dried. If the prescribed dose is > 24

mg, which will require that part or all of a second sachet be used, then the suspension can be prepared by adding the contents of the two sachets to 19.0 mL of water, and then following the steps outlined above. The water has to be drawn by using the 10cc syringe twice, and similarly dosing has to occur by using the same 10cc syringe twice. If the dosage requires more than two sachets, then for each sachet used 9.5 mL of water will be added, and then following the steps outlined above. The water has to be drawn by using the same 10cc syringe for each sachet used, and similarly dosing has to occur by using the same 10cc syringe. A fresh dose is prepared everyday just prior to the dosing and no storage of the reconstituted suspension is allowed.

Handling and Storage of Study Treatment
Eltrombopag PfOS sachets will be stored at a controlled room temperature of 20-25°C (68-77°F); excursions between 15-30°C (59-86°F) are permissible.

*Shipping:*The NIH Pharmaceutical Development Services will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is

National Institutes of Health/CC/PHARM/PDS
10 Center Drive, MSC 1196, Building 10, Room 1C230
Bethesda, Maryland 20892-1196
Shipping Designee Name: Judith Starling, RPh
Shipping Designee Phone No: (301) 496-1031
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: jstarling@NIH.gov

12.2 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)

Supply: Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.

Preparation: For parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. The recommended liquids for dilution of the oral solution to improve palatability include milk, chocolate milk or orange juice, preferable at room temperature.

Storage and Stability: Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

Administration: Cyclosporine may be given intravenously or orally.

Toxicities: see section 11.3.2 Risks related to CsA

12.3 ANTI-THYMOCYTE GLOBULIN (equine) sterile solution (ATGAM®)

Other: Antithymocyte Gammaglobulin, Antithymocyte Globulin, ATGAM, Antithymocyte Immunoglobulin, lymphocyte immune globulin and h-ATG

Supply / availability: commercially available (Pharmacia & Upjohn Company)

Product description: Anti-thymocyte globulin (equine) sterile solution (ATGAM®) is available in 5 ml ampoules containing 50 mg of horse gamma globulin/mL (250 mg per ampoule).

Preparation: The calculated dose of anti-thymocyte globulin should be diluted in 0.9% sodium chloride injection to a concentration not to exceed 4 mg/mL.

Storage / stability: Anti-thymocyte globulin (equine) ampoules should be stored in a refrigerator at 2^{o} to 8^{o} C. Once diluted, anti-thymocyte globulin (equine) is physically and chemically stable for up to 24 hours at concentrations of up to 4 mg/mL in the recommended diluents. It is recommended that diluted anti-thymocyte globulin (equine) be stored in a refrigerator if it is prepared prior to the time of infusion.

Administration: Anti-thymocyte globulin (equine) should be administered into a high-flow central vein through an in-line filter with a pore size of 0.2 to 1 micron. The dose should be infused over no less than 4 hours. Infusion times may be extended to up to 24 hours for intolerance. Patients should be closely monitored for infusion / allergic reactions.

Compound: Principally monomeric IgG, prepared from plasma or serum of healthy horses hyperimmunized with human thymus lymphocytes.

Action: Immunosuppressive agent. Exact mechanism of immunosuppression of ATGAM has not been fully elucidated but may involve elimination of antigen-reactive T-cells in peripheral blood and/or alteration of T-cell function

Side effects: see section 11.3.1 Risks related to h-ATG

12.4 PENTAMIDINE

Supply: Commercially available (NebuPent®, American Pharmaceutical Partners, Inc.)

Product description: Pentamidineisethionate is available as a 300 mg single dose vial containing 300 mg of lyophilized powder in a 15 mL capacity vial. The contents of one vial must be dissolved in 6 mL of sterile water for injection, USP. It is important to use only sterile water; saline solution will cause the drug to precipitate.

Storage and stability: Store dry product at controlled room temperature 15-30^{o}C (59-86^{o}F).

Route of administration: Inhalation; Once reconstituted, the entire contents of a vial should be placed into the Respigard® II nebulizer (Marquest) reservoir for administration by inhalation Do not mix the pentamidine solution with any other drugs.

Toxicities: see section 11.3.9Risks related to Concomitant medications.

12.5 VALACYCLOVIR

Generic name: valacyclovir
Brand Name: Valtrex
Supply: Commercially available.
Pharmacology: Valacyclovir is the hydrochloride salt of L-valyl ester of the antiviral drug acyclovir. After oral administration, valacyclovir is rapidly absorbed from the GI tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal or hepatic metabolism.
Product description: Valacyclovir is available in 500mg tablets and 1gm tablets. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines.
Storage and Stability: Oral tablets should be stored at 15^{o} to 25^{o}C (59^{o} to 77^{o}F).
Route of administration: Oral
Toxicities: see section 11.3.9 risks related to concomitant medications.

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, Platanias 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. Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin Hematol.* Jan 2000;37(1):30-42.
11. Storb R, Etzioni R, Anasetti C, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. *Blood.* Aug 1 1994;84(3):941-949.
12. Deeg HJ, Leisenring W, Storb R, et al. Long-term outcome after marrow transplantation for severe aplastic anemia. *Blood.* May 15 1998;91(10):3637-3645.
13. Gupta V, Eapen M, Brazauskas R, et al. Impact of age on outcomes after transplantation for acquired aplastic anemia using HLA-identical sibling donors. *Haematologica.* September 17, 2010 2010:haematol.2010.026682.
14. Marsh J, Schrezenmeier H, Marin P, et al. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of

patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. *Blood.* Apr 1 1999;93(7):2191-2195.

15. Frickhofen N, Kaltwasser JP. Immunosuppressive treatment of aplastic anemia: a prospective, randomized multicenter trial evaluating antilymphocyte globulin (ALG) versus ALG and cyclosporin A. *Blut.* Apr 1988;56(4):191-192.

16. Young N, Griffith P, Brittain E, et al. A multicenter trial of antithymocyte globulin in aplastic anemia and related diseases. *Blood.* Dec 1988;72(6):1861-1869.

17. Raefsky EL, Gascon P, Gratwohl A, Speck B, Young NS. Biological and immunological characterization of ATG and ALG. *Blood.* Sep 1986;68(3):712-719.

18. Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. *Transplantation.* Apr 27 1995;59(8):1194-1200.

19. Sloand E, Kim S, Maciejewski JP, Tisdale J, Follmann D, Young NS. Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. *Blood.* Aug 15 2002;100(4):1185-1191.

20. Zeng W, Nakao S, Takamatsu H, et al. Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia. *Blood.* May 1 1999;93(9):3008-3016.

21. Gascon P, Zoumbos NC, Scala G, Djeu JY, Moore JG, Young NS. Lymphokine abnormalities in aplastic anemia: implications for the mechanism of action of antithymocyte globulin. *Blood.* Feb 1985;65(2):407-413.

22. Barrett AJ, Longhurst P, Rosengurt N, Hobbs JR, Humble JG. Crossreaction of antilymphocyte globulin with human granulocyte colony-forming cells. *Journal of clinical pathology.* Feb 1978;31(2):129-135.

23. Greco B, Bielory L, Stephany D, et al. Antithymocyte globulin reacts with many normal human cell types. *Blood.* Nov 1983;62(5):1047-1054.

24. Shevach EM. The effects of cyclosporin A on the immune system. *Annual review of immunology.* 1985;3:397-423.

25. Nussenblatt RB, Palestine AG. Cyclosporine: immunology, pharmacology and therapeutic uses. *Survey of ophthalmology.* Nov-Dec 1986;31(3):159-169.

26. Bacigalupo A, Bruno B, Saracco P, et al. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midolio Osseo (GITMO). *Blood.* Mar 15 2000;95(6):1931-1934.

27. Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA.* Mar 5 2003;289(9):1130-1135.

28. Frickhofen N, Kaltwasser JP, Schrezenmeier H, et al. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group. *N Engl J Med.* May 9 1991;324(19):1297-1304.

29. Brodsky RA, Sensenbrenner LL, Smith BD, et al. Durable treatment-free remission after high-dose cyclophosphamide therapy for previously untreated severe aplastic anemia. *Ann Intern Med.* Oct 2 2001;135(7):477-483.

30. Tisdale JF, Dunn DE, Geller N, et al. High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial. *Lancet.* Nov 4 2000;356(9241):1554-1559.

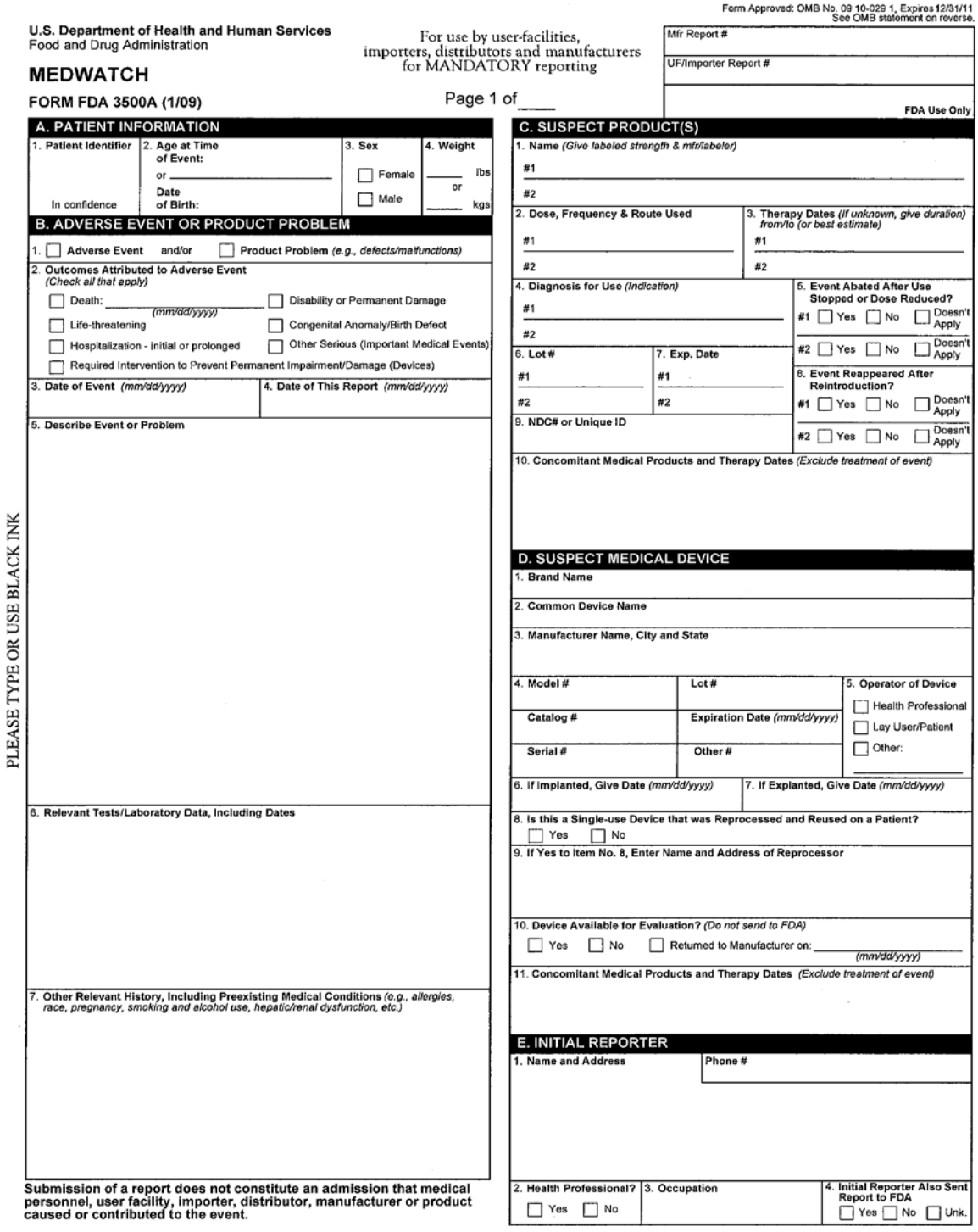
31. Tisdale JF, Maciejewski JP, Nunez O, Rosenfeld SJ, Young NS. Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial. *Blood.* Dec 15 2002;100(13):4668-4670.

32. Scheinberg P, Nunez O, Wu C, Young NS. Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil. *Br J Haematol.* Jun 2006;133(6):606-611.

33. Scheinberg P, Nunez O, Weinstein B, Biancotto A, Wu CO, Young NS. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med.* Aug 4 2011;365(5):430-438.
34. Tichelli A, Schrezenmeier H, Socie G, et al. A randomized controlled study in newly-diagnosed severe aplastic anemia patients receiving antithymocyte globulin (ATG), cyclosporine, with or without G-CSF: a study of the SAA Working Party of the EBMT. *Blood.* Jan 13 2011.
35. Kojima S, Hibi S, Kosaka Y, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. *Blood.* Sep 15 2000;96(6):2049-2054.
36. Marsh JC, Ganser A, Stadler M. Hematopoietic growth factors in the treatment of acquired bone marrow failure states. *Semin Hematol.* Jul 2007;44(3):138-147.
37. Gurion R, Gafter-Gvili A, Paul M, et al. Hematopoietic growth factors in aplastic anemia patients treated with immunosuppressive therapy-systematic review and meta-analysis. *Haematologica.* May 2009;94(5):712-719.
38. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood.* Aug 1 2002;100(3):799-803.
39. Kaito K, Kobayashi M, Katayama T, et al. Long-term administration of G-CSF for aplastic anaemia is closely related to the early evolution of monosomy 7 MDS in adults. *Br J Haematol.* Nov 1998;103(2):297-303.
40. 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.
41. 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.
42. 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.
43. 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.
44. 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.
45. Wang W, Matsuo T, Yoshida S, et al. Colony-forming unit-megakaryocyte (CFR-meg) numbers and serum thrombopoietin concentrations in thrombocytopenic disorders: an inverse correlation in myelodysplastic syndromes. *Leukemia.* Oct 2000;14(10):1751-1756.
46. Garnock-Jones KP. Eltrombopag: a review of its use in treatment-refractory chronic primary immune thrombocytopenia. *Drugs.* Jul 9 2011;71(10):1333-1353.
47. Imbach P, Crowther M. Thrombopoietin-receptor agonists for primary immune thrombocytopenia. *The New England journal of medicine.* Aug 25 2011;365(8):734-741.
48. 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.
49. 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.
50. 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.
51. 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.
52. 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.
53. 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.
54. 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.
55. 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.
56. Locasciulli A, Oneto R, Bacigalupo A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica.* Jan 2007;92(1):11-18.
57. Maciejewski JP, Anderson S, Katevas P, Young NS. Phenotypic and functional analysis of bone marrow progenitor cell compartment in bone marrow failure. *Br J Haematol.* Jun 1994;87(2):227-234.
58. Dusheiko G, Afdhal NH, Giannini E, et al. Final Results of Open-Label Treatment with Eltrombopag During ENABLE 1: A Study of Eltrombopag As An Adjunct for Antiviral Treatment of Hepatitis C Virus Associated with Thrombocytopenia. *ASH Annual Meeting Abstracts.* November 18, 2011 2011;118(21):2232-.
59. Afdhal NH. Final Results of ENABLE 1, a Phase 3, Multicenter Study of Eltrombopag as an Adjunct for Antiviral Treatment of Hepatitis C Virus-Related Chronic Liver Disease Associated With Thrombocytopenia. *Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, 2011*2011.
60. Olnes MJ, Scheinberg P, Calvo K, et al. Eltrombopag Can Stimulate Trilineage Hematopoiesis with Transfusion Independence in Patients with Refractory Severe Aplastic Anemia: Results From a Phase II Trial. *ASH Annual Meeting Abstracts.* November 18, 2011 2011;118(21):54-.
61. Johnson JR, Temple R. Food and Drug Administration requirements for approval of new anticancer drugs. *Cancer Treat Rep.* Oct 1985;69(10):1155-1159.
62. . In: Adler NE, Page AEK, eds. *Cancer Care for the Whole Patient: Meeting Psychosocial Health Needs.* Washington (DC)2008.
63. PROMIS. Patient-Reported Outcomes Measurement Information System: Dynamic Tools to Measure Health Outcomes From the Patient. 2011; Perspective. Available at. Accessed January 31, 2011.
64. D C. *Manual of the Functional Assessment of Chronic Ilness Therapy (FACIT Scales)(Version 4.1)*2004.
65. 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.
66. Nancy L. Geller, Dean Follmann, Eric S. Leifer, and Shelly L. Carter "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach" Chapter 2, pp 41-52, Advances in Clinical Trial Biostatistics, 2004, Marcel Dekker: New York
67. Scheinberg P, Rios O, Scheinberg P, Weinstein B, Wu CO, Young NS. Prolonged cyclosporine administration after antithymocyte globulin delays but does not prevent relapse in severe aplastic anemia. Am J Hematol. 2014 Jun;89(6):571-4.
68. Novick AC, Hwei HH, Steinmuller D, Streem SB, Cunningham RJ, Steinhilber D, Goormastic M, Buszta C. Detrimental effect of cyclosporine on initial function of cadaver renal allografts following extended preservation. Results of a randomized prospective study. Transplantation. 1986;42(2):154.
69. Tostivint I, du Montcel ST, Jaudon MC, Mallet A, Le Hoang P, Bodaghi B, Deray G, Bagnis CI. Renal outcome after ciclosporin-induced nephrotoxicity. Nephrol Dial Transplant. 2007;22(3):880.

APPENDIX A MEDWATCH FORM



Form Approved: OMB No. 09 10-029 1, Expires 12/31/11
See OMB statement on reverse.

U.S. Department of Health and Human Services
Food and Drug Administration

MEDWATCH

FORM FDA 3500A (1/09)

For use by user-facilities, importers, distributors and manufacturers for MANDATORY reporting

Page 1 of ____

Mfr Report #

UF/Importer Report #

FDA Use Only

PLEASE TYPE OR USE BLACK INK

A. PATIENT INFORMATION

1. Patient Identifier — In confidence
2. Age at Time of Event: or Date of Birth:
3. Sex — ☐ Female ☐ Male
4. Weight — ____ lbs or ____ kgs

B. ADVERSE EVENT OR PRODUCT PROBLEM

1. ☐ Adverse Event and/or ☐ Product Problem *(e.g., defects/malfunctions)*

2. Outcomes Attributed to Adverse Event *(Check all that apply)*
☐ Death: _____ *(mm/dd/yyyy)*
☐ Life-threatening
☐ Hospitalization - initial or prolonged
☐ Required Intervention to Prevent Permanent Impairment/Damage (Devices)
☐ Disability or Permanent Damage
☐ Congenital Anomaly/Birth Defect
☐ Other Serious (Important Medical Events)

3. Date of Event *(mm/dd/yyyy)*

4. Date of This Report *(mm/dd/yyyy)*

5. Describe Event or Problem

6. Relevant Tests/Laboratory Data, Including Dates

7. Other Relevant History, Including Preexisting Medical Conditions *(e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.)*

Submission of a report does not constitute an admission that medical personnel, user facility, importer, distributor, manufacturer or product caused or contributed to the event.

C. SUSPECT PRODUCT(S)

1. Name *(Give labeled strength & mfr/labeler)*
#1
#2

2. Dose, Frequency & Route Used
#1
#2

3. Therapy Dates *(if unknown, give duration)* from/to *(or best estimate)*
#1
#2

4. Diagnosis for Use *(Indication)*
#1
#2

5. Event Abated After Use Stopped or Dose Reduced?
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

6. Lot #
#1
#2

7. Exp. Date
#1
#2

8. Event Reappeared After Reintroduction?
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

9. NDC# or Unique ID

10. Concomitant Medical Products and Therapy Dates *(Exclude treatment of event)*

D. SUSPECT MEDICAL DEVICE

1. Brand Name

2. Common Device Name

3. Manufacturer Name, City and State

4. Model # | Lot # | Catalog # | Expiration Date *(mm/dd/yyyy)* | Serial # | Other #

5. Operator of Device
☐ Health Professional
☐ Lay User/Patient
☐ Other: _____

6. If Implanted, Give Date *(mm/dd/yyyy)*

7. If Explanted, Give Date *(mm/dd/yyyy)*

8. Is this a Single-use Device that was Reprocessed and Reused on a Patient?
☐ Yes ☐ No

9. If Yes to Item No. 8, Enter Name and Address of Reprocessor

10. Device Available for Evaluation? *(Do not send to FDA)*
☐ Yes ☐ No ☐ Returned to Manufacturer on: _____ *(mm/dd/yyyy)*

11. Concomitant Medical Products and Therapy Dates *(Exclude treatment of event)*

E. INITIAL REPORTER

1. Name and Address

Phone #

2. Health Professional? ☐ Yes ☐ No

3. Occupation

4. Initial Reporter Also Sent Report to FDA ☐ Yes ☐ No ☐ Unk.

APPENDIX B NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES v. 2.5.2013

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

B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine 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 myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No

C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1 ,TERC, TERT, SBDS, NOp10 , NHP2.*	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high densitycDNA 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 microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

APPENDIX C SUPPLEMENTAL FIGURES

Figure 1: Study Design

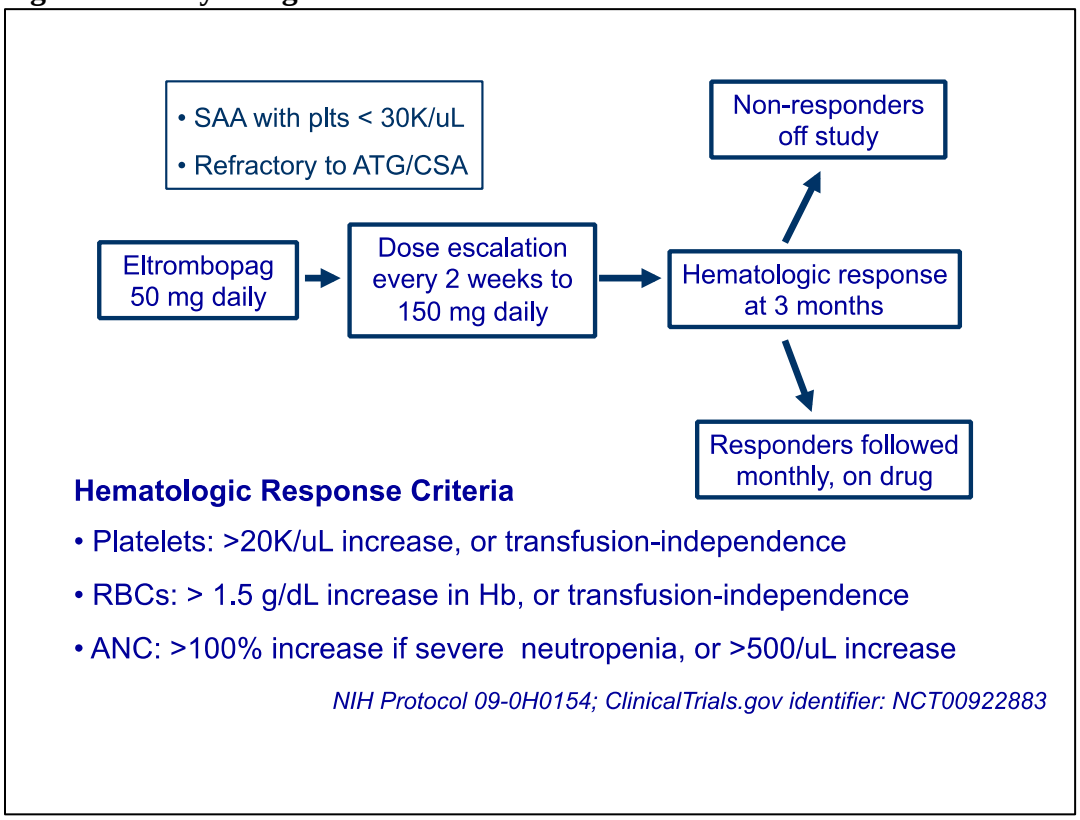


Figure 2: Study results as defined by response criteria at 12 weeks. Achievement of further lineage responses during extension phase at greater than 12 weeks are also indicated.

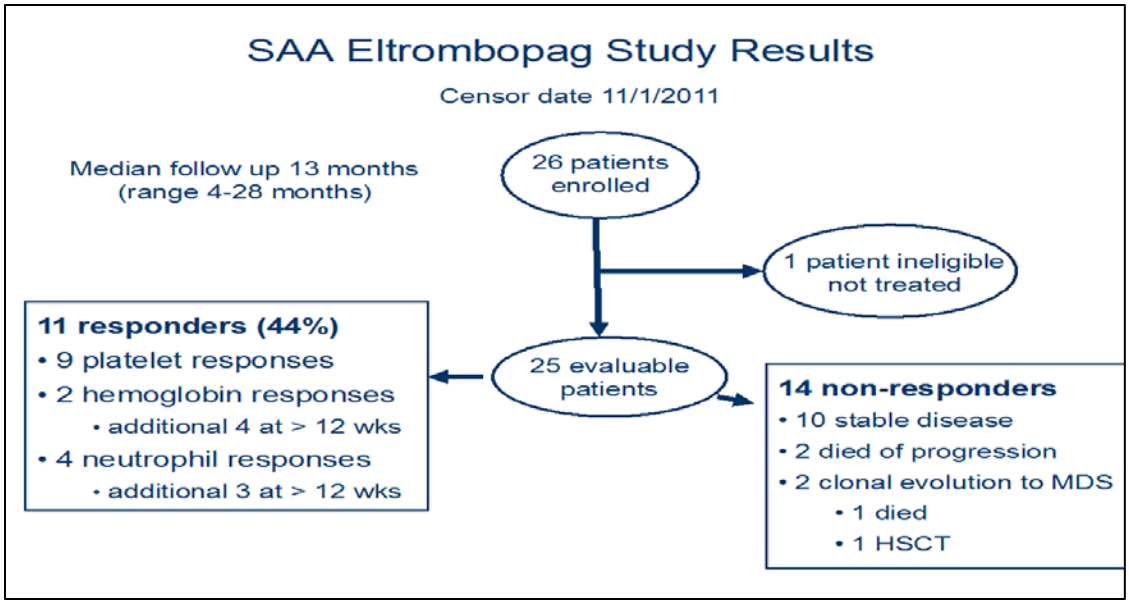
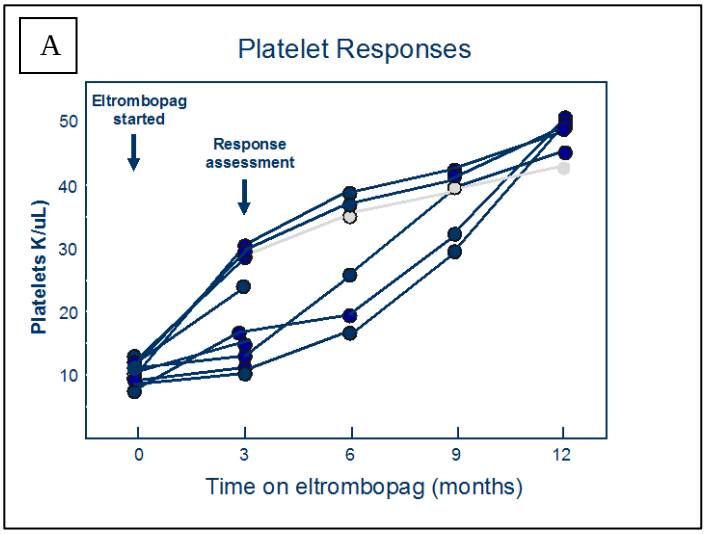
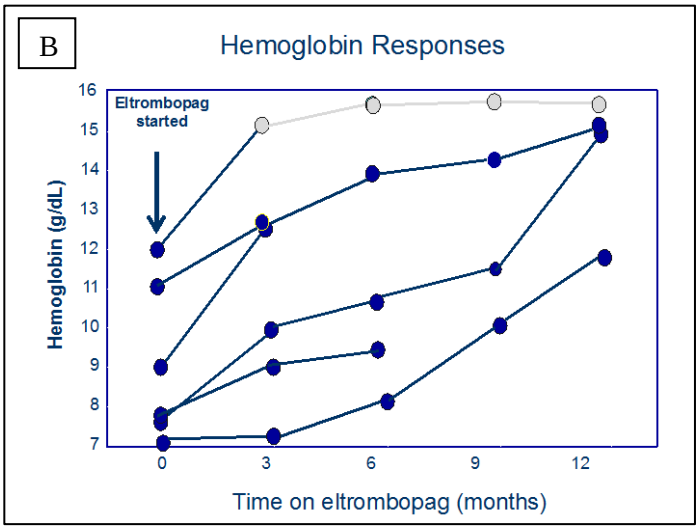


Figure 3: Responses to eltrombopag in responders over time. For each lineage, individual patients reaching response criteria are shown (A. Platelets, B. Hemoglobin, C. Neutrophils). Black lines indicate patients remaining on drug.
Gray lines indicate the patient taken off drug at three months due to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


A
Platelet Responses
Eltrombopag started
Response assessment
Platelets K/uL)
50
40
30
20
10
0
3
6
9
12
Time on eltrombopag (months)


B
Hemoglobin Responses
Eltrombopag started
Hemoglobin (g/dL)
16
15
14
13
12
11
10
9
8
7
0
3
6
9
12
Time on eltrombopag (months)

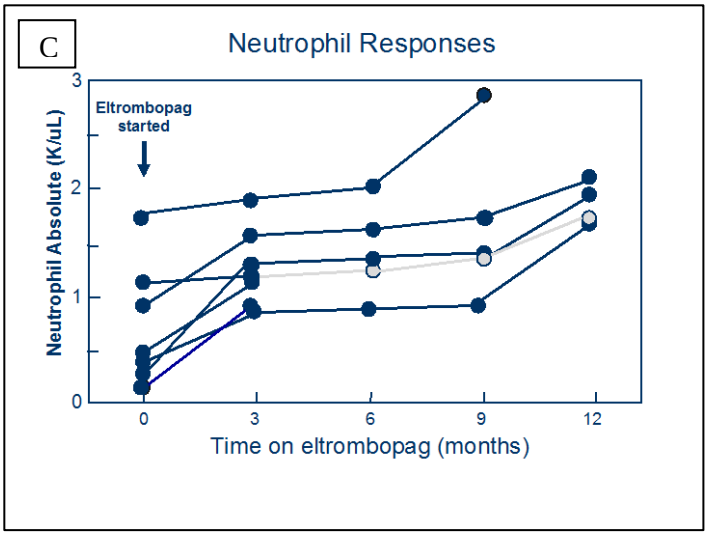
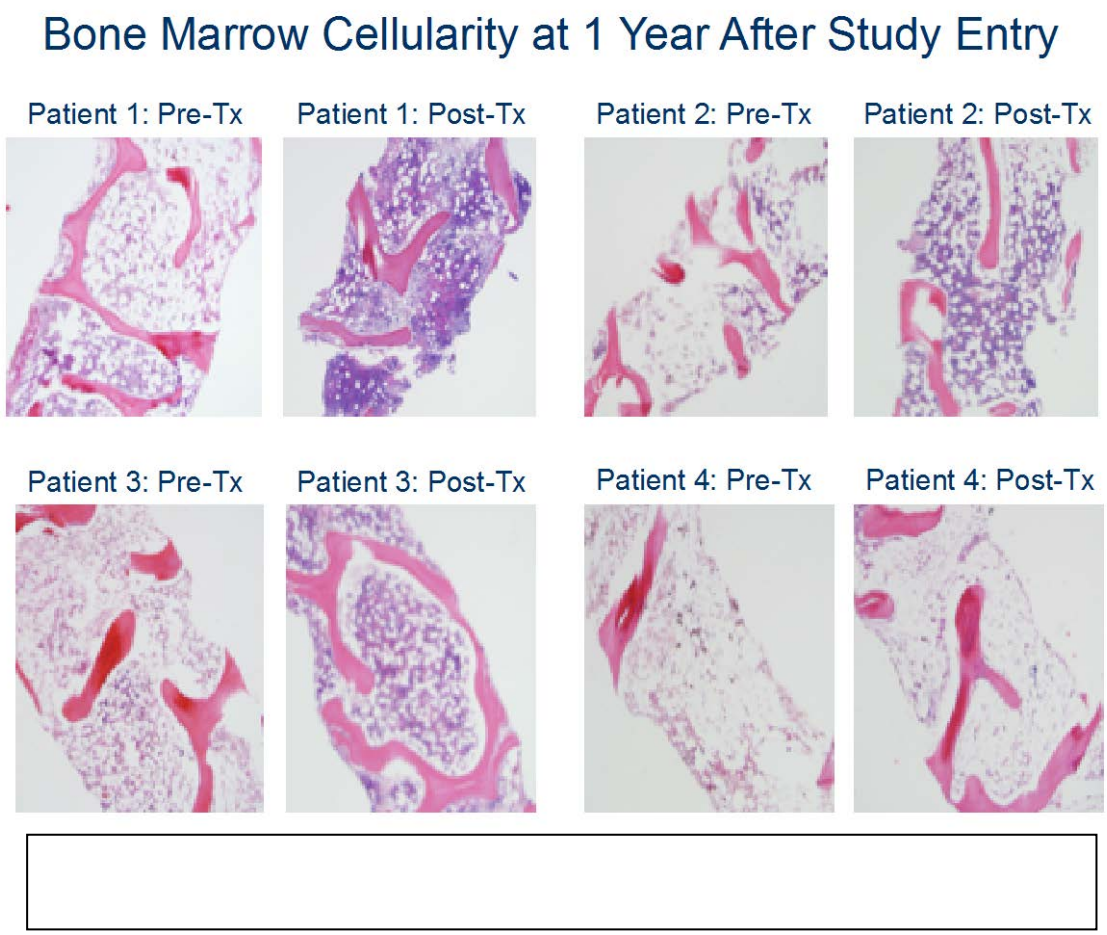

C
Neutrophil Responses
Eltrombopag started
Neutrophil Absolute (K/uL)
3
2
1
0
0
3
6
9
12
Time on eltrombopag (months)

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



APPENDIX D– PHARMACOKINETIC STUDIES

Collection of samples for PK Assessments

Subjects enrolled in Cohort 1, 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, 0.5-1mL 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:

Maria Edwards
PPD
2244 Dabney Road
Richmond VA, 23230, USA
Tel: (804) 254.8430
Fax: (804) 254.1104
e-mail:Maria.Edwards@ppdi.com

APPENDIX E – PROMIS QUESTIONNAIRE

PROMIS V.1.0/1.1 – Global

Global Items

Please respond to each item by marking one box per row.

		Excellent	Very good	Good	Fair	Poor
Global01	In general, would you say your health is:.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global02	In general, would you say your quality of life is:	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global03	In general, how would you rate your physical health?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global04	In general, how would you rate your mental health, including your mood and your ability to think?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global05	In general, how would you rate your satisfaction with your social activities and relationships?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global09	In general, please rate how well you carry out your usual social activities and roles. (This includes activities at home, at work and in your community, and responsibilities as a parent, child, spouse, employee, friend, etc.).....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Completely	**Mostly**	**Moderately**	**A little**	**Not at all**
Global06	To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
Global10	How often have you been bothered by emotional problems such as feeling anxious, depressed or irritable?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

		None	Mild	Moderate	Severe	Very severe
Global08	How would you rate your fatigue on average?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Global07	How would you rate your pain on average?.....	☐ 0 No pain	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10 Worst imaginable pain

FACT-An (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-An (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-An (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless (washed out)	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An6	I have trouble walking	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An9	I feel lightheaded (dizzy)	0	1	2	3	4
An10	I get headaches	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
An11	I have pain in my chest	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
An13	I am motivated to do my usual activities	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

FACT-Th11 (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
Th1	I bleed easily	0	1	2	3	4
Th2	I bruise easily	0	1	2	3	4
Th3	I worry about problems with bruising or bleeding	0	1	2	3	4
Th5	I am bothered by nosebleeds	0	1	2	3	4
Th7	I am bothered by pinpoint bruising beneath my skin	0	1	2	3	4
Th8	I am bothered by blood in my urine or stool	0	1	2	3	4
Th 10	I avoid or limit physical activity (because of concern with bleeding or bruising)	0	1	2	3	4
Th 12	I am frustrated by not being able to do my usual activities	0	1	2	3	4
Th 13	I worry that my treatment will be delayed (because of low blood counts)	0	1	2	3	4

FACT-N (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	None of the time	A little of the time	Some of the time	Most of the time	All of the time
N1	I worry about getting sick due to low blood counts	0	1	2	3	4
N2	I avoid public places for fear of getting an infection	0	1	2	3	4
P1	I get aches and pains that bother me	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
N3	I worry about getting infections	0	1	2	3	4
N4	I worry my condition will not improve if my treatment is delayed	0	1	2	3	4
An5	I have energy	0	1	2	3	4
BRM3	I am bothered by fevers (episodes of high body temperature)	0	1	2	3	4
BRM2	I am bothered by the chills	0	1	2	3	4
ES 3	I have night sweats	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4
MS10	I need to rest during the day	0	1	2	3	4
An1	I feel listless (washed out)	0	1	2	3	4
An13	I am motivated to do my usual activities	0	1	2	3	4
N6	I have mouth sores	0	1	2	3	4
N7	My partner worries about me when my blood counts are low	0	1	2	3	4
N8	My low blood counts interfere with my intimate relationships	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
MS3	I am bothered by headaches	0	1	2	3	4

Emotional Distress-Depression – Short Form 4a

Please respond to each question or statement by marking one box per row.

	In the past 7 days...	Never	Rarely	Sometimes	Often	Always
EDDEP04 1	I felt worthless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP06 2	I felt helpless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP29 3	I felt depressed	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP41 4	I felt hopeless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Emotional Distress-Anxiety – Short Form 4a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	Never	Rarely	Sometimes	Often	Always
EDANX01 1	I felt fearful.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX40 2	I found it hard to focus on anything other than my anxiety	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX41 3	My worries overwhelmed me.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDANX63 4	I felt uneasy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Sleep Disturbance – Short Form 4a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	Very poor	Poor	Fair	Good	Very good
Sleep109 1	My sleep quality was	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
	In the past 7 days…	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
Sleep116 2	My sleep was refreshing.	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Sleep20 3	I had a problem with my sleep.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Sleep44 4	I had difficulty falling asleep	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Applied Cognition-Abilities-Short Form 8a

Please respond to each item by marking one box per row.

	In the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
PC43_2	My mind has been as sharp as usual.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC44_2	My memory has been as good as usual...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC45_2	My thinking has been as fast as usual.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC47_2	I have been able to keep track of what I am doing, even if I am interrupted.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC6	I have been able to concentrate.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC-CaPS3	I have been able to think clearly without extra effort.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC29_2	I have been able to pay attention and keep track of what I am doing without extra effort.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
PC-CaPS14	I have been able to remember things as easily as usual without extra effort	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5