CLINICAL RESEARCH PROJECT

Protocol # 12-H-0150
Drug Name: eltrombopag (Promacta®)
IND: 104877
IND holder: NHLBI OCD

Date: March 18, 2020

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 (enrolled only 31)	either	≥ 2 years and weight >12 kg
Cohort 3	31	either	≥ 2 years and weight >12 kg
Extension Cohort	87	either	≥ 2 years and weight >12 kg
Laboratory Exploratory Cohort	25	either	≥ 2 years and weight >12 kg
Total	207		

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/□L 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.

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

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

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.

2.3.3 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 industry in the mid1990s, 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)

Number of patients	26
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

Category	Event	N	Dose (mg)	Related to eltrombopag
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 thrombosis. In 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

An Extension Cohort of 87 subjects was added to the protocol once enrollment was 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, and to obtain more precise data on secondary endpoints, and more pharmacokinetic data with the treatment regimen. We anticipate enrollment will be completed before we have a new SAA treatment naïve protocol developed and approved.

2.9.4 Justification for adding Laboratory Exploratory Cohort

We currently have accrued 178/182 patients to our protocol, 12-H-0150. Among these patients, 86 compose an extension cohort; the primary aim of extension of the study was to obtain more precise data regarding important secondary endpoints and to collect more pharmacokinetic samples.

We now propose an extension to add 25 subjects to perform novel laboratory experiments using *fresh* bone marrow samples obtained at the time of diagnosis and prior to institution of definitive therapy. **These experiments will not be feasible in our upcoming treatment naïve SAA protocol because we plan to initiate treatment remotely, and thus will not be collecting baseline marrow/blood samples for research.**

T cell mediated immune destruction of hematopoietic stem and progenitor cells (HSPCs) is the pathophysiology of SAA, but precise mechanisms have not been characterized, nor have biomarkers been identified. We intend to elucidate the pathophysiology of SAA using advanced single-cell methodologies (scRNA-seq), a technique that allows high resolution whole transcriptome profiling of the individual cells, and time of flight cytometry (CyTOF), a novel deep-phenotyping technique using multiparameter mass cytometry. ScRNA-seq will be used to study the transcriptome of HSPCs and enriched T cells, and T cell clonality in patients with newly diagnosed severe aplastic anemia (SAA). CyTOF will enable high resolution characterization of HSPCs, immune cells especially regulatory T cell subsets, and other marrow populations simultaneously based on a range of cell surface and intracellular markers, to explore the relationship of bone marrow microenvironment and hematopoiesis in patients with SAA. If a biomarker or genetic signature is identified from these experiments, further experiments could be performed on larger cohort of frozen samples using simpler methods, such as standard flow cytometry or multiplex polymerase chain reaction (PCR).

Our aims are to:

(1) Identify characteristic cell population in treatment naïve SAA, provide a comprehensive view of active marrow process in SAA patients at both cellular and molecular levels
(2) Infer potential autoantigens from the expanded clonal T cells, perform integrated analysis of receptors and ligands of T cells and HSPCs
(3) Expand our understanding of the interaction between HSPCs and the microenvironment of the marrow of SAA patients by interrogating immune mechanisms in detail (cytotoxic T cells, T regs, MDSC, macrophages)
(4) Characterize transcriptomes programs in HSPCs in treatment naïve SAA
(5) Identify biomarkers for clinical response and long term outcomes, particularly clonal evolution
(6) Explore mechanisms by which eltrombopag (EPAG) stimulates hematopoiesis and modulates the immune system

We plan to perform this experiment with *fresh* bone marrow samples from 25 subjects at baseline and at 6 months' time point. These patients will not be included in the analysis on secondary endpoints of the current extension cohort. Fresh samples will be used for flow cytometry, particularly mass spectrometry, due to the impact of cryopreservation on fragile cell types and certain proteins. A study with human tumor specimen found

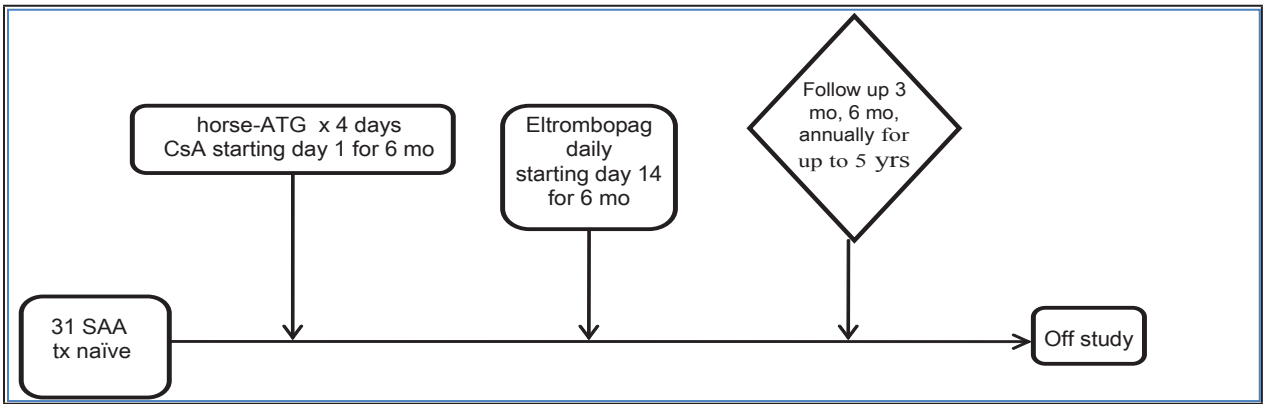
significant reduction of expression levels of multiple markers upon cryopreservation [70]. Fresh samples are desirable in single cell experiments using CD34 cells from SAA patients due to low cell numbers, which are inevitably decreased with cryopreservation. Additionally, there are data suggesting that cryopreservation results in molecular alterations in some cells including DNA damage, gene expression, and epigenetic changes[72, 73].

3. STUDY DESIGN

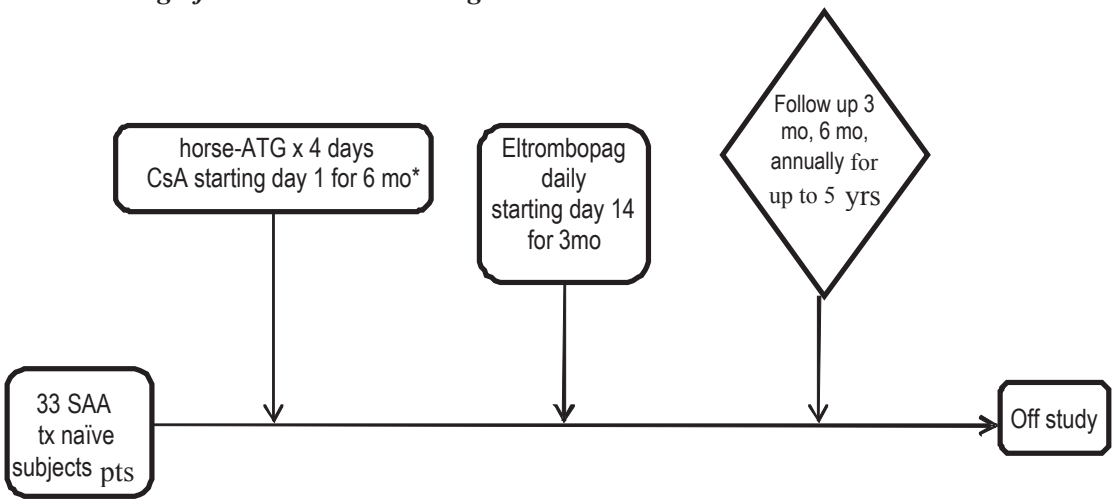
This study is designed as a non-randomized, pilot phase I/II study in which 182 subjects with severe aplastic anemia who have not received prior definitive immunosuppressive therapy will be treated with a standard regimen of h-ATG/CsA, combined with eltrombopag as experimental therapy. The first 31 subjects will be enrolled to cohort 1. Then up to 33 subjects will be enrolled to cohort 2 while 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 87 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. In addition, 25 subjects will be enrolled for Laboratory Exploratory Cohort. The treatment schedule for both the Extension & Laboratory cohorts will be the same as Cohort 3.

Protocol Design for Cohort 1

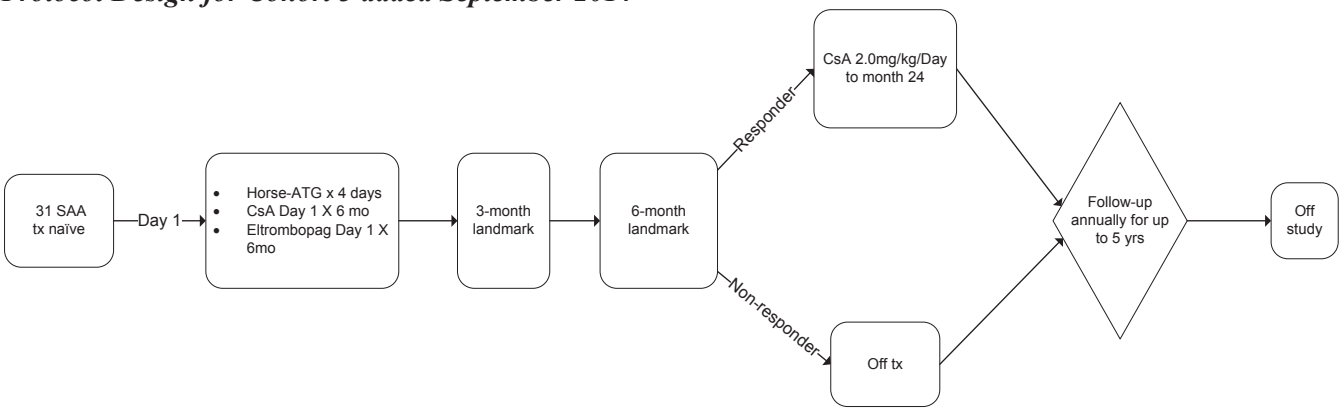


Protocol Design for Cohort 2 added August 2013

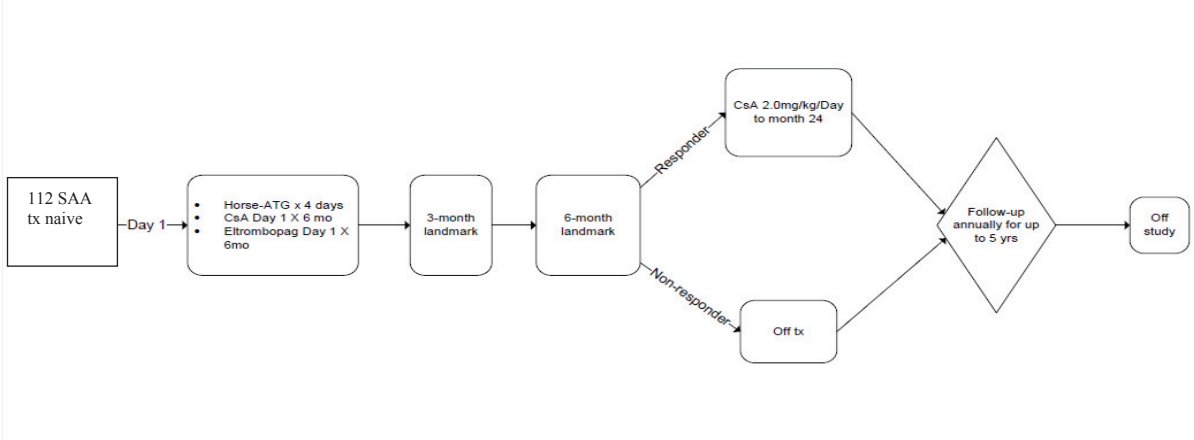


*Cohort 2 will continue CsA administration month 6 to month 24 at 2.0mg/kg per amendment number 15

Protocol Design for Cohort 3 added September 2014



Protocol Design for Extension Cohort and Laboratory Exploratory 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 National Heart Lung and Blood Institute (NHLBI) screening protocol (97-H-0041) or any other 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 60-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 (or intravenous methylprednisolone as clinically indicated) 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).

Cyclosporine dosing will be adjusted, at the investigator's discretion, to aim for a therapeutic trough level between 200 and 400 mcg/L. 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 or adjusted as clinically indicated at the discretion of the investigator for side effects. Interruptions will only be recorded in the medical record.

In between NIH landmark visits, only CsA levels will be tracked and recorded into the clinical database.

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.

Medication dosing errors, dose delays or dosing interruptions:
Interruptions such as delays in request for medication refills or medication errors by subjects will be recorded in the medical record.

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 25 mg Pfos sachet contains eltrombopag olamine equivalent to 25 mg of eltrombopag free acid. If a child's dose is based on body weight and needs a dose of 28 mg, then dose only single sachet that provides 25 mg dose. However, if the child needs a dose of 29 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 25 mg where a patient needs 28 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

Age groups	Daily dose
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 be recorded in the medical record.

Cohort 2, 3, Extension Cohort and Exploratory Laboratory 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.

Medication dosing errors, dose delays or dosing interruptions:
Interruptions such as delays in request for medication refills or medication errors by subjects will be recorded in the medical record.

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 Extension Cohort and Exploratory Laboratory 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

5.6.1 Thrombosis/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 (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.

Laboratory Exploratory Cohort:
Follow up for subjects in this cohort will be the same as Cohort 3 and Extension Cohort (above).

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 Medications:

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 anion transporter polypeptide, OATP1B1, but is an inhibitor of this transporter in vitro and as evidenced by increased plasma rosuvastatin levels when eltrombopag and rosuvastatin were co-administered in a clinical drug interaction study. When co-administered with eltrombopag, a reduced dose of rosuvastatin should be considered and careful monitoring should be undertaken. In clinical trials with eltrombopag, a dose reduction of rosuvastatin by 50% was recommended for co-administration of rosuvastatin and eltrombopag. Concomitant administration of eltrombopag and other OATP1B1 substrates should be undertaken with caution.

Inhibitors of cytochrome p450: In vitro studies demonstrate that CYP1A2 and CYP2C8 are involved in the oxidative metabolism of eltrombopag. Trimethoprim, gemfibrozil, ciprofloxacin, fluvoxamine and other moderate or strong inhibitors of CYPs may therefore theoretically result enhanced activity of eltrombopag, however these interactions have not yet been established in clinical studies. 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 total for 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), or adhere to a restricted diet of low calcium (dairy products) and polyvalent cations at least 4 hours apart from co-administration of eltrombopag.

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 (Surveys will not be required for the Extension and Exploratory Laboratory Cohort)
- 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 the following, unless the hospitalization is interrupted due to the participant leaving the hospital "on pass" but not to exceed more than 7 days:

- 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. 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. Standard of care tests will be done as needed and may include the tests listed below. Changes in frequency of the tests below will be performed as clinically indicated at the investigators discretion.

- Complete blood counts with differential every 1-2 weeks. Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: absolute neutrophil count (ANC), or neutrophil percentage and white blood cell count if an absolute neutrophil count is not provided, hemoglobin, platelets, and absolute reticulocyte count (or reticulocyte percentage and red blood cells [RBC] if an absolute reticulocyte count is not provided).
- 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). Only the following parameters will be recorded in the clinical database because of their relevance to the underlying disease and study treatment: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and direct bilirubin, creatinine, blood urea nitrogen (BUN), glucose, sodium, potassium, CO2 and chloride.
- CsA blood level will be monitored every week for the first month and then every other week 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 unless participants cannot tolerate therapeutic dosing of CsA. More frequent drug serum levels may be obtained as needed to achieve target therapeutic levels and avoid toxicity, and conversely less frequent levels may be obtained as needed in individuals unable to tolerate therapeutic dosing of CsA. The dose may not necessarily be adjusted following every out of range CsA level, rather the decision to adjust the dose will be determined by the clinician judgement based on multiple data sources, that could include but not limited to time of measurement, prior trend of CsA levels, estimation of steady state, concomitant medications that may affect CsA metabolism, and renal function. 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)

(Surveys will not be required for the Extension and Exploratory Laboratory Cohort.)

Landmark 3-month and 6-month monitoring

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

- History and physical examination
- Complete blood counts with differential
- Acute care, 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, cohort 1; also, samples may be drawn any day between day 8 through day 30 on the extension cohort starting with subject #120). Pharmacokinetic sampling will not be collected for Exploratory Laboratory Cohort.
- HRQL survey administration (Surveys will not be required for the Extension and Exploratory Laboratory Cohort.)
- 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 (+/- 90 days for Years 1 & 2; +/- 1 Year for Years 3 & 4). 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. If a subject is unable to present to CC for landmark Year 3 or 4 follow up due to circumstances beyond their control and their peripheral blood counts are available and stable compared to last visit at CC, the subject may remain on the study and follow up as soon as able but no longer than 1 year from the missed visit.

- 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 (Surveys will not be required for the Extension and Exploratory Laboratory Cohorts.)

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 as clinically indicated while on extended access for relapse, but no less than annually until 5 years. 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. The date of relapse will be recorded into the clinical database as the date the participant resumed immunosuppression with CsA or required higher doses of CsA if on the low dose prior to the 2-year landmark visit. A description of how the clinical assessment was made by the PI will be recorded in the clinical database. To corroborate the relapse, the most recent blood counts collected prior to the date of relapse will be recorded into the clinical database with the following parameters: absolute neutrophil count, or neutrophil percentage and white blood cell count if ANC is not available, hemoglobin, absolute reticulocyte count (or reticulocyte percentage and RBC if absolute reticulocyte count is not available) and platelets.

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. Up to an additional 10 cc of blood (3 ml/kg not to exceed 10 ml of blood for pediatric subjects) may be collected during follow up every 1-6 months (according to how often they get their counts checked) for correlative laboratory studies. The protocol will cover shipment materials/costs for these additional samples to be returned to the NIH since they are for research use (no results given). 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
- Evaluation for the presence of abnormalities of genes associated with inherited bone marrow failure
- 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 and extension cohort starting with subject # 120 (Appendix D). Pharmacokinetic sampling will not be collected for Exploratory Laboratory Cohort.
- Single cell RNAseq on bone marrow specimens to detect chromosomal aneuploidy before and after treatment
- Telomere length on peripheral blood leukocytes by flow-FISH before and after treatment
- 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 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. Coded/linked biospecimens to be shared outside of NIH for future research use requires an executed Material Transfer Agreement (MTA) and may require IRB approval if results will be returned and re-identified. Identifiable samples will not be sent outside of NIH without IRB approval and an 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. BIOSTATISTICAL 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≤10%, versus the alternative, H1: p ≥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≤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≤10%, versus the alternative, H1: p ≥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≤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

Starting with Amendment V, an extension cohort was added of 55 subjects in order to have data to improve precision of exploratory analysis of secondary endpoints, and to obtain more pharmacokinetic data on the

combined regimen. Then with Amendment FF, the extension cohort ceiling is raised to 87 subjects with the aim of having an additional 32 subjects to provide fresh research samples for 1) detection of cytogenetic abnormalities by single cell RNAseq methods in our laboratory, 2) measurement of telomere length before and after treatment by flow-FISH, and 3) additional pharmacokinetic data. The entire extension cohort (n=87) will be included in the analysis of secondary endpoints, but not in the primary endpoint analysis of cohort 3. We plan to use descriptive statistics, not hypothesis testing, in the analysis of the extension cohort.

In this revision, we have estimated our sample size (n=32) on the need for fresh samples for ancillary studies. Specifically, using our newly developed method to detect clonal evolution using RNAseq (will provide manuscript), we predict that we will require at least 3 patient samples with clonal evolution. We estimate that between 10% to 40% of patients will clonally evolve (Townsley et al. NEJM 2017). Assume that the expected proportion of patients with clonal evolution is 21%. Based on the exact calculation using Binomial distributions, the requested sample size of (n=32) will lead to a probability of 97.6% to obtain 3 or more samples. The true proportions of clonal evolution and their precision will be estimated using sample proportions, standard errors and confidence intervals.

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

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.

Cohorts 1, 2 & 3

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
≤ 37	11
≤ 41	12
≤ 45	13
≤ 49	14
≤ 54	15

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.

Extension Cohort

Following Amendment FF, we updated the TRSAE stopping rule for this expanded cohort of N=87 patients (55 patients in the original cohort, 32 additional patients) the TRSAE stopping rule is updated using the

same Bayesian approach (Geller et al., 2004) with the same assumptions. Specifically, the assumptions are:

a) The rate of developing one or more of the specified TRSAEs is 20% or less.
b) The stopping boundary is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAEs exceeds this benchmark rate of 20% is at least 90%.
c) The prior distribution is 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.
d) The safety monitoring starts when 3 or more subjects have developed the 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
≤ 20	7
≤ 24	8
≤ 29	9
≤ 33	10
≤ 37	11
≤ 41	12
≤ 45	13
≤ 49	14
≤ 54	15
≤ 58	16
≤ 62	17
≤ 66	18
≤ 71	19
≤ 75	20
≤ 80	21
≤ 84	22
≤ 87	23

We performed a simulation study to verify the appropriateness of this stopping rule. In each simulation run, we generated a study with 87 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 several different scenarios for the true value of p:

Probability of "Monitored" TRSAE= p	5%	10%	15%	20%	25%	40%

Proportion of stopped studies	0.27%	2.38%	10.35%	32.83%	66.73%	99.90%
Average number of Tx naive SAA subjects	86.78	86.13	79.57	66.31	47.15	12.58
Average number of Tx naive SAA with a specified TRSAE	4.34	8.50	11.93	13.27	11.79	5.04

These results suggest that the above stopping rule has 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 rule has satisfactory statistical properties.

9.6 Off Study/Off Treatment 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
- 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 (if beyond 6 months then mycophenolate mofetil can also be administered per PI discretion in patients who cannot tolerate cyclosporine and who require immunosuppression) or if beyond 2 years, sirolimus per protocol 17-H-0019
- 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.

There are risks to pregnancy for patients with aplastic anemia or a history of aplastic anemia, and it is generally advised against, regardless of participation in a research trial. For this protocol, if pregnancy occurs when a patient is not actively receiving medical drugs, it will not be cause for withdrawal from study.

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.

Death: Death of Subject

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. Identifiable data will not be sent outside of the NIH without IRB approval and an executed agreement.

All human subjects personally identifiable information (PII) will be recorded according to the NIH policy. 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.

10. DATA AND SAFETY MONITORING

10.1 Safety Monitoring

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

NIH Intramural IRB: 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, in follow up of subjects or in data analysis.

***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:

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
Clinical Research Manager
Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080
USA
Phone +1 862 778 3640
Mobile +1 201 452 8479
Fax +1 973 781 2116
kelly.haines@novartis.com

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, PDs, 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.

10.2.1 Assessment of Safety

Definitions

Please refer to Policy 801 "Reporting Research Events" for current definitions.

10.2.2 Protocol Deviation (PD) Characterization

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 be captured in the database. If the number of events per subject or per cumulative enrolled subjects exceeds 50%, which is a frequency greater than what is expected, this will be reported at time of continuing review. 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.

Interruptions in eltrombopag dosing that are clinically indicated per section 5.3, will be recorded in the medical record.

In addition, CsA interruptions, such as delay in request for medication refills or medication errors by subjects, will be recorded in the medical record.

10.2.3 Adverse Events (AEs) Characterization

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.

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 collection: The collection and recording of events in the database 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 or recorded in the database or toxicity tables.

AEs that are captured in the database will be followed until satisfactory resolution as long as the subject remains on study.

Exclusions to AE data capturing:

- 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 captured in the research database. 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 the research database.

- In addition, the following non-hematologic AEs will be captured only in the source documents and will not be recorded in the database:
    - 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 captured in the research database unless (1) the adverse event is more severe or occurs at a higher frequency than on the package insert; or (2) 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 during the first week of h-ATG treatment.
- Cohort 3 the Extension Cohort, and Laboratory Exploratory Cohort: Because grade 3 and 4 adverse events 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 only be recorded in the database 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 per Policy 801.
- Cohort 3, the Extension Cohort, and Laboratory Exploratory Cohort: Grade 3 and 4 LFTs will be recorded in the medical record, and only recorded in the database as adverse events from the start of h-ATG to 14 days post treatment.
- All grade 1 events listed as expected in the protocol, consent forms, or other applicable protocol documentation.

10.2.4 Serious Adverse Events Characterization

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 be captured in the database.

Duration of Serious Adverse Event collecting and reporting: The collection or recording of SAEs in the database 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 recording for events that are unexpected and/or definitely, probably or possibly related to the study drug will continue as long as the subject remains on study.

10.2.5 Event Reporting

All events will be reported to Neal S. Young, M.D., Principal Investigator of this study:

Neal S. Young, M.D.
Bldg. 10, Room CRC 3E-5142
Phone: (301) 496-5093
E-mail: youngns@nhlbi.nih.gov

10.2.5.1 NIH Intramural IRB and NHLBI Clinical Director (CD) Reporting

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per HRPP Policy 801 "Reporting Research Events".

Reports to the IRB at the time of continuing review:

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements.
In addition, the following commonly occurring disease specific Serious Adverse Events (SAEs) will be captured in the database but are not required to be reported to the CD per NHLBI guidelines:

- Neutropenic fever
- Minimal bleeding (such as nose bleed or menorrhagia)
- Transfusion reactions to red blood cells and/or platelets

10.2.5.2 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.

10.2.5.3 Sponsor and FDA Reporting

IND: 104877

IND Sponsor Representative: 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:

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

10.2.5.4 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 Novartis within 24 hours of the research team learning of the event. A copy of the SAE report will be forwarded as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events. If the SAE is unexpected and determined possibly, probably or definitely related to the study drug the SAE report (in the appropriate format, e.g., NIH Reportable Events 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.'

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, the 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 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.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population.

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.

There are risks to pregnancy for patients with aplastic anemia or a history of aplastic anemia, and it is generally advised against, regardless of participation in a research trial. For this protocol, if pregnancy occurs when a patient is not actively receiving medical drugs, it will not be cause for withdrawal from study. In the event of relapse during pregnancy, patient will not be retreated on the protocol

but recommendations will be provided to the local treating physician.

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.

Post marketing 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 than .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)

Potential Serious Adverse Effects:

WARNING: RISK FOR HEPATIC DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C RISK OF HEPATOTOXICITY See full prescribing information for complete boxed warning

In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation.

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

Warnings and Precautions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Adverse Reaction	**PROMACTA (n = 43) (%)**
Nausea	33
Fatigue	28
Cough	23
Diarrhea	21
Headache	21
Pain in extremity	19
Dyspnea	14

Pyrexia	14
Dizziness	14
Oropharyngeal pain	14
Febrile neutropenia	14
Abdominal pain	12
Ecchymosis	12
Muscle spasms	12
Transaminases increased	12
Arthralgia	12
Rhinorrhea	12

Rash was reported in 7% of patients.

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

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category C

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

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

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

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

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

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

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

The safety and efficacy of PROMACTA in pediatric patients with thrombocytopenia associated with chronic hepatitis C and severe aplastic anemia have not been established.

Adverse reactions:

Severe cutaneous reaction
There is a risk that subject may develop a severe cutaneous reaction that may require hospitalization and discontinuation of eltrombopag. As of December 2015, there have been three occurrences of this event in subjects enrolled on this protocol.

Investigator Brochure, version 13, dated 4/13/2016 - "Adverse Events considered to be Expected for Reporting Purposes"
Below are lists of "Adverse Events considered to be Expected for Reporting Purposes" for each chronic ITP and SAA. This list is based upon evaluation of the available clinical safety information, including data from all global clinical trials (phase I-III) and the Novartis safety database, Argus (cut-off date of 29 February 2016).

Adverse reactions are listed below for each indication by MedDRA body system organ class and by frequency. Frequency category for each adverse drug reaction is based on the following convention (CIOMS III). The frequency categories used are:

Very common: ≥1 in 10 (≥10%)
Common: ≥1 in 100 and <1 in 10 (≥1% and <10%)
Uncommon: ≥1 in 1,000 and <1 in 100 (≥0.1% and <1%)
Rare: ≥1 in 10,000 and <1 in 1,000 (≥0.01% and <0.1%)

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

Infections and infestations	
Common:	Pharyngitis
	Urinary tract infection
Gastrointestinal disorders	
Very Common:	Nausea
	Diarrhea
Common:	Dry mouth
	Vomiting
Hepatobiliary disorders	
Common:	Increased aspartate aminotransferase
	Increased alanine aminotransferase
	Blood bilirubin unconjugated increased

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

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

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

Adverse Events considered to be expected for Reporting Purposes in SAA

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

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

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

** Leukocytosis and white blood cell count increased occur individually with a frequency of common, however the terms were grouped as they represent the same medical concept, giving a revised frequency of very common.
*** Serum discoloration has been reported in investigator sponsored studies in MDS/AML, and can lead to analytical interference with some colorimetric analytical methods

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.

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.

11.6 Informed Consent Processes and Procedures

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

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient during the initial clinic evaluation. The Principal Investigator, Dr. Young, or the Associate Investigators on this protocol delegated to obtain informed consents 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.

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

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

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

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

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

(1) The research involves no more than minimal risk to the subjects;
   a) Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;

a) Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.

(3) The research could not practicably be carried out without the waiver or alteration; and
   a) Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
   a) We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up.

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.

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 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.

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) (Category B per SOP 14E). See Appendix F for details.

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.

11.8 FWA Coverage Agreement

Dr. Townsley is currently working at Medimmune and will be analyzing identifiable data as an Non-NIH, Non-Enrolling Engaged Investigator in this protocol. Dr. Townsley's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity has been executed by Dr. Townsley and Dr. Young.

11.9 Collaboration(s)

Phillip Scheinberg, M.D. will continue to collaborate on trial with protocol related questions and interpretation of study data. Dr. Scheinberg has no access to PII and is no longer participating in direct patient care.

Between NHLBI (Neal S. Young, MD) and Satu Mustjoki, M.D., Ph.D. Professor of Translational Hematology in University of Helsinki (Medicum) and Helsinki University Hospital. Upon execution of the MTA, de-identified (coded) peripheral blood and plasma samples of Severe Aplastic Anemia patients will be sent for sequencing of DNA using Next Generation Sequencing (NGS), analyze results, seeking somatic mutations in lymphoid cells, T-cell receptor (TCR) usage, and autoantibody identification by imputation. Collaborator will return the results of the analysis to NHLBI for further analysis and correlation of results with NHLBI's clinical data, especially clinical outcome. Both parties intend to jointly design experiments and analyze the results and data, and publish the results in co-authored articles.

Between NHLBI (Neal S. Young, M.D.) and Shahram Kordasti, M.D., Ph.D. at Kings College London. Upon execution of the MTA, de-identified (coded) bone marrow samples of Severe Aplastic Anemia patients will be sent to investigate freshly stained bone marrow cells, HSPCs, and supporting cells (stromal cells, macrophages, myeloid-derived suppressor cells, and their inflammation cytokine production). Additionally, the samples collected after the treatment will be analyzed similarly to explore mechanisms by which eltrombopag stimulates hematopoiesis and modulates the immune system. Collaborator will return the results of the analysis to NHLBI for further analysis and correlation of results with NHLBI's clinical data.

12. PHARMACEUTICALS

12.1 ELTROMBOPAG (PROMACTA®)

Supply: The drug Novartis is providing for this study may be either investigational or commercial material, based on their supply, and is available in tablets and as a powder for oral suspension. Each sachet contains eltrombopag olamine equivalent to 25 mg of eltrombopag. The tablets are available as 12.5, 25, 50, and 75 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 11.4.3 Risks related to eltrombopag.

Tablets:

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

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

Eltrombopag Powder for Oral Suspension:

Eltrombopag powder for oral suspension (Eltrombopag PfOS) is a reddish-brown to yellow powder contained inside an elongated sachet. A 25 mg PfOS strength is available, containing eltrombopag olamine equivalent to 25 mg of eltrombopag free acid. The powder blend composition contains eltrombopag olamine equivalent to 20 mg of eltrombopag free acid. The powder fill weight is 1.25 gram for PfOS 25 mg.

PfOS sachets will be packaged in a carton. PfOS may be supplied as bulk stickpacks (sachets) or as commercial kit. If bulk stickpacks are supplied, the clinical site will also receive reconstitution bottle, syringe-adapta cap, and 20mL oral dosing syringes. If commercial kit is provided, each carton pack will hold 30 sachets along with a plastic reconstitution container, a syringe-adapt cap and 30 single-use 20mL oral dosing syringes. The sachet should not be opened until ready to use. Add 9.5 mL of water drawn using a 20mL 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 cap are rinsed with water and dried. If the prescribed dose is > 29 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 20cc syringe, and similarly dosing has to occur by using the same 20cc syringe. 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 20cc syringe for each sachet used, and similarly dosing has to occur by using the same 20cc 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 Investigational Drug Management and Research Section (IMDRS) will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is:

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

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 11.4.2 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° to 8° 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 11.4.1 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 11.4.9 Risks 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 11.4.9 risks related to concomitant medications

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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)*
   - ☐ Disability or Permanent Damage
   - ☐ Life-threatening
   - ☐ Congenital Anomaly/Birth Defect
   - ☐ Hospitalization - initial or prolonged
   - ☐ Other Serious (Important Medical Events)
   - ☐ Required Intervention to Prevent Permanent Impairment/Damage (Devices)

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
2. Phone #
3. Health Professional? ☐ Yes ☐ No
4. Occupation
5. 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) – No longer active as of July 1, 2018**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA, protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	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 serum chemokines 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

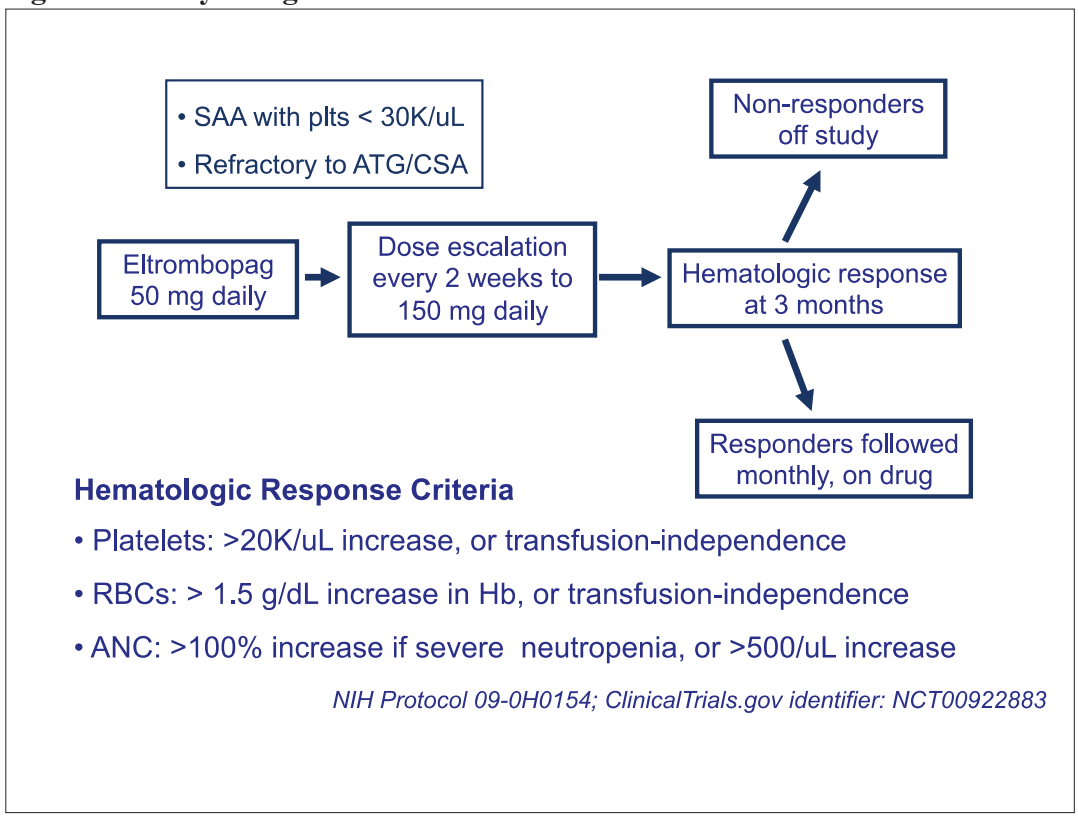


Figure 2: Study results as defined by response criteria at 12 weeks. Achievement of further lineage responses during extension phase at greater than 12 weeks are also indicated.

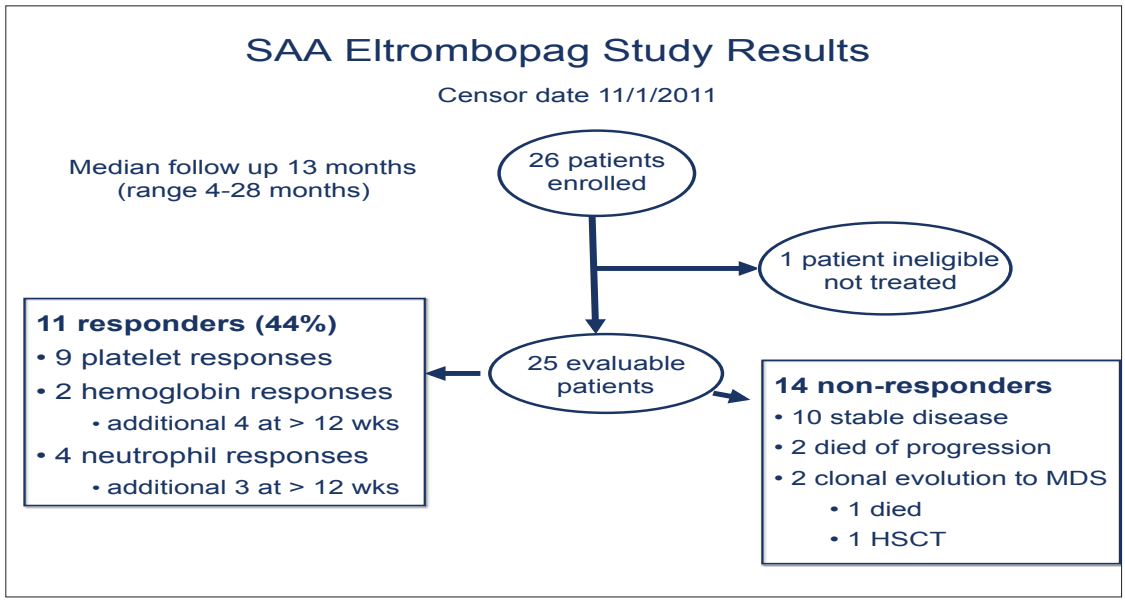
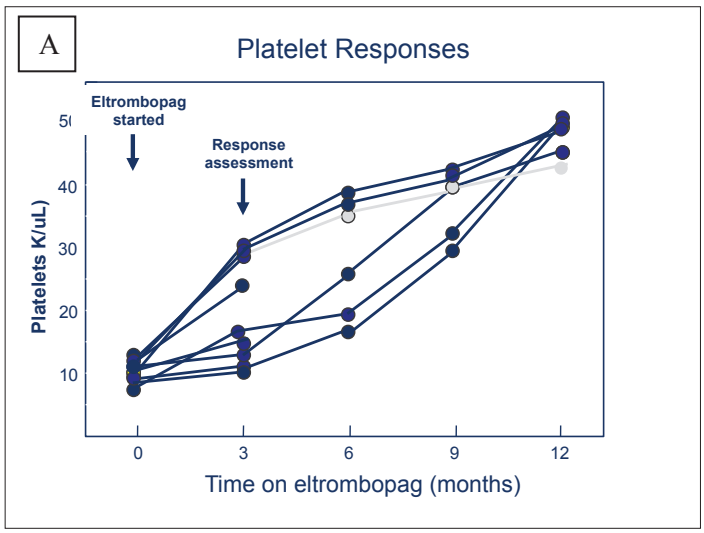
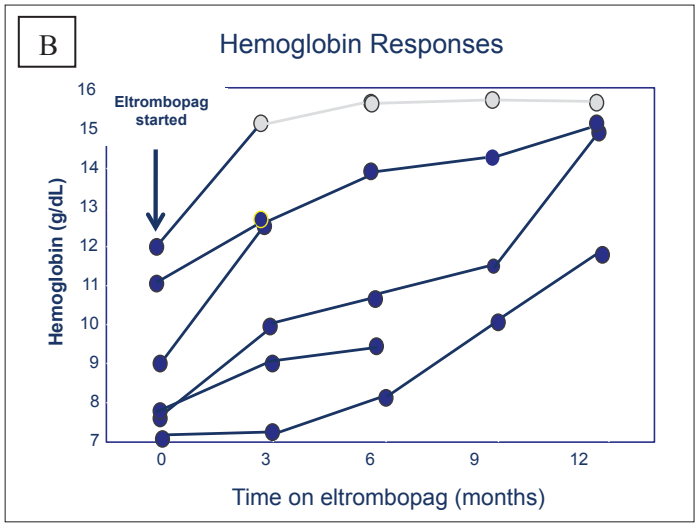


Figure 3: Responses to eltrombopag in responders over time. For each lineage, individual patients reaching response criteria are shown (A. Platelets, B. Hemoglobin, C. Neutrophils). Black lines indicate patients remaining on drug.
Gray lines indicate the patient taken off drug at three months due to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





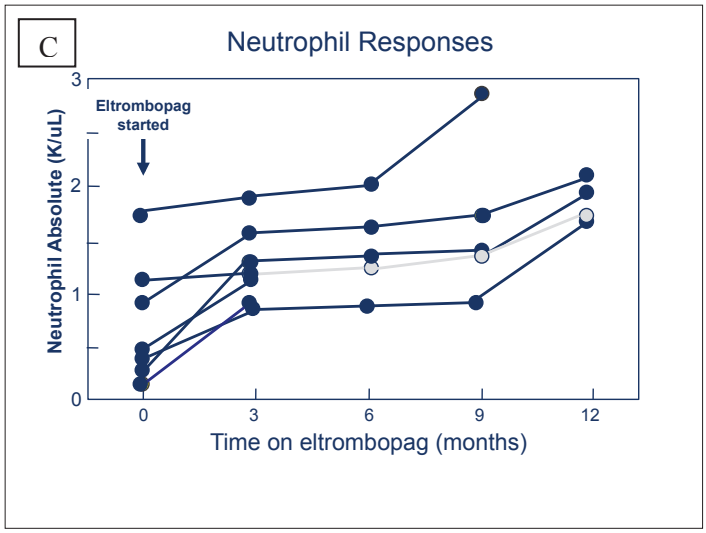
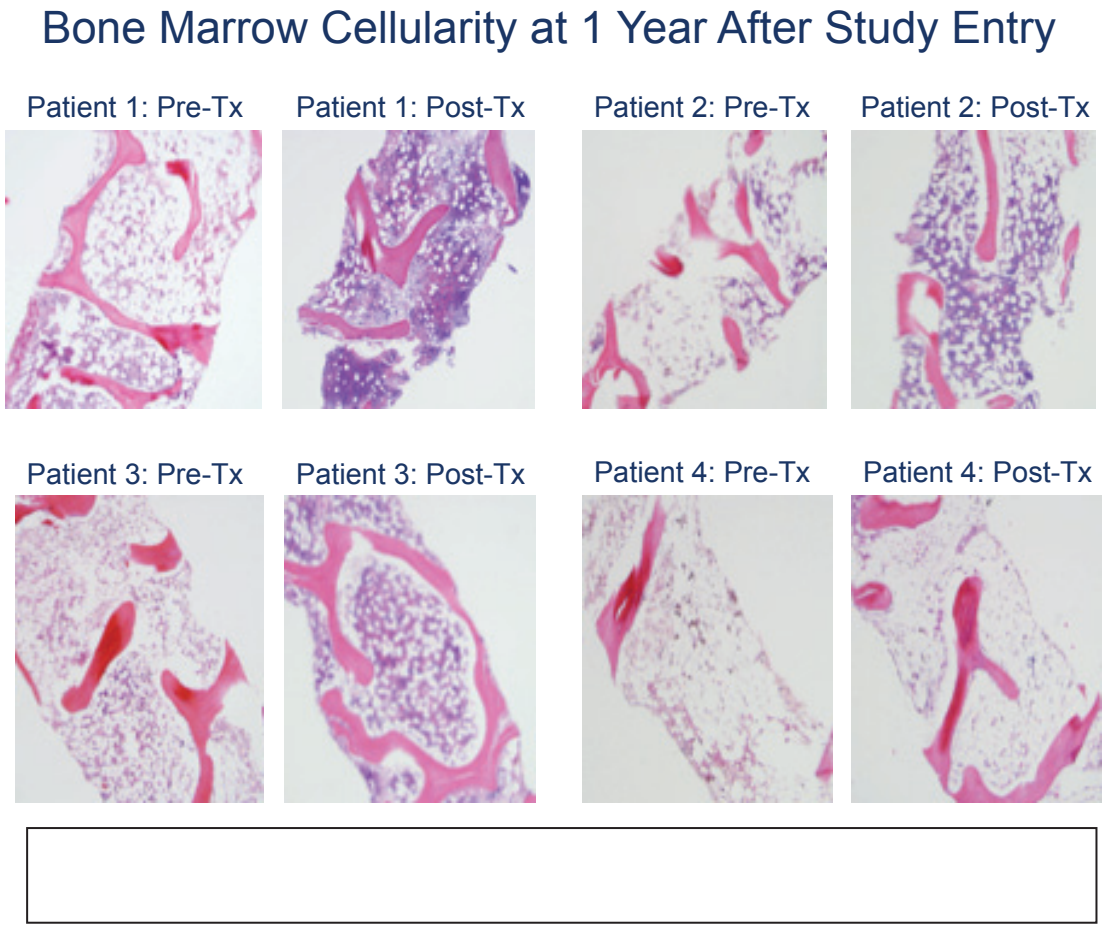


Figure 4: Bone marrow biopsies stained with hemotoxylin and eosin and shown at 100X magnification pre-treatment and 12 months following study entry.



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. Subjects enrolled into the extension cohort, starting with subject # 120, may have their PK assessment between day 8 and day 30. An additional trough collection (within 30 min prior to eltrombopag dosing) may also be obtained in subjects on the extension cohort at the 3 or 6 month visit. Subjects must have received once daily eltrombopag for at least 7 days prior to PK assessment (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 assessment (because of a dose interruption) or eltrombopag has been reinitiated after a dose interruption within the 7 days prior to this assessment, PK assessments will be deferred until the landmark 6-month study visit for Cohort 1. 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:

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

APPENDIX E: PROMIS QUESTIONNAIRE

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 <u>past 7 days</u>.**

	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 <u>past 7 days</u>.

	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 <u>past 7 days</u>.

	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 <u>starting</u> things because I am tired	0	1	2	3	4
An4	I have trouble <u>finishing</u> 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 <u>past 7 days</u>.

	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
EDDEP041	I felt worthless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP062	I felt helpless	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP293	I felt depressed	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EDDEP414	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
EDANX53 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

APPENDIX F: SOP 14E, APPENDIX B, TABLE 1

REQUIREMENTS FOR THE DETERMINATION OF AN LAR'S APPROPRIATENESS TO CONSENT TO RESEARCH NOT INVOLVING GREATER THAN MINIMAL RISK (CATEGORY A) AND FOR RESEARCH INVOLVING GREATER THAN MINIMAL RISK BUT PRESENTING THE PROSPECT OF DIRECT BENEFIT TO THE INDIVIDUAL SUBJECTS (CATEGORY B)
First preference is #1. If not possible, go to option #2. If #2 is not possible, go to option #3.)

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

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

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

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

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