CLINICAL RESEARCH PROJECT

Protocol #09-H-0154
Drug Name: eltrombopag (Promacta®)
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To: Richard Canon, M.D.; Chairman, NHLBI IRB

Title: A Pilot Study of a Thrombopoietin-receptor Agonist (TPO-R agonist), Eltrombopag, in Aplastic Anemia Patients with Immunosuppressive-therapy Refractory Thrombocytopenia

Other Identifying Words: Hematopoiesis, autoimmunity, thrombocytopenia, neutropenia, stem cells, cytokine, Promacta® (eltrombopag)

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Estimated duration of the study: indefinite
Subjects of Study: Number : 50 Sex: Either Age-range > 12 years Project
Involves Ionizing Radiation? No (only when medically indicated)
Off-Site Project? No

Multi center trial?	No
DSMB Involvement?	Yes

PRECIS

Severe aplastic anemia (SAA) is a life-threatening blood disease which can be effectively treated with immunosuppressive drug regimens or allogeneic stem cell transplantation. However, 20-40% of patients without transplant options do not respond to immunosuppressive therapies, and have persistent severe thrombocytopenia. Even patients that respond to immunosuppressive therapies with an improvement in their life-threatening neutropenia sometimes have persistent thrombocytopenia. Both groups of patients (i.e. non-responders to immunosuppressive therapy and responders with persistent thrombocytopenia) require regular platelet transfusions, which are expensive and inconvenient, and are a risk for further serious bleeding complications.

Thrombopoietin (TPO) is the principal endogenous regulator of platelet production. On binding to the megakaryocyte progenitor TPO receptor, TPO initiates a number of signal transduction events to increase the production of mature megakaryocytes and platelets. Thrombopoietin also has stimulatory effects on more primitive multilineage progenitors and stem cells in vitro and in animal models. A 2nd generation small molecule TPO-agonist, eltrombopag (Promacta®) has been shown to increase platelets in healthy subjects and in thrombocytopenic patients with chronic immune thrombocytopenic purpura (ITP) and hepatitis C virus (HCV)-infection. Eltrombopag is administered orally and has been well-tolerated in clinical trials. Unlike recombinant TPO, it has not been found to induce autoantibodies. Eltrombopag received FDA accelerated approval on Nov 20, 2008 for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy.

Because a paucity of megakaryocytes and decreased platelet production is responsible for thrombocytopenia in aplastic anemia patients, we now propose this Phase 2, non-randomized pilot study of eltrombopag in aplastic anemia patients with immunosuppressive therapy refractory thrombocytopenia.

Subjects will initiate study medication at an oral dose of 50 mg/day (25 mg/day for East Asians), which will be increased or decreased as clinically indicated to the lowest dose that maintains a stable platelet count $\geq$ 20,000/μL above baseline while maximizing tolerability. Platelet treatment response is defined as platelet count increases to 20,000/μL above baseline at three months, or stable platelet counts with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a reduction in the units of transfusions by an absolute number of at least 4 PRBC transfusions for eight consecutive weeks weeks compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L. Subjects with a platelet, erythroid, and/or neutrophil response at 12 weeks may continue study medication (extended access) until they meet an off study criteria. Subjects with platelet, erythroid, or neutrophil response at 12 weeks may continue study medication for an additional 4 weeks (to ensure eligibility) prior to being consented for entry into the extended access part of the trial. Patients may remain on the extended access until they met an off study criteria.

The ***primary objective*** is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in aplastic anemia patients with immunosuppressive-therapy refractory thrombocytopenia.

Secondary objectives include the analysis of the incidence and severity of bleeding episodes, and the impact on quality of life.

The ***primary endpoint*** will be the portion of drug responders as defined by changes in the platelet count and/or platelet transfusion requirements, hemoglobin levels, number of red blood cell transfusions, or neutrophil counts

as measured by International Working Group criteria and the toxicity profile as measured using the CTCAE criteria. Platelet treatment response is defined as platelet count increases to 20,000/μL above baseline at three months, or stable platelet counts with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL or a reduction in the units of PRBC transfusions by an absolute number of at least 4 PRBC transfusions for eight consecutive weeks - compared with the pretreatment transfusion number in the previous 8 weeks . Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase in ANC, or an ANC increase $> 0.5 \times 10^9$/L.

Secondary **endpoints** will include incidence of bleeding; changes in serum thrombopoietin level (as measured by enzyme-linked immunosorbent assay, R&D Systems), and health related quality of life (as measured by the Medical Outcomes Study 36-Item Short Form General Health Survey, version 2 [SF36v2]; Quality-Metric) measured at 12 weeks.

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Investigator's Brochure for SB-497115-GR (eltrombopag olamine) April 15, 2012
General Quality of Life - SF-36 questionnaire

1. OBJECTIVES

The ***primary objective*** is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in aplastic anemia patients with immunosuppressive-therapy refractory thrombocytopenia.

Secondary objectives include the analysis of the incidence and severity of bleeding episodes, and the impact on quality of life.

2. BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 Pathophysiology of thrombocytopenia in aplastic anemia patients

Thrombocytopenia is a major cause of morbidity and mortality in patients with aplastic anemia. At presentation, virtually all patients with aplastic anemia are thrombocytopenic: platelet counts of less than 50,000/μL or 20,000/ μL are diagnostic criteria for moderate and severe aplastic anemia, respectively. Thrombocytopenia in aplastic anemia patients is caused by decreased hematopoietic stem and progenitor cell numbers and function, resulting in decreased megakaryocytes that can produce mature platelets. The clinical efficacy of immunosuppression and a myriad of laboratory data suggest that the ultimate mechanism leading to hematopoietic stem and progenitor destruction is autoimmune attack [1;2] The regulation of endogenous TPO production is not yet fully understood. The major site of production is the liver, and the primary determinant of circulating TPO levels appears to be platelet and megakaryocyte mass, with low numbers resulting in higher than baseline TPO levels (for review see [3]). In aplastic anemia, TPO levels are significantly increased, in contrast to TPO levels in ITP, which are generally within the normal range or only moderately increased ([4]; [5]).

2.2 Clinical consequences of thrombocytopenia: The major symptom of thrombocytopenia in aplastic anemia patients is bleeding: petechiae of the skin and mucous membranes, epistaxis and gum bleeding. Bleeding can be brisk in the presence of accompanying physical lesions related to the underlying aplastic anemia or treatment with immunosuppression, such as corticosteroid-related gastritis, or neutropenia-related fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage which is life threatening if not promptly treated. In long-term follow-up studies in the modern era, up to 10% of patients presenting with aplastic anemia still eventually die of bleeding, and in patients that do not respond to immunosuppression, more than half of subsequent deaths are due to bleeding.([6])

2.3 Treatment of thrombocytopenia in aplastic anemia patients

The only current treatment available for aplastic anemia patients who have refractory thrombocytopenia despite immunosuppression, is platelet transfusions. Patients with a platelet count less than 10,000/ μL are routinely transfused to avoid significant bleeding. Due to the short half-life of platelets in the circulation, many aplastic anemia patients with severe thrombocytopenia require transfusions as frequently as two or three times per week. Platelet transfusions are associated with a number of side effects including febrile or allergic transfusion reactions, transmission of bacterial and viral infections, circulatory congestion, transfusion-related acute lung injury and allo-immunization. The possible increased demands on the blood supply in the future may further limit the feasibility of chronic platelets transfusions as therapy for aplastic anemia.

In patients responding to immunosuppression, platelet counts improve weeks to months after treatment,

allowing discontinuation of platelet transfusions. However, 30-40% of aplastic anemia patients do not respond robustly to immunosuppressive treatment; and some patients with good improvement in red cell and neutrophil counts have persistent severe thrombocytopenia necessitating regular platelet transfusions. The reason for lack of platelet response despite robust improvements in other lineages is unclear, but these patients generally lack marrow megakaryocytes, suggesting a persistent problem with platelet production. The management of aplastic anemia patients with persistent thrombocytopenia remains unsatisfactory and novel therapy approaches are needed.

Despite the fact that TPO levels are generally already increased in aplastic anemia, we believe there is sufficient justification for a clinical trial testing the hypothesis that supraphysiologic pharmacologic levels of a TPO-R agonist could result in improved platelet production in patients with aplastic anemia. Despite elevated erythropoietin levels, some patients with persistent anemia following immunosuppressive therapy for aplastic anemia respond to combination therapy with erythropoietin and G-CSF ([7]). In ITP, despite ongoing platelet production and normal TPO levels, pharmacologic dosing of TPO-R agonists can result in overcoming the impact of autoimmune platelet destruction. It is reasonable to ask whether TPO-R agonists could similarly overcome autoimmune destruction or loss of more primitive hematopoietic stem and progenitor cells in aplastic anemia.

2.4 The Investigational Agent Eltrombopag (Promacta), a 2nd Generation Thrombopoietin Receptor (TPO-R) Agonist (excerpted from the Investigator Brochure, GlaxoSmithKline (GSK), April 2011. Version 8 of the Investigator' brochure was distributed April 2011.

2.4.1 Description of the drug

Eltrombopag (SB-497115-GR), the bis-monoethanolamine salt form, is an orally bioavailable, small molecule thrombopoietin receptor agonist developed for the treatment of thrombocytopenia. Promacta® received FDA approval for the treatment of adults with chronic ITP in November 2008.

2.4.2 Nonclinical pharmacology

Studies conducted in vitro have shown that eltrombopag (SB-497115-GR) is an effective agonist binding to the thrombopoietin receptor (TPO-R) to stimulate thrombopoiesis. In vivo, eltrombopag increases platelet number in the chimpanzee (the only nonclinical species which is pharmacologically responsive to eltrombopag). These findings, coupled with supporting clinical efficacy data, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous thrombopoietin (TPO).

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

Comprehensive nonclinical pharmacokinetic, distribution, metabolism and excretion studies were conducted in the mouse, rat and dog with eltrombopag. Absorption of eltrombopag was low to moderate and plasma clearance was generally low with moderate to long half-lives. Eltrombopag-related material was widely distributed into peripheral tissues in the mouse and rat but the concentrations in most tissues were lower than in the blood. Drug-related material did not extensively penetrate into the central nervous system or the lens of the eye, nor was it selectively retained in melanin containing tissues. There was no evidence of tissue accumulation of drug-related material in mice, including eyes, kidneys and skin. Eltrombopag was highly bound to plasma proteins in nonclinical species as well as in human plasma (>99%), with low association with blood cells. Eltrombopag was the predominant circulating component in all species. Minor metabolites in circulation included products of oxidation or glucuronidation. Eltrombopag was primarily eliminated as unchanged drug in the feces with renal elimination of cleavage products contributing a minor route. Qualitatively, all of the major metabolites of eltrombopag observed in humans were observed in the nonclinical species. In vitro, eltrombopag inhibited cytochrome P450 (CYP) enzymes CYP2C8 and

CYP2C9 and several uridine diphosphate glucuronosyl transferase (UGT) enzymes at potentially clinically relevant concentrations. Eltrombopag was neither an inhibitor nor a substrate of human P-glycoprotein (Pgp) and was not a substrate of human organic anion transporting polypeptide (OATP1B1), although it was an inhibitor of this transporter with the potential for such an interaction confirmed clinically.

2.4.4 Nonclinical toxicology

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

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

Cataracts (mice and rats): In vitro phototoxicity (3T3 and CHO cells) was observed. In mice and rats, the development of cataracts was dose- and time-dependent and the rapidly developing lenses of young mice were shown to be more susceptible. Exposure at the no observed effect level (NOEL) was 1.4- and 0.6-fold clinical exposure in patients with idiopathic thrombocytopenia purpura (ITP) and hepatitis C (HCV)-related thrombocytopenia, respectively. Cataract development was not associated with drug accumulation in ocular tissues. No treatment-related ocular abnormalities were evident in dogs following oral administration for 52 weeks at 2.9- and 1.3-fold clinical exposure in ITP and HCV patients, respectively. There was no evidence of acute photo-ocular toxicity in albino or pigmented rats. An apparent delay in onset and a lower incidence of cataracts in albino mice housed in subdued versus ambient light was observed suggesting that light may contribute to cataract development in young mice. However, there was no evidence of ocular phototoxicity in young albino or pigmented mice given eltrombopag and exposed to repeated doses of solar-simulated ultraviolet radiation (UVR). B6C3F1 mice (a pigmented strain) given eltrombopag with or without UVR exposure appeared to be more susceptible than albino mice to eltrombopag-induced cataractogenesis. However, given that eltrombopag has not been shown to be selectively retained in melanin-containing tissues, this likely represents a strain difference in sensitivity to cataract induction.

Renal toxicity (mice and rats). In mice, renal proximal tubular toxicity was observed following repeated oral administration of eltrombopag in a 2 year carcinogenicity study at 1.4- and 0.6-fold clinical exposure in ITP and HCV patients, respectively. Renal toxicity was not observed in mice in a 13 week study at a greater exposure (4.5- and 2.0-fold clinical exposure in ITP and HCV patients, respectively) than that achieved at the lowest dose in the 2 year study, suggesting that the renal effects are time-dependent. In rats, an increase in the incidence or severity of spontaneous, age-related chronic progressive nephropathy was observed at a similar exposure level, but not at lower exposures. The relationship of this finding to the renal effects observed in mice is unknown. No renal toxicity was observed following repeated oral administration to rats for 28 weeks or to dogs for 52 weeks at exposures up to 4.5- and 2.9-fold clinical exposure in ITP patients and up to 2.0- and 1.3-fold clinical exposure in HCV patients, respectively.

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

fold clinical exposure in ITP patients and up to 2.0- and 1.3-fold clinical exposure in HCV patients, respectively.

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

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

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

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

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

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

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

Elimination: The predominant route of eltrombopag excretion is via feces (59%), and 31% of the dose is found in the urine. Unchanged eltrombopag in feces accounts for approximately 20% of the dose;

unchanged eltrombopag is not detectable in urine. The plasma elimination half-life of eltrombopag is approximately 21 to 32 hours in healthy subjects and 26-35 hours in ITP patients.

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

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

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

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

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

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

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

- No dose-dependent pattern of adverse events (AEs) was observed across the eltrombopag 30 mg, 50 mg, and 75 mg treatment groups.
- No clinically meaningful differences in incidence or severity of the most common (≥5%) AEs were observed between subjects treated with eltrombopag 50 mg compared to placebo.

- Similar incidences of serious adverse events (SAEs) (12% and 11%) and discontinuations due to AEs (7% and 5%) were observed in the placebo and eltrombopag 50 mg treatment groups, respectively.
- Increases in hepatobiliary values (ALT, AST, bilirubin, alk phos) were seen in 16/164 subjects (9.7%) in the eltrombopag group (all doses), compared with 5/67 (7.5%) in the placebo group. These elevations in liver aminotransferase were generally asymptomatic and returned to baseline after discontinuation of therapy.
- One case of thromboembolism was observed (platelet count 108,000/uL) in the eltrombopag 50 mg treatment group in a subject who died from sepsis of pulmonary origin.
- Preclinical findings that indicated potential for phototoxicity, cataracts and renal tubular toxicity did not appear to translate to clinical consequences during short-term use.
- Transient decreases in platelet counts to levels below baseline were observed in both treatment groups after eltrombopag treatment ended. However, the decreases in platelet count were not accompanied by a clinically meaningful increase in bleeding symptoms.

TRA105325 (EXTEND) (chronic ITP Study): An open-label, dose-modification, Phase 3 extension study to evaluate the safety and efficacy of eltrombopag for the treatment of 299 subjects with ITP who were previously enrolled in an eltrombopag trial.

TRA108057 (REPEAT) (chronic ITP study): Data as of 10 December 2008 an ongoing, Phase II, multi-center, open label single group repeat dose study to evaluate the efficacy, safety and tolerability of repeated, short term administration of eltrombopag initially administered as 50 mg tablets once daily in subjects with previously treated chronic ITP (66 subjects with ongoing enrollment). In general, the results from the ongoing REPEAT and EXTEND studies confirmed the safety and efficacy profile noted in the completed TRA100773A and TRA100773B and are summarized below:

- The incidence of SAEs was 0% and 14% in REPEAT and EXTEND, respectively and discontinuations due to AEs were ≤6% across the 2 studies
- 2/66 (3%) subjects in REPEAT and 8/109 (7%) subjects in EXTEND developed elevations of hepatobiliary laboratory values. The majority of events were asymptomatic and resolved following drug discontinuation.
- The proportion of Asians who had hepatobiliary laboratory abnormalities (transaminases >3x ULN, bilirubin ≥1.5x ULN or ALP ≥1.5x ULN) was 15.8%, 16.7%, and 20.8%, as compared to 10.2%, 7.5%, and 4.5% of White-Caucasian subjects, in TRA100773A, TRA100773B, and EXTEND, respectively. High plasma eltrombopag concentrations were noted in 2 subjects who had ALT and AST elevations (>3x ULN).
- Four eltrombopag treated subjects developed thromboembolic events (4 in EXTEND, none in REPEAT). Although risk factors were present in all subjects, a causal relationship with eltrombopag cannot be ruled out.

With the exception of the hepatobiliary findings in Asian subjects, no clinically meaningful differences in the safety profile of eltrombopag were found with regard to age, sex and race.

TRA102537/RAISE (chronic ITP study): RAISE was a blinded Phase III study designed to determine the efficacy and safety of eltrombopag in comparison to placebo when administered once daily for 6 months. Subjects initiated treatment with either 50 mg eltrombopag or matching placebo once daily and followed specific instructions for dosing modifications based upon their individual platelet count response. 196 subjects received at least one dose of study medication.

- Overall, 92% of subjects in the placebo group and 87% in the eltrombopag group experienced at least 1 on-therapy AE, regardless of causality.

- 5 subjects (8%) experienced 8 hepatobiliary AEs in the placebo group and 16 subjects (12%) experienced 43 hepatobiliary AEs in the eltrombopag group.
- No thromboembolic events were reported in the placebo treatment group. On-therapy, 2 subjects in the eltrombopag treatment group experienced thromboembolic events, considered related to study medication by the investigator.

- A similar number and percentage of subjects treated with placebo and eltrombopag had a report of cataract during the study, and all of the subjects with a report of cataract had documented, pre-existing risk factors for cataractogenesis including corticosteroid use. These data provide strong evidence that there is no significant excess risk of cataract formation in patients on eltrombopag.

TPL102357 (Hepatitis C Study): A phase II, double blind, randomized, placebo-controlled, multi-center, dose ranging, parallel group study in 56 subjects with hepatitis C viral infection and platelet counts of 20 to < 70 K/ul were treated with 30 mg, 50 mg 75 mg or placebo once daily for 12-16 weeks.

- No dose-dependent pattern of AEs was observed across the eltrombopag 30 mg, 50 mg, and 75 mg treatment groups.
- 44/74 (59%) of subjects reported AEs during the 4-week pre-antiviral treatment phase (eltrombopag alone, up to 75 mg daily). The occurrence of AEs was similar across all treatment groups. Headache (20%) was the most common AE in all treatment groups.
- 57% of subjects reported an AE during the antiviral treatment phase (eltrombopag, up to 75 mg daily, in combination with pegylated interferon and ribavirin antiviral therapy). The most commonly reported AEs were influenza-like illness (31%), fatigue (25%), depression (12%) and headache (15%); all except headache are known side effects of peginterferon-based therapy. The incidence of these symptoms, during the antiviral phase, was lower in the placebo-treated group, only four of whom initiated antiviral therapy.
- Seven (9%) subjects reported 8 SAEs.
- Two hepatobiliary AEs were reported; both occurred while on-therapy. One subject had biliary tract disorder and 1 subject had hyperbilirubinemia. Both subjects were receiving 50 mg eltrombopag.
- There were no thromboembolic events reported.
- Analysis of the subjects' safety data provided no evidence of renal injury related to eltrombopag treatment.
- Overall, there was no clear association between the onset of any skin- and subcutaneous-related event and study medication.
- There were 4 reports of cataract (from 63 subjects assessed). All 4 subjects had documented risk factors for development of cataracts. These data do not appear to demonstrate an increased risk of cataract development in subjects treated with eltrombopag.

SB-497115/003 (CIT Study): A Phase II randomized, double-blind, four arm, parallel group, placebo-controlled, multi-center study to evaluate the efficacy, safety, and pharmacokinetics of oral eltrombopag in 134 cancer subjects with an advanced solid tumor who were receiving multiple cycles of carboplatin/paclitaxel and evaluate the effects of eltrombopag on chemotherapy induced thrombocytopenia (CIT).

- 156/180 (87%) of subjects reported AEs. The incidence and severity of AEs were similar in all the active eltrombopag arms and in the placebo arm. Neutropenia (28%) was the most common hematologic AE reported, where nausea (36%) and alopecia (27%) were the most common non-hematologic AEs reported.

- 30 subjects (17%) experienced SAEs during the entire study; 28 subjects (16%) experienced on-therapy SAEs and 2 subjects (1%) experienced post-therapy SAEs.
- 10 SAEs were reported as related to study medication and were distributed across the placebo and eltrombopag treatment groups. Despite the numeric increase in SAEs in the eltrombopag treatment groups, the number of events reported as related to the study drug was less in each of the eltrombopag groups than that reported in the placebo group.
- 34 hepatobiliary AEs occurred on-therapy in 15 subjects. Hepatobiliary AEs were predominantly Grade 1 or Grade 2 with a greater incidence in the placebo compared to the active and treatment groups.
- 16 on-therapy thromboembolic events occurred in 13 subjects (7%) across all four treatment groups, including placebo. The majority of the events (13 events, 81%) were not considered by the investigators as related to study medication.
- 19 on-therapy renal events in 16 subjects across all four treatment groups. The incidence of renal AEs was similar in the placebo and eltrombopag groups.
- 46 on-therapy skin disorder and subcutaneous-related events in 31 subjects were identified. Events were distributed across all treatment groups, most of the AEs were Grade 1 or Grade 2 and most were considered not related to study treatment.
- 32 reports of cataract (of 176 subjects assessed); 25 subjects had cataracts prior to beginning the study; 20 remained stable; 5 were reported to progress. Most subjects had documented baseline risk factors for cataracts. These data do not appear to demonstrate an increased risk of cataract development in subjects treated with eltrombopag.

2.4.7 Efficacy findings from completed and ongoing studies

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

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

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

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

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

Table 1. Primary Endpoint in Studies TRA100773A and TRA100773B

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

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

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

Figure 1

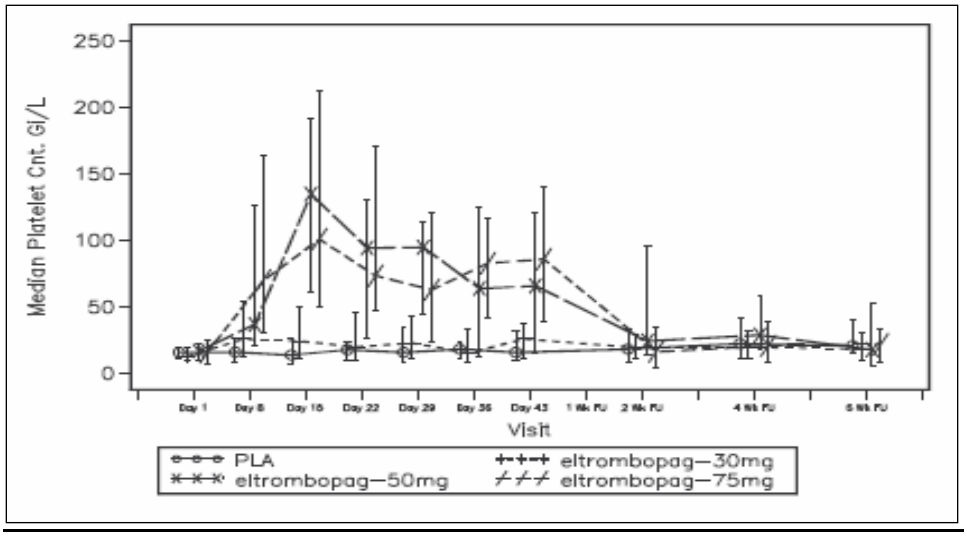
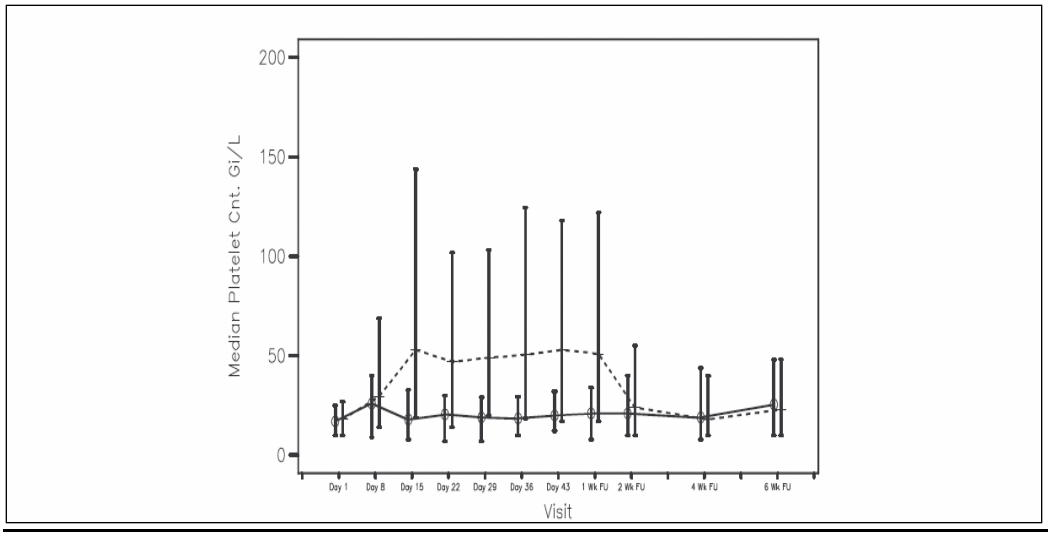


Figure 2



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

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

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

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

These data indicate a reduction in the percentage of subjects with any bleeding compared to baseline in the eltrombopag treatment groups. This reduction was not statistically significant in Study TRA100773A.

However, in TRA100773B, the odds of any bleeding in the eltrombopag arm were significantly lower than that of placebo at Day 43 (Odds Ratio [OR]=0.27, p=0.029). In addition, a lower proportion of eltrombopag subjects had any bleeding (as indicated by WHO Bleeding Grade 1-4) at any point in time over the course of their treatment (Day 8 up to Day 43) compared to subjects in the placebo group (OR=0.49, p=0.021).

Table 2 WHO Bleeding Scale Assessment

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

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

TRA105325 (EXTEND) (chronic ITP Study): An ongoing open-label, dose-modification, Phase 3 extension study to evaluate the safety and efficacy of eltrombopag for the treatment of 299 subjects with ITP who were previously enrolled in an eltrombopag trial. Data from the EXTEND study shows clinically meaningful continuous platelet count elevations ≥50 K/uL for at least 10 consecutive weeks in the majority of subjects, with 24% achieving continuous elevation of platelet counts >50 K/uL for more than 6 months and a decrease in bleeding symptoms.

TRA108057 (REPEAT) (chronic ITP study): An ongoing, Phase II, multi-center, open label single group repeat dose study to evaluate the efficacy, safety and tolerability of repeated, short term administration of eltrombopag initially administered as 50 mg tablets once daily in subjects with previously treated chronic ITP (66 subjects with ongoing enrollment). Across all three cycles, the median platelet counts at baseline of each cycle were below 35 K/ μL. Elevation in median platelet counts was observed by Day 8 of each cycle, with the median platelet counts of 74, 110 and 102.5 K/ μL observed in Cycles 1, 2 and 3, respectively. By Day 15, median platelet counts were 124, 132 and 156 K/ μL in each cycle, respectively. One week after discontinuation of eltrombopag, median platelet counts remained >100 K/uL across all three cycles of treatment. Two weeks after discontinuation, platelet counts in each cycle returned to near baseline levels. These results are similar to those from TRA100773A and TRA100773B in which median platelet counts in the eltrombopag treatment groups showed an elevation of platelet counts as early as Day 8 and continued to rise to Day 15, and in which the median platelet levels remain elevated.

TRA102537 (RAISE) (chronic ITP study): A phase 3, double-blind, placebo-controlled study in adults with previously treated immune thrombocytopenia of more than 6 months' duration who had baseline platelet counts lower than 30 000 per μL197 patients were randomly allocated to treatment groups and were included in the intention-to-treat analysis (135 eltrombopag, 62 placebo). 106 (79%) patients in the eltrombopag group responded to treatment at least once during the study, compared with 17 (28%) patients in the placebo group. The odds of responding were greater in patients in the eltrombopag group compared with those in the placebo group throughout the 6-month treatment period (odds ratio 8·2, 99% CI 3·59—18·73; p<0·0001). 37 (59%) patients receiving eltrombopag reduced concomitant treatment versus ten (32%) patients receiving placebo (p=0·016). 24 (18%) patients receiving eltrombopag needed rescue treatment compared with 25 (40%) patients receiving placebo (p=0·001). Three (2%) patients receiving eltrombopag had thromboembolic events compared with none in patients on placebo. Nine (7%) eltrombopag-treated patients and two (3%) in the placebo group had mild increases in alanine aminotransferase concentration, and five (4%) eltrombopag-treated patients (*vs* none allocated to placebo) had increases in total bilirubin. Four (7%) patients taking placebo had serious bleeding events, compared with one (<1%) patient treated with eltrombopag.

TPL102357 (Hepatitis C Study): A phase II, double blind, randomized, placebo-controlled, multi-center, dose ranging, parallel group study in subjects with Hepatitis C viral infection and platelet counts of 20 to < 70 K/μl were treated with 30 mg, 50 mg 75 mg or placebo once daily for 12-16 weeks (56 subjects). The primary endpoint was the proportion of subjects with a shift from baseline platelet count (between 20 K/μL and <70 K/μL) to ≥100 K/μL after 4 weeks (Part 1) treatment with study drug prior to receiving antiviral therapy. At the Day 28 Visit (Week 4), there were no responders in the placebo treatment group. None of the placebo subjects achieved a platelet count of ≥100 K/μL at any time during Part 1. The percentage of responders at the Day 28 Visit increased with eltrombopag treatment in a dose-dependent manner. By Day 15, the majority of subjects in each eltrombopag treatment group achieved a response, with the highest response rate observed in the eltrombopag 75 mg group (20/21, 95%). At all subsequent treatment visits, the highest response rate was also observed in the eltrombopag 75 mg group. The p-value for an overall treatment effect at Week 4 was statistically significant (p<0.0001). At all time points during Part 2, the median platelet counts were higher in the eltrombopag treatment groups compared to the placebo treatment group. As expected, the median platelet counts decreased during the antiviral treatment phase compared to the pre-antiviral phase, however, they remained above the median baseline platelet counts in each eltrombopag treatment group. Of note, subjects could have had their eltrombopag treatment interrupted if their platelet counts had risen above 200 K/μL.

SB-497115/003 (Chemotherapy-induced thrombocytopenia Study): A Phase II randomized, double-blind, four arm, parallel group, placebo-controlled, multi-center study to evaluate the efficacy, safety, and pharmacokinetics of oral eltrombopag in cancer patients with an advanced solid tumor who were receiving multiple cycles of carboplatin/paclitaxel was performed to evaluate the effects of eltrombopag on chemotherapy induced thrombocytopenia (CIT) (134 subjects) The primary endpoint was the change in platelet count in Cycle 2 measured by the difference in the platelet count from Day 1 in Cycle 2 to platelet nadir in Cycle 2. When mean platelet counts for Cycle 2 were plotted versus time, all three eltrombopag treatment groups clearly began Cycle 2 at higher mean platelet counts than the placebo treatment group. This was an unexpected effect as the assumption was that this increase would happen at the nadir rather than at the end of Cycle 1/beginning of Cycle 2. Although the eltrombopag treatment groups had larger absolute decreases in mean platelet counts, the mean platelets remained higher than the placebo treatment group and also rose more rapidly in the latter part of Cycle 2.

2.4 FDA approval: On November 20, 2008 Promacta® (eltrombopag) received accelerated approval for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The new drug application for eltrombopag was supported by the largest database of randomized clinical trial information on investigational therapies for chronic ITP patients. Eltrombopag is the first oral thrombopoietin (TPO) receptor agonist approved for adult patients with chronic ITP.

2.5 Rationale for dose selection:

Eltrombopag 50 mg once daily has been selected as the starting dose for this study because this regimen has been safe and effective in increasing platelet counts in patients with HCV and chronic ITP. A starting dose of 25mg once daily in East Asian patients appears to be equivalent. After two weeks the dose can be increased every 2 weeks in incremental doses up to a maximum dose of 150 mg (East Asians 75 mg) once daily as detailed in the treatment plan (Section 5) based on the following considerations:

- The effective dose in SAA subjects is unknown.
- 300 mg per day is the maximum dose that is currently being studied in an eltrombopag protocol for patients with Sarcoma.
- In healthy subjects, a clear dose and exposure response was seen for eltrombopag doses of 10 mg to 200 mg once daily for 5 days, with geometric mean $AUC_{(0-\tau)}$ values of 302 μg.h/mL for the 200 mg once daily regimen. Eltrombopag was well tolerated in healthy subjects at all dose levels.
- In ITP subjects, a dose response was seen for eltrombopag doses of 30 mg to 75 mg once daily, with geometric mean $AUC_{(0-\tau)}$ values of 146μg.h/mL for the 75 mg once daily regimen. There was no significant difference between the safety profile of ITP subjects receiving 30, 50 or 75 mg of eltrombopag.
- In HCV subjects, a dose and exposure response was seen for eltrombopag doses of 30 mg to 75 mg once daily, with geometric mean $AUC_{(0-\tau)}$ values of 333μg.h/mL for the 75 mg once daily regimen (approximately 2-fold the exposures observed in ITP patients at the same dose). There was no significant difference between the safety profile of HCV subjects receiving 30, 50 or 75 mg of eltrombopag, and the frequency of adverse events in these subjects did not increase in a dose-dependent manner.
- Up to 100 mg eltrombopag was studied in subjects with chemotherapy-induced thrombocytopenia, with geometric mean $AUC_{(0-\tau)}$ values of 191 μg.h/mL. No apparent safety issues at 100 mg were identified.
- Thrombocytosis is a theoretical risk of eltrombopag treatment when high dosages are administered. Thrombocytosis has been observed in healthy volunteers as well as in subjects with ITP, hepatitis C or chemotherapy-induced thrombocytopenia. None of these subjects experienced an AE related to thrombocytosis. The likelihood that aplastic anemia patients would develop thrombocytosis, given the underlying pathophysiology of their marrow disease, is likely to be very low.
- There is evidence that higher doses of growth factors are required in MDS subjects: the effective erythropoietin (EPO) dose in MDS is several times higher than the dose used in renal anemia [8].
- To ensure subject safety, the current study uses a dose escalation scheme in which subjects are exposed to the lowest dose necessary to achieve the desired platelet count target or decrease in bone marrow blast count. Only subjects who have tolerated the previous dose will be considered for the next highest dose, dependent on their last bone marrow blast and platelet count. This approach minimizes potential risks while allowing the subject the maximum potential for benefit.

Modified dosing for subjects of East Asian heritage (i.e., Japanese, Chinese, Taiwanese and Korean) has been implemented for the following reasons. In healthy Japanese subjects, plasma eltrombopag $AUC_{(0-\tau)}$ was approximately 80% higher when compared to non-Japanese healthy subjects who were predominantly Caucasian. Similarly, in patients with ITP, plasma eltrombopag exposure was approximately 70% higher in East Asian (i.e., Japanese, Chinese, Taiwanese and Korean) subjects as compared to non–East Asian subjects who were predominantly caucasian.

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.

2.6 Rationale for permitting dose interruption:

The effect of dose interruption is unknown in the aplastic anemia population. 31% (34 ITP subjects) on the GSK long term extension study (TRA105325/EXTEND) required 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, and in some patients there was a brief swing to below baseline, although not associated with any bleeding complications. We anticipate some patients on the current trial will be hospitalized for other disease-related issues such as fever and neutropenia during the study, and may require suspension of the study drug temporarily.

2.7 Rationale for extended access to study medication

In patients with refractory cytopenias due to aplastic anemia, there is little evidence for spontaneous recovery. There is also no evidence that cytokine drugs such as erythropoietin, G-CSF, or TPO-R agonists have efficacy sustained beyond the treatment period. As this class of agents is cleared from the circulation and metabolized or excreted, new hematopoietic progenitor cells are being produced in the bone marrow and are not exposed to the drug. The impact on production of end-stage cells with life-spans in the circulation, such as red cells, platelets or neutrophils, therefore does not last more than days to weeks beyond cessation of therapy. GSK study TRA105325 is an open label dose modification extension study evaluating the safety and efficacy of extended therapy of eltrombopag in ITP subjects. As of 2/8/2008 (date last IB) the extent of exposure in this populations was as follows: the median daily dose was 50 mg, the median number of days on treatment was 194 days (6.5 months) and the median cumulative dose was 6725 mg. ITP patients have return of their platelet counts to baseline within 1-2 weeks of discontinuation of drug.

We will continue treatment beyond the primary endpoint at 12 weeks in the current study, in patients responding to the drug until they reach blood count normalization sufficient for tapering as detailed below.. Toxicity and efficacy data will continue to be collected during that time to help identify the secondary endpoints of efficacy, duration of response and toxicities with extended duration of therapy.

We hypothesize, however, based on interim results, that once hematopoietic stem and primitive progenitor cells are normalized in number by exposure to eltrombopag, that this increase in number may be able to maintain more normal hematopoiesis without continued exposure to drug, or with exposure to lower doses of drug.

In the first 25 patients treated, responses only began to be observed beginning at the 3 month time point, and with continued exposure to drug, blood counts of all lineages improved towards the normal range gradually over time periods up to 31 months to date. Bone marrow cellularity began to normalize by 9-15 months.

2.8 Rationale for tapering eltrombopag during the extension protocol

Clonal evolution is a serious complication of aplastic anemia. Rates are in the range of 10-15% over 10 years (Maciejewski JP, Selleri C. Leuk Lymphoma. 2004 Mar;45(3):433-40). Such patients may go on to develop worstening of their disease or acute myeloid leukemia. There is a theoretical concern that TPO-R agonists, being growth factors, may cause clonal evolution although across the clinical trials in ITP (n = 493) no difference in the incidence of malignancies or haematological malignancies was demonstrated between placebo and eltrombopag treated patients. This is consistent with information derived from non-clinical research, where no malignant cell proliferation has been demonstrated upon co-incubation of eltrombopag with MDS cell lines, multiple leukemic cell lines and solid tumour cell lines (colon, prostate, ovary and lung).

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

2.9 Scientific and clinical justification of the protocol

Currently, patients with severe aplastic anemia not eligible or suitable for allogeneic stem cell transplantation, receive immunosuppressive therapy (IST) with horse or rabbit antithymocyte globulin and cyclosporine, or Campath. The response rate to initial IST is approximately 60%, with another approximately 10% of patients refractory to the first IST treatment responding to treatment with the same or an alternative IST regimen. However, even with repeated courses of immunosuppression, the response rate at best approaches 70%(Rosenfeld, 2003 1045 /id)(Scheinberg, 2006 1017 /id; Scheinberg, 2006 1018 /id). Almost 30% of patients have persistent severe thrombocytopenia after repeated IST, requiring regular platelet transfusions; or in alloimmunized patients unable to receive platelets, life-threatening bleeding. Quality of life is severely impacted by the necessity to come to a treatment center as frequently as every other day to receive platelet transfusions. Patients requiring only red cell transfusions can go one to four weeks between transfusion sessions. Thus decreasing or abrogating the need for platelet transfusions could significantly improve the quality of life and ability to carry out normal daily activities even in patients with refractory aplastic anemia.

New treatment modalities are needed for this population.

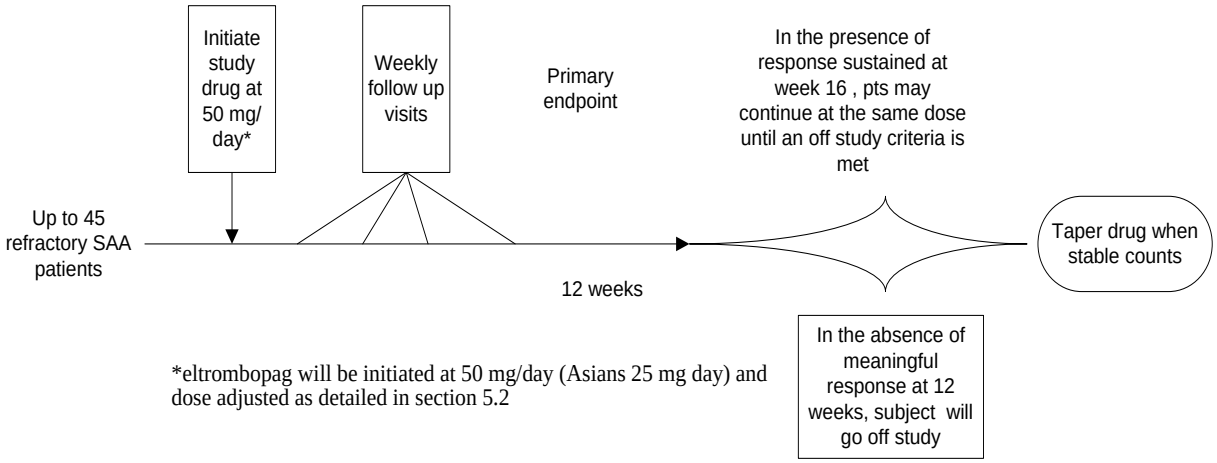
Thrombopoietin (TPO) is a potent endogenous cytokine and the principal regulator of platelet production. On binding to TPO receptors on megakaryocyte progenitors, TPO initiates a number of signal transduction events to increase the production of mature megakaryocytes and platelets. A 2nd generation TPO-agonist,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

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

Because, the severe paucity of megakaryocytes is the cause of thrombocytopenia in aplastic anemia patients and because of the efficacy demonstrated in ongoing ITP clinical trials and subsequent FDA approval for use in ITP, we now propose this Phase 2, non-randomized pilot study of eltrombopag in aplastic anemia patients with immunosuppressive therapy refractory thrombocytopenia. We hope that the drug will stimulate more robust platelet production from the depleted megakaryocyte pool, and also potentially help drive primitive hematopoietic stem and progenitor cells to produce more megakaryocytes.

3.0 STUDY DESIGN

The study is designed as a non-randomized, Phase II, dose modification study of the oral TPO-R agonist eltrombopag in severe aplastic anemia subjects with immunosuppression-refractory thrombocytopenia. The primary endpoint is measured at 12 weeks. Subjects who cannot tolerate the medication or fail to respond by 12 weeks will go off study. Subjects with response may continue study medication for an additional 4 weeks to ensure eligibility to enter the extended access portion of the trial. Drug dose during extended access will be at the lowest dosage that maintains a stable platelet count until they meet off study criteria or the study is closed.



4.0 ELIGIBILITY ASSESSMENT

4.1 Inclusion criteria

4.1.1 **Diagnosis of aplastic anemia,** with refractory thrombocytopenia following at least one treatment course of horse or rabbit ATG/cyclosporine.

4.1.2 Platelet count ≤ 30,000/μL

4.1.3 Age ≥ 12years old

4.2 Exclusion criteria

4.2.1 Diagnosis of Fanconi anemia
4.2.2 Infection not adequately responding to appropriate therapy
4.2.3 Patients with a PNH clone size in neutrophils of ≥50%
4.2.4 HIV positivity
4.2.5 Creatinine > 2.5
4.2.6 Bilirubin > 2.0
4.2.7 SGOT or SGPT >5 times the upper limit of normal
4.2.8 Hypersensitivity to eltrombopag or its components
4.2.9 Female subjects who are nursing or pregnant or are unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential
4.2.10 History of malignancy other than localized tumors diagnosed more than one year previously and treated surgically with curative intent (for instance squamous cell or other skin cancers, stage 1 breast cancer, cervical carcinoma in situ, etc)
4.2.11 Unable to understand the investigational nature of the study or give informed consent
4.2.12 History of congestive heart failure, arrhythmia requiring chronic treatment, arterial or venous thrombosis (not excluding line thrombosis) within the last 1 year, or myocardial infarction within 3 months before enrollment
4.2.13 ECOG Performance Status of 3 or greater
4.2.14 Treatment with horse or rabbit ATG or Campath within 6 months of study entry. Concurrent stable treatment with cyclosporine or G-CSF is permitted.

5.0 TREATMENT PLAN

5.1 Administration of study drug (eltrombopag)

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

5.2 Dose adjustments of eltrombopag (See section 2.5)

If after 2 weeks, the platelet count has not increased by 20,000/ul from baseline or platelet transfusion requirements have not decreased, the dose will be increased by 25 mg every 2 weeks (+/- 3 days) to a maximum of 150 mg (in the Asian population the dose may be escalated to a maximum of 75 mg). Depending on tolerability, the daily dose may be increased or decreased according to the following rules:

Platelet Count	Dose Adjustment or Response
<20,000/μL above baseline or platelet transfusion requirement has not decreased following at least 2 weeks of eltrombopag	Increase daily dose every 2 weeks (+/- 3 days) to maximum 150 mg/day for non- East Asians (75 mg/day for East Asians).
≥20,000/ μL above baseline but ≤100,000/ μL following at least 2 weeks of-eltrombopag	Keep at current dosage.
>100,000/ μL (untransfused) at any time on study	Decrease dosage every 2 weeks (+/- 3 days) to lowest dosage that maintains platelet count ≥20,000/ μL above baseline.
>200,000/ μL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets < 50,000/ μL; restart at 25 mg/day, or next lowest dose

If after 12 weeks there is no response, treatment will be discontinued and subjects will go off study per section 8.6.

5.3 Dose adjustments in extension protocol

Once platelets > 50,000, Hb > 10 in the absence of RCC transfusion and neutrophils > 1,000 for more than 8 weeks the dose of Eltrombopag will be cut by 50% (to 75 mg/day, or to 25 mg/day for East Asians). After 8 weeks (+/- 3 days) at this dose if counts stay greater than the above limits, then drug will be discontinued. If platelets drop to <30,000/ul, Hb <9gr/dL or ANC<500/ul while at 50% dose level, the dose can be increased to 150 mg/day. If counts drop below these levels off drug, the drug can be restarted at 75mg/day, (25 mg/day for East Asians), if no response by 8 weeks (+/- 3 days) at 75 mg/day, the dose can be increased to 150 mg/day, (75 mg/day for East Asians).

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

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

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

5.5 Dose delays, modifications or discontinuation for hematologic side effects

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

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

5.6 Extended access to study drug

Subjects with response at 12 weeks may continue study medication for an additional 4 weeks (to ensure eligibility) prior to being consented for entry into the extended access part of the trial. Per dosing criteria given in section 5.3, patients may remain on the extended access as long as they maintain a treatment response

5.7 Permitted supportive care

- Transfusional supportive care (e.g., blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) as clinically indicated.
- Estrogens or combination OCPs as indicated for uterine bleeding.

5.8 Concurrent mediations:

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

Rosuvastatin: In vitro studies demonstrated that eltrombopag is not a substrate for the organic anion transporter polypeptide, OATP1B1, but is an inhibitor of this transporter in vitro and as evidenced by increased plasma rosuvastatin levels when eltrombopag and rosuvastatin were co-administered in a clinical drug interaction study. When co-administered with eltrombopag, a reduced dose of rosuvastatin should be

considered and careful monitoring should be undertaken. In clinical trials with eltrombopag, a dose reduction of rosuvastatin by 50% was recommended for co-administration of rosuvastatin and eltrombopag. Concomitant administration of eltrombopag and other OATP1B1 substrates should be undertaken with caution.

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

Other medications: Subjects may continue on any of the medications that they were prescribed prior to study enrollment for co-morbid conditions, and standard anti-infectious prophylaxis medications including pentamidine, valacyclovir, and voriconazol**5.9Instructions to patients**

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

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

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

6 CLINICAL MONITORING

6.1 Pre-study evaluation

Baseline status will be evaluated as follows:

- Medical History and physical examination
- Concurrent medication review
- Baseline assessments (done at screening or diagnostic workup, not repeated on study)
  - Folate level
  - B12 level
  - Iron panel (ferritin, transferrin, % saturation)
- Baseline laboratory studies (evaluations designated with an * must be repeated within 72 hours of the first dose of study drug)
  - Complete blood count with differential*
  - Reticulocyte count*
  - Chem 20 panel*
  - Pregnancy test (urine HCG in women of child bearing potential)*
  - Serum thrombopoietin level (contract through R&D systems)
  - Coagulation screens (PT, PTT)
  - Thyroid function tests

Peripheral blood smear
Viral serologies for HIV, hepatitis B, C, HSV, EBV and CMV
HLA typing (if not already available)
DAT (direct antiglobulin test)
Flow cytometry of the peripheral blood for GPI-cells
Lymphocyte phenotyping (TBNK flow cytometry)

- Bone marrow aspirate and biopsy with reticulin and collagen fiber staining and cytogenetic analysis (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis by PCR) within three months of first dose of study drug
- General Quality of Life - SF-36 questionnaire within 72 hours of first dose of study drug

6.2 Monitoring study drug initiation through primary endpoint (13 weeks +/- 4 days)

Subjects will be monitored weekly so long as they remain on study drug through week 12. At a minimum, subjects must be evaluated at the NIH Clinical Center at the beginning of weeks 5, 9 and 13 (+/- 4 days). Subjects must have interim weekly blood tests drawn by their referring health care provider or at the NIH. If subjects are to be followed at home, progress notes and laboratory results from their health care provider and laboratory must be faxed to the study research nurse, Kinneret Broder, RN 301-402-3088. The following assessments will be done:

- Clinical assessment (at weeks 5, 9and 13 [+/- 4 days])
- Medication review with attention to compliance with eltrombopag so that early discontinuation and subsequent rebound exacerbation is carefully monitored (weeks 5, 9 and 13 +/- 4 days)
- CBC with differential (weekly +/- 4 days)
- Peripheral blood smear (weekly +/- 4 days)
- Chem 20 panel (weekly +/- 4 days) (Home MDs: electrolytes, transaminases, urea nitrogen [BUN], serum creatinine clearance, total bilirubin)
- Reticulocyte count (weekly +/- 4 days) (If seen at home, only if available)
- Thrombopoietin level (at the 13 week visit [+/- 4days])
- Coagulation screens (PT, PTT) (at the week 13 visit [+/- 4days])
- DAT (direct antiglobulin test) (as clinically indicated)
- Type and screen (as clinically indicated)
- Pregnancy test (at the week 5, 9, and 13 visits [+/- 4 days])
- Flow cytometry of the peripheral blood for GPI-cells (at the week 13 visit [+/- 4days])
- Bone marrow aspirate and biopsy with reticulin and collagen fiber staining and cytogenetic analysis at primary end point (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis by PCR) (after 12 weeks of medication [+/- 4days])
- Lymphocyte phenotyping (TBNK flow cytometry) (week 13 visit [+/- 4 days])
- General Quality of Life - SF-36 questionnaire (at the week 13 visit [+/- 4days])

6.3 Monitoring Weeks 13-16 and during extended access

Responding subjects who opt to remain on study drug (extended access) will be monitored monthly so long as they do not meet off study criteria, per Section 8.6. At a minimum, subjects must be evaluated at the NIH Clinical Center every 6 months (+/-1 week). Subjects may be seen for monthly interim visits at the NIH or at their referring home health care provider. If subjects are to be followed at home, progress notes and laboratory results from the home health care provider and laboratory must be faxed to the study research nurse, Kinneret Broder, RN 301-402-3088.

- Interim clinical assessment (monthly +/- 1 week)

- Concurrent medication review (with each clinical assessment)
- CBC with differential (monthly +/- 1 week)
- Peripheral blood smear (monthly +/- 1 week)
- Chem 20 panel (monthly +/- 1 week) (Home MDs: electrolytes, transaminases, urea nitrogen [BUN], serum creatinine, total bilirubin)
- Reticulocyte count (monthly +/- 1 week) (If seen at home, only if available)
- Pregnancy test (monthly +/- 1 week)
- Thrombopoietin level (every six months +/- 1 week)
- Coagulation screens (PT, PTT) (as clinically indicated)
- DAT (direct antiglobulin test) (as clinically indicated)
- Type and screen (as clinically indicated)
- Imaging studies (only as medically indicated)
- Bone marrow examination with reticulin and collagen staining and aspiration with cytogenetics (every six months +/- 1 week or more frequent if clinically indicated)
- Lymphocyte phenotyping (TBNK flow cytometry) (every 6 months [+/- 4 days])

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

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

- Clinical assessment and vital signs (1 and 6 months [+/- 1 week])
- CBC with differential (weekly for 1 month, and at 6 months [+/- 1 week])
- Chem 20 panel (weekly for 1 month, and at 6 months [+/- 1 week])
- Reticulocyte count (weekly for 1 month, and at 6 months [+/- 1 week])
- Peripheral blood smear (weekly for 1 month, and at 6 months [+/- 1 week])
- Thrombopoietin level (weekly for 1 month, and at 6 months [+/- 1 week])
- Coagulation screens (PT, PTT) (1 month [+/- 1 week])
- Bone marrow biopsy with reticulin and collagen staining and aspiration with cytogenetics (6 months [+/- 1 week]
- Lymphocyte phenotyping (TBNK flow cytometry) (6 months [+/- 4 days]

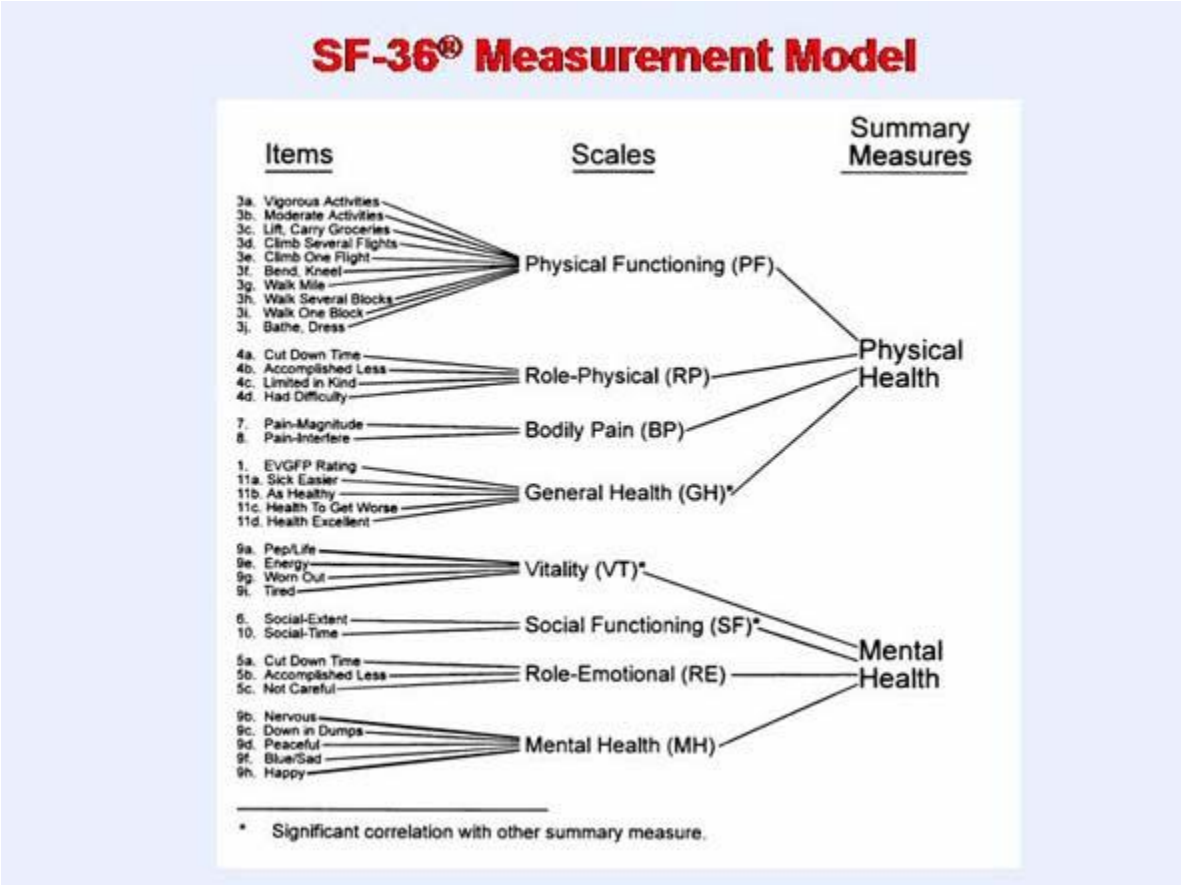
6.5 Extended access off study drug with robust response

Subjects who go off drug because of robust response will be monitored according to the following schedule. At a minimum, subjects must be evaluated at the NIH Clinical Center every 6 months. The following studies will be performed:

- Clinical assessment and vital signs (6 months [+/- 1 week])
- CBC with differential (monthly [+/- 1 week])
- Chem 20 panel (monthly [+/- 1 week])
- Reticulocyte count (monthly [+/- 1 week])
- Peripheral blood smear (monthly [+/- 1 week])
- Thrombopoietin level (every 6 months [+/- 1 week])
- Coagulation screens (PT, PTT) (as clinically indicated)
- Bone marrow biopsy with reticulin and collagen staining and aspiration with cytogenetics (yearly [+/- 1 week])
- Lymphocyte phenotyping (TBNK flow cytometry) (6 months [+/- 4 days])

6.6 General quality of life (SF-36)

SF-36 is a generic health assessment instrument with high validity and reliability which has been used extensively in outcome research [9]. It has been shown to be sensitive to treatment effects. SF-36 contains questions grouped in 8 categories assessing both physical and mental health status. General quality of life measure (SF-36) will be completed at baseline and the end of the study (12 weeks).



7 ANCILLARY LABORATORY RESEARCH STUDIES

Samples are stored in compliance with the NHLBI BSI Policy.

7.1 Collection of samples

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

7.2 Intended use: These specimens will not be read by a pathologist or be used for diagnostic purposes. Studies will not be used in assessing the primary endpoint but will be undertaken for descriptive or exploratory ancillary research. The following laboratory research studies may be done and if done, may be correlated with the presence or absence of response. Additional studies which are approved by the NHLBI IRB and listed in the Appendix of the protocol may be done on stored samples.

- Assay for cytokines/chemokines and their receptors
- Hematopoietic progenitor colony formation and long term-culture-initiating cell assays
- Serum (or plasma) and cells for DNA/RNA extraction

7.3 **Tracking:** Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.

7.4 **Storage:** Research samples will be stored with identifiers in the secure laboratory of the principal investigator.

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

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

8.0 BIOSTATISTICAL CONSIDERATIONS

8.1 Objectives

The ***primary objective*** is to assess the safety and efficacy of the oral thrombopoietin receptor agonist (TPO-R agonist) eltrombopag in aplastic anemia patients with immunosuppressive-therapy refractory thrombocytopenia.

Secondary objectives include the analysis of the incidence and severity of bleeding episodes, and the impact on quality of life.

8.2 Endpoints

The ***primary endpoint*** will be the portion of drug responders as defined by changes in the platelet count and/or platelet transfusion requirements, hemoglobin levels, number of red blood cell transfusions, or neutrophil counts as measured by International Working Group criteria and the toxicity profile as measured using the CTCAE criteria. Platelet treatment response is defined as platelet count increases to 20,000/ μL above baseline at three months, or stable platelet counts with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL or a reduction in the units of PRBC transfusions by an absolute number of at least 4 PRBC transfusions for eight consecutive weeks compared with the pretreatment transfusion number in the previous 8 weeks . Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase in ANC, or an ANC increase $> 0.5 \times 10^9$/L.

Criteria for response: Platelet treatment response is defined as platelet count increases to 20,000/ μL above baseline at three months, or stable platelet counts with transfusion independence for a minimum of 8 weeks. Erythroid response for subjects with a pretreatment hemoglobin of less than 9 g/dL will be defined as an increase in hemoglobin by $\geq$ 1.5g/dL without packed red blood cell (PRBC) transfusion support, or a

reduction in the units of transfusions by an absolute number of at least 4 PRBC transfusions for eight consecutive weeks compared with the pretreatment transfusion number in the previous 8 weeks. Neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase or an absolute increase $> 0.5 \times 10^9$/L.

Secondary endpoints will include change in platelet count (continuous variable), incidence of bleeding; changes in serum thrombopoietin level (as measured by enzyme-linked immunosorbent assay, R&D Systems), and health related quality of life (as measured by the Medical Outcomes Study 36-Item Short Form General Health Survey, version 2 [SF36v2]; Quality-Metric) measured at 12 weeks.

General Quality of Life (SF-36). SF-36 (Appendix E) is a generic health assessment instrument with high validity and reliability which has been used extensively in outcome research (Larson, 1997 2214 /id). It has been shown to be sensitive to treatment effects. SF-36 contains questions grouped in 8 categories assessing both physical and mental health status. General quality of life measure (SF-36) will be completed at baseline and after 12 weeks.

8.3 Sample size

Because the efficacy of eltrombapag in this patient population is unknown, we would like to reject the treatment as quickly as possible with a small number of patients if the treatment is not effective. We will use the Two-Stage Minimax Design outlined in Table 1 of Simon ([10]) with a response probability of 10% or less to terminate the treatment and the hypothesized actual response probability of 30% or more. The sample size is determined by testing the null hypothesis H0: $p \leq 10\%$ versus the alternative H1: $p \geq 30\%$ at a significance level of 0.05 and a power of 0.8. At the first stage, 15 subjects will be accrued and the null hypothesis will not be rejected if no more than 1 subject responds to the treatment within 12 weeks. If 2 or more subjects respond to the treatment within 12 weeks at the first stage, then an additional 10 subjects will be accrued, bringing the total number of subjects to n=25. The null hypothesis of $p \leq 10\%$ will be accepted if the total number of responders within 12 weeks is 5 or less. The additional 5 patients together with the 20 patients from the last amendment will be included in the exploratory analysis of the primary endpoint and secondary endpoints. The original statistical analysis plan for the primary endpoint will not be changed, i.e. the primary endpoint will be formally evaluated using the 25 patients in the original protocol.

Subjects who discontinue the study drug prematurely (before 12 weeks):

Platelet count measurement will be attempted even if a subject discontinues study drug. Subjects who withdraw from the study for reasons other than lack of efficacy or toxicity (rendering platelet count missing) may be replaced. All other subjects should be evaluable for efficacy. Based on the assumed drop out rate of 15-20% and the goal of having 25 evaluable subjects, 5 additional subjects may be enrolled.

Amendment:

Primary Endpoint and Sample Size:
We request to expand the same treatment of this protocol to 20 additional patients. By doing this we will narrow the confidence intervals and get more safety data.

Secondary Endpoint and Sample Size:

In addition to the primary endpoint of response to eltrombapag at 12 weeks, an important secondary endpoint, which has not been formally considered in the study design of this protocol is clonal evolution to monosomy 7 or complex cytogenetics. Clonal evolution is observed in these patients at a rate of 10-15% and currently no predictive factors are known. Evolution to monosomy 7 or complex cytogenetics was seen

more often in patients with very short telomeres treated with immunosuppressive therapy (Scheinberg 2010 JAMA). It is not known whether Eltrombopag therapy increases the rate of progression and as this agent shows good efficacy in this group of patients it is imperative this issue is explored. Therefore, as a preliminary study to explore the rate of clonal evolution for this patient population, we wish to add as an exploratory secondary endpoint clonal evolution to monosomy 7 or complex cytogenetics at 9 months.

Sample Size Justification:
Adding 25 patients will narrow the confidence intervals and get more safety data.

Stopping Rules for Safety: The same stopping rules for safety as described in Section 8.5 will be used to monitor safety for the 25 newly accrued patients. In this case, the stopping boundary for the additional 25 patients is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAE's exceeds this benchmark rate of 20% is at least 90%, and we take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (0.6, 2.4)$. We will start safety monitoring when 3 or more subjects have developed a TRSAE. The threshold numbers for stopping the study are given by:

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 6	3
≤ 9	4
≤ 13	5
≤ 17	6
≤ 20	7
≤ 25	8

8.4 Statistical methods

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

The time to responses and the progression-free survival will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate and Cox regression type analysis for covariates, which takes consideration of both death without the event of interest as a complete risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their corresponding 95% confidence intervals. Methods based on multiple regression, analysis of variance, and logistic regression will also be employed if deemed appropriate.

8.5 Stopping rules

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

1. Death

2. Any Grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities

3. Grade IV thrombosis/embolism

We anticipate the rate of these specified TRSAEs within the 3 month study period to be 20% or less. Following Geller et al. ([11]), our stopping rule is determined by a Bayesian approach. The stopping boundary for an experiment is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAE's exceeds this benchmark rate of 20% is at least 90%. We take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (0.6, 2.4)$. The parameter are chosen so that the mean $\alpha / (\alpha + \beta) = 0.2$ as the expected proportion of specified TRSAE's and the sum $\alpha + \beta = 3$ as the "worth" we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is 3/30=10% of the weight we will place on the results of the new study. Hence when we make decisions about stopping the study, the data from the study will dominate over the prior opinion. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 or more subjects have developed a TRSAE. The following table summarizes the threshold numbers for stopping the study.

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 6	3
≤ 9	4
≤ 13	5
≤ 17	6
≤ 20	7
≤ 24	8
≤ 28	9
≤ 30	10

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 30 independent Bernoulli trials, each had a probability p for having TRSAE and q=1-p for not having TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for p:

Probability of TRSAE = p	0.10	0.20	0.25	0.30	0.40
Proportion of Stopped Studies	2.4%	22.3%	41.3%	62.4%	90.7%
Average number of subjects	29.5	25.7	22.4	18.5	11.6
Average number TRSAEs	2.95	5.1	5.6	5.5	4.6

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

8.6 Off study criteria

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

8.6.2 Per principal investigator decision: Should any of the following adverse events occur during the 12 week study period, or in the extension treatment arm in responders, eltrombopag will be discontinued. The subject will be followed until resolution of the event. Labs will be monitored through 30 days off study drug time point or until he/she initiates alternative disease directed therapy at which time the subject's participation on this study will be considered complete and the subject will go off study.

- Intolerance of eltrombopag not resolved by dose reduction
- Life threatening acute hypersensitivity reaction
- Thrombosis/embolism (DVT, PE, stroke or TIA, myocardial infarction) other than central line thrombosis
- Persistent hepatotoxicity as defined in section 5.3.2
- New or worsening morphological abnormalities or cytopenia(s) as defined in section 5.4.2
- No treatment response by week 12
- Any Grade IV toxicity considered related to the study medication excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities
- Significant progression of disease or a concomitant condition that would make the subject ineligible for further protocol participation
- Pregnancy or unwillingness to use acceptable forms of contraception
- Initiation of non-protocol therapy for aplastic anemia
- Development of study related cataracts

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

9 DATA AND SAFETY MONITORING

9.1 Data and Safety monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Ronan Desmond M.D. MRCPI, FRCPath

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

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

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

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

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

IND 104,877: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to

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

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

Eileen Starrs, RN, BS, CCRP

Manager, Clinical Development
GlaxoSmithKline, North America R&D/Medical Affairs
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Fax: 610-917-6715

9.2 Adverse events

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs, symptoms or diseases which either occurs during the study, having been absent at baseline or if present at baseline appear to worsen. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 3.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Abnormal laboratory findings used to evaluate the safety of this protocol regimen will be collected to include any change from laboratory assessments done prior to first dose of study medication that result in a progression to a grade 3 or 4 laboratory toxicity. In view of the underlying illness (bone marrow failure), many patients may enter study with compromised hematologic indices that would be classified as toxicities. Therefore, we will look at the relative decline of hematologic parameters and length of time to recovery to the patient's baseline as more significant than absolute values of these parameters. The laboratory toxicities will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 3.0.
Thirty days after the last dose of study drug, adverse event reporting will be limited to those events considered possibly, probably, or definitely related to study drug and those deemed an SAE regardless of attribution.

Grading of Adverse events:

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

Attribution of Adverse Events:

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment		
1	**Not related**	This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).

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

9.3 Serious adverse events

Any serious adverse events as defined in the "NIH Guidelines for Adverse Event Reporting" and include any untoward medical occurrence that:

- results in death,
- is life threatening
- requires (or prolongs) hospitalization
- causes persistent or significant disability/incapacity,
- results in congenital anomalies or birth defects, or
- any other condition which in the judgment of the investigators represent significant hazards.

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

Treatment related SAEs (TRSAEs) are those attributed as definitely, probably, or possibly related that will be monitored and considered for early stopping of the study according to statistically determined criteria. These include death and any grade IV toxicity considered to be probably or definitely related to

study medication. John Tisdale, MD, NIDDK will serve as the independent monitor who reviews the attribution of TRSAEs .

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

9.4 Reporting of serious adverse events

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

Ronan Desmond, M.D MRCPI, FRCPath.
HB, NHLBI, NIH, Clinical Center
10 Center Dr. Building 10, Room CRC 3-5256
Bethesda, MD 20892-1452
Tel: 301-451-7143 Fax: 301-402-3405
E-mail: ronan.desmond@nih.gov

NHLBI IRB*:* Serious adverse events will be reported to the NHLBI IRB/Office of Clinical Affairs (OCA), through the NHLBI Protocol Tracking Management System (PTMS) 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 using the Serious Adverse Event Form (see Appendix A). If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held.

NHLBI DSMB: Reports of serious adverse events that are unexpected and thought to be related to the experimental drug will also be forwarded 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 SAEs will be included for review by the DSMB.

FDA: IND # 104,877: A summary of all SAEs will be submitted to the FDA with the annual progress report. SAEs that are unexpected and thought to be related to the experimental drug will be forwarded within than 7 calendar days in the case of death or life-threatening serious adverse events or within 15 calendar days after the occurrence of all other forms of serious adverse events using an NHLBI SAE report form and a Medwatch form to

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

GlaxoSmithKline: 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 GlaxoSmithKline

within 24 hours of the research team learning of the event. A copy of the SAE report (NHLBI SAE report form, Appendix A) will be forwarded as soon as possible, but no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events. If the SAE is unexpected and determined possibly, probably or definitely related to study drug the SAE report (NHLBI SAE report form and Medwatch form, Appendix B) will be forwarded to GSK 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 a full written summary detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports will be included. The investigator will always provide an assessment of causality at the time of the initial report as described in 'Assessment of Causality'.

Reports will be forwarded to

GlaxoSmithKline Oncology MDC Fax: +1-610-422-2527
For medical emergencies contact:
Katie Dawson, MD, MPH, Office: 610-917-6992, cell: 610-864-7246
Toll Free Number: 1 (800) 877-7074, ext 5731 or 6862
After Hours or Weekends: (800) 366-8900, ask for physician on call

GlaxoSmithKline UP4420
1250 S. Collegeville Road,
P.O. Box 5089
Collegeville, PA 19426-0989

9.5 Reporting of pregnancy

Subjects who become pregnant during the study should discontinue the study immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her designee, will submit pregnancy information to GSK within two weeks of learning of a subject's pregnancy. Information on the status of the mother and child will be forwarded to GSK. 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 GSK as an AE or SAE.
A spontaneous abortion is always considered to be an SAE and will be reported to GSK. 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 GSK. While the investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting.

9.6 Data management

Data collection and distribution: The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All human subjects personally

identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded in DIR's Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. 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., study-specific identifying number (SSPIN) generated by CDS or other unique code, or minimum PII required for subject identification.

GlaxoSmithKline will receive quarterly accrual and toxicity information as detailed in the CTA. In order to maintain patient confidentiality, all communications relating to the study will identify participants by assigned subject study numbers. No personally identifiable information will be sent to GSK. In accordance with local and federal regulations, the Investigator will allow GlaxoSmithKline 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.

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

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

10.0 HUMAN SUBJECT PROTECTION

10.1 Rationale for subject selection

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

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

gender: 60% males and 40% females;

race/ethnicity: approximately 55% White, 15% Black, 6% Asian and 24% Hispanic;
age: will range between 2 and 82 (median age of 30) and that 15% of patients will be under the age of 18.

For subjects of Asian heritage: : Plasma eltrombopag exposure was approximately 70% higher in East Asian (i.e. Japanese, Chinese, Taiwanese and Korean) subjects as compared to non-East Asian subjects who were predominantly Caucasian. Therefore subjects of Asian heritage will be included but they will be initiated at a lowered dose and monitored closely.

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

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

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

Recruitment efforts: The study will be listed on the clinicaltrials.gov, Clinical Center research studies, The Aplastic Anemia Foundation, and the National Heart, Lung and Blood Institute patient recruitment websites. If recruitment goals are not met, recruitment plan will be developed by the Clinical Center Office of Patient Recruitment. Hematologists and Oncologists throughout the country will be informed about the protocol by letter. Because many aplastic anemia patients may respond to initial immunosuppressive treatment with a response that is sufficient to prevent serious infections, but have persistent thrombocytopenia, we will also be able to rapidly recruit study patients who have completed other trials for aplastic anemia therapy within the Branch.

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

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: $0

Travel (air/train/bus): Travel from home for the first NIH visit will not be reimbursable. If the patient consents to the research protocol, travel home following the first visit will be reimbursable. Subjects will be reimbursed 100% of government rate for travel once the subject has been determined eligible to participate and signs consent.

Local Travel (car/taxi/shuttle/train/bus): Subjects will be reimbursed for local train/bus and/or shuttle costs. Car mileage will be reimbursed $0.55 /mile when the distance from home is greater than 50 miles. Reimbursement for mileage less than 50 miles from home is not provided. Subjects will not be reimbursed for rental car cost beyond the car mileage rate. Taxi will be paid only when medically necessary and authorized by the PI.

Meals: Subjects will not be reimbursed for meals.

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

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

10.2 Participation of pediatric patients

Aplastic anemia with severe thrombocytopenia is seen in children, and 15% of all severe aplastic anemia patients referred to the NIH for protocol participation are <18. In the combination immunosuppression with Eltrombopag study (12-H-0150), headed by Dr. Danielle Townsley, pediatric patients are included and those over 12 are treated with adult doses (150mg). Thus, we propose enrolling pediatric patients 12 years and older into this protocol and they will be treated according to the adult dosing schedule.

10.3 Risks and Discomforts: (excerpted from Promacta product label, dated March 2010)

10.3.1 Boxed warnings related to Promacta® (eltrombopag):

WARNING: RISK FOR HEPATOTOXICITY
See full prescribing information for complete boxed warning
PROMACTA may cause hepatotoxicity:
● Measure serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin prior to initiation of PROMACTA, every 2 weeks during the dose adjustment phase and monthly following establishment of a stable dose. If bilirubin is elevated, perform fractionation.
● Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until the abnormality(ies) resolve, stabilize, or return to baseline levels.
● Discontinue PROMACTA if ALT levels increase to ≥3X upper limit of normal (ULN) and are:
● progressive, or
● persistent for ≥4 weeks, or

● **accompanied by increased direct bilirubin, or**
● **accompanied by clinical symptoms of liver injury or evidence for hepatic decompensation**.

10.3.2 Precautions:

Hepatotoxicity: Eltrombopag administration may cause hepatotoxicity. In the controlled ITP studies one patient experienced Grade 4 elevations in serum liver test values during therapy, worsening of underlying cardiopulmonary disease, and death. No patients in the placebo group experienced Grade 4 liver test abnormalities. Overall, serum liver test abnormalities (predominantly Grade 2 or less in severity) were reported in 10% and 8% of the eltrombopag and placebo groups, respectively. In the controlled ITP studies, two patients (1%) treated with eltrombopag and two patients in the placebo group (3%) discontinued treatment due to hepatobiliary laboratory abnormalities. Seven of the patients treated with eltrombopag in the ITP controlled studies with hepatobiliary laboratory abnormalities were re-exposed to eltrombopag in the extension study. Six of these patients again experienced liver test abnormalities (predominantly Grade 1) resulting in discontinuation of eltrombopag in one patient. In the extension study, one additional patient had eltrombopag discontinued due to liver test abnormalities (≤Grade 3).

Measure serum ALT, AST, and bilirubin prior to initiation of eltrombopag, every 2 weeks during the dose adjustment phase and monthly following establishment of a stable dose. If bilirubin is elevated, perform fractionation. Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until the abnormality(ies) resolve, stabilize, or return to baseline levels. Discontinue eltrombopag if ALT levels increase to ≥3X the upper limit of normal (ULN) and are:
• progressive, or
• persistent for ≥4 weeks, or
• accompanied by increased direct bilirubin, or
• accompanied by clinical symptoms of liver injury or evidence for hepatic decompensation.

Reinitiating treatment with eltrombopag is not recommended. If the potential benefit for reinitiating eltrombopag treatment is considered to outweigh the risk for hepatotoxicity then cautiously reintroduce eltrombopag and measure serum liver tests weekly during the dose adjustment phase. If liver tests abnormalities persist, worsen or recur, then permanently discontinue eltrombopag. Exercise caution when administering eltrombopag to patients with hepatic disease. Use a lower starting dose of eltrombopag in patients with moderate to severe hepatic disease and monitor closely

Bone marrow reticulin formation and bone marrow fibrosis: Eltrombopag may increase the risk for development or progression of reticulin fiber deposition within the bone marrow.

In the ITP extension study, seven patients had reticulin fiber deposition reported in bone marrow biopsies, including two patients who also had collagen fiber deposition. The fiber deposition was not associated with cytopenias and did not necessitate discontinuation of eltrombopag. However, clinical studies have not excluded a risk of progression to clinically-significant bone marrow fibrosis with cytopenias.

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

Worsening thrombocytopenia and hemorrhage risk after stopping drug (withdrawal and rebound): Discontinuation of eltrombopag may result in thrombocytopenia of greater severity than was present prior to therapy with eltrombopag . This worsened thrombocytopenia may increase the patient's risk of bleeding, particularly if eltrombopag is discontinued while the patient is on anticoagulants or antiplatelet agents. In the controlled clinical studies, transient decreases in platelet counts to levels lower than baseline were observed following discontinuation of treatment in 10% and 6% of the eltrombopag and placebo groups, respectively. Serious hemorrhagic events requiring the use of supportive ITP medications occurred in 3 severely thrombocytopenic patients within one month following the discontinuation of eltrombopag; none were reported among the placebo group. Following discontinuation of eltrombopag, obtain weekly CBCs, including platelet counts for at least 4 weeks and consider alternative treatments for worsening thrombocytopenia, according to current treatment guidelines.

Thrombotic/thromboembolic complications. Thrombotic/thromboembolic complications may result from excessive increases in platelet counts. Excessive doses of eltrombopag or medication errors that result in excessive doses of eltrombopag may increase platelet counts to a level that produces thrombotic/ thromboembolic complications. In the controlled ITP clinical studies, one thrombotic/ thromboembolic complication was reported within the groups that received eltrombopag and none within the placebo groups. Seven patients experienced thrombotic/ thromboembolic complications in the ITP extension study. Use caution when administering eltrombopag to patients with known risk factors for thromboembolism (e.g., Factor V Leiden, ATIII deficiency, antiphospholipid syndrome, etc). To minimize the risk for thrombotic/ thromboembolic complications, do not use eltrombopag in an attempt to normalize platelet counts. Follow the dose adjustment guidelines to achieve and maintain a platelet count of $\geq 50 \times 10^9$/L but less than 100 X 10^9/L.

Malignancies and progression of malignancies. Eltrombopag stimulation of the TPO receptor on the surface of hematopoietic cells may increase the risk for hematologic malignancies. In the controlled clinical studies, patients were treated with eltrombopag for a maximum of 6 weeks and during this period no hematologic malignancies were reported. One hematologic malignancy (non- Hodgkin's lymphoma) was reported in the ITP extension study. Eltrombopag is not indicated for the treatment of thrombocytopenia due to causes of thrombocytopenia (e.g., myelodysplasia or chemotherapy) other than chronic ITP.

Cataracts In the controlled clinical studies, cataracts developed or worsened in five (5%) patients who received 50 mg eltrombopag daily and two (3%) placebo-group patients. In the ITP extension study, cataracts developed or worsened in 4% of patients who underwent ocular examination prior to therapy with eltrombopag. Cataracts were observed in toxicology studies of eltrombopag at high doses in one study in rats. GSK were previously performing ocular exams before treatment and routinely on treatment in all of their eltrombopag studies, but has stopped this in the ongoing phase I/II MDS studies, given the lack of any increased incidence of cataracts in the eltrombopag versus the control groups in the long-term chronic ITP RAISE study. The GSK Ocular Clinical Experts Committee (which consists of external experts) have reviewed all the data from the ocular exams and for the NDA and EU submissions and have concluded that there is no evidence that eltrombopag increases the risk of cataracts

10.3.3 Adverse reactions:

As detailed on the product label: In clinical studies, hemorrhage was the most common serious adverse reaction and most hemorrhagic reactions followed discontinuation of eltrombopag.

The data described below reflects the experience of 313 patients with chronic ITP aged 18 to 85, of whom 65% were female who participated in randomized, placebo controlled studies in which patients received the drug for no more than 6 weeks. Eltrombopag was also studied in an open label single arm study in which

patients received the drug over an extended period of time. Overall, eltrombopag was administered to 81 patients for at least 6 months and 39 patients for at least 1 year.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Table 2 presents the most common adverse drug reactions (experienced by more than 1 patient receiving eltrombopag) from the placebo-controlled studies, with a higher incidence in eltrombopag versus placebo.

Table 2. Adverse Reactions Identified in Two Placebo-Controlled Studies in ITP patients per product label

Adverse effect	**Eltrombopag 50mg n = 106 (%)**	**Placebo n = 67 (%)**
Nausea	6	4
Vomiting	4	3
Menorrhagia	4	1
Myalgia	3	1
Paresthesia	3	1
Cataract	3	1
Dyspepsia	2	0
Ecchymosis	2	1
Thrombocytopenia	2	0
Increased ALT	2	0
Increased AST	2	0
Conjunctival hemorrhage	2	1

As detailed in Version 8 of the Investigator's brochure dated 4/2011: The safety and efficacy of eltrombopag has been demonstrated in two randomized, double blind placebo controlled studies in adults with previously treated ITP. Most undesirable reactions associated with eltrombopag were mild to moderate in severity, early in onset and rarely treatment limited. Adverse events among the 311 patients who received either eltrombopag or placebo included: (very common $\geq$ 10%; common $\geq$ 1% but less than 10%)

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

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

will be discontinued and the pregnancy followed and outcome reported (see section 2.4.4, non clinical toxicology).

10.3.3 Additional Eltrombopag risks

Patients with aplastic anemia are at risk of developing chromosomal abnormalities and progressing to another form of bone marrow failure syndrome called myelodysplasia. Eltrombopag stimulates stem cells and because of this there may be a risk of this process being accelerated.

10.3. 4 Related to bone marrow aspirate and biopsy: No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

10.3. 5 Related to blood draws: No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws. Infections may rarely occur.

10.3. 6 Related to SF26 questionnaire: The only anticipated adverse consequences associated with the SF36 will be the time required for the participants to complete the questionnaire.

10.4 Risks in Relation to Benefit

10.4.1 For adult subjects:

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

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

10.4.2 For children:

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

(a) the risk is justified by the anticipated benefit to the subjects: We are offering pediatric subjects, with a probably lethal hematological disease, an alternative to symptomatic therapy.

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

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

Therefore, participation of pediatric subjects on this protocol involves greater than minimal risk but

presents the prospect of direct benefit to the individual subjects (45 CFR 46.405).

10.5 Informed Consent Processes and Procedures

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient during the initial clinic evaluation. The PI, Dr. Desmond and an associate investigator on this protocol (Drs. Dunbar, Aue, Larochelle, Young, Townsley, or Dumitriu) from the Hematology Branch will lead this discussion and obtain the informed consent. The consent form will be signed in the presence of the investigator and a witness prior to commencement of the treatment plan. The treatment plan and risks will be discussed again and in detail during their hospital visit for treatment.

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

- Ideally, a copy of the consent document will be provided at the time of screening so in the event the subject is found eligible, there is sufficient time to make an informed decision or come up with questions to bring up during the telephone consent process.
- Informed consent will be obtained by Dr. Desmond or Dr. Dunbar. If not already done, a copy of the consent document will be sent to the potential subject via fax or e-mail or the U.S. Postal Service, if fax & e-mail options are not available. .
- Either the PI or the potential subject may initiate the phone call for discussion of the study after a reasonable amount of time is given to participants to review the consent document prior to telephone consent. A conference call is recommended and both parties will properly identify themselves and the purpose of the telephone call followed by a thorough explanation of the protocol by the investigator with ample time for questions related to participation.
- The potential subject will be instructed to sign and date the consent document along with the signature of an adult witness during the conference call.
- The original signed informed consent document may be faxed back (301-402-3088) or e-mailed to the PI followed by delivery of the original signed document via the US Postal Service or FedEx to Ronan Desmond, M.D., Hematology Branch, NHLBI, NIH, Building 10, Room CRC 4-5130, Rockville Pike, Bethesda, MD, 20892.
- The telephone informed consent process will be documented in the progress note by the investigator obtaining consent and a copy of the note and the original fully signed consent document will be filed in the subject's medical records with a copy provided to the subject.

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

If the subject is a minor, the parent who signs the consent for the minor must be a legally authorized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. If the minor subject is a female of childbearing age, she will be informed about pregnancy testing and will be told that if her pregnancy test is positive, we will counsel her and help her tell her parents or we will tell her parents. Is she does not agree she will be advised not to sign the assent. If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or

prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

Non-English speaking research participants: We anticipate enrolling non-English speaking research participants into this study. The IRB approved full consent document will be translated into the subject's native language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use a short form oral consent process as described in MAS Policy M77-2, 45CFR46.117(b)(2) and 21CFR50.27(b)(a). The summary that will be used is the English version of the extant IRB approved consent document.

10.6 Conflict of Interest

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

A CTA between GlaxoSmithKline and NHLBI was executed on 4/1/2009.

11.0 PHARMACEUTICALS

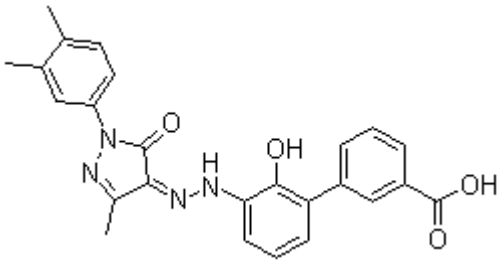
11.1 Eltrombopag (Promacta®): will be supplied by GSK as 50mg and 25 mg tablets

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

Molecular formula: C25H22N4O4.2(C2H7NO).

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

Chemical and structural formula:



Physical form: red/brown solid.

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

Supply: The drug GSK is providing for this study is investigational material (white to off-white 10.3mm standard bi-convex tablets). GSK packs investigational material in 35 tablet counts - a few days overage in case a patient is delayed returning to clinic. 25, 50 and 75 mg tablets were shipped, the 75 mg tablets as a convenience to the patient - those patients at the 75 mg level would be relieved of the pill burden of a 25 mg + 50 mg tablet.

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

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

National Institutes of Health
PHARM DEV SVC, Room 1D35
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196
Shipping Designee Name: Judith Starling, RPh
Shipping Designee Phone No: (301) 496-1031
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: jstarling@NIH.gov

Accountability Procedures: Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials which are destroyed, and copies of these documents will be provided to the Sponsor and GSK. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the Sponsor and/or GSK at the end of the study after drug accountability is performed by the study monitor.

12. REFERENCES

Reference List

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(7) Matsuda A, Kishimoto K, Yoshida K et al. Long-term follow-up of patients with aplastic anemia and refractory anemia responding to combination therapy with recombinant human granulocyte colony-stimulating factor and erythropoietin. *Int J Hematol* 2002;76:244-250.

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(10) Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1-10.

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See also: Promacta (eltrombopag) full prescribing information (March 2010) and the Investigator's brochure for compound SB-497115 (eltrombopag olamine) 2009.

APPENDIX A NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES

NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES v. 2/5/2013

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

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

C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1 ,TERC, TERT, SBDS, NOp10 , NHP2.*	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No

E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture 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