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CLINICAL RESEARCH PROJECT

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Principal Investigator:
David J Young, M.D., Ph. D., TSCBB, NHLBI (E) 301-827-7823 Bldg. 10, CRC 5-3150

Medically Advisory and Accountable Investigator:
Cynthia E. Dunbar, M.D., Chief, TSCBB, NHLBI (E) 301-496-5093 Bldg. 10, CRC 5-3332

Subjects in study:	Number	Gender	Age range
	25 eligible subjects (Accrual Ceiling 30)	M/F	2 and older

Multicenter trial:	No
Ionizing Radiation:	No (Medically indicated only)
Off-Site Project:	No
DSMB Involvement:	Yes
Tech Transfer:	Yes (Novartis, CRADA)
IND:	Yes

Synopsis

Diamond-Blackfan anemia (DBA) is a heritable bone marrow failure (BMF) syndrome characterized by selective erythroid defects typically presenting within the first year of life as a normochromic, macrocytic anemia with reticulocytopenia. More than half of all DBA cases are associated with either inherited or spontaneous mutations in ribosomal proteins, making DBA a prototypic "ribosomopathy". Furthermore, although the primary presentation is isolated anemia, as life expectancy has improved, progressive defects in other lineages have now been identified, consistent with a long-term stem cell defect. Current standard of care for DBA is the use of systemic corticosteroids, the mechanism of which is unclear, although only half show an initial response. Even when a response to steroids is observed, long-term steroid therapy carries significant morbidity, especially in children or in combination with transfusion-associated iron overload, and thus most cannot tolerate high-dose steroids long-term. Responses are rare with second-line immunomodulatory agents. Yet other than allogeneic hematopoietic stem cell transplantation in those patients with healthy matched donors, there are no alternative therapies.

In one model for DBA pathogenesis, the defects lead to an overabundance of the iron-carrying moiety heme in primitive erythroid cells, unbound by protein. Free heme is toxic to cells, likely exacerbated over time by iron overload due to transfusions. Ongoing work with eltrombopag (EPAG) has shown that it is capable of acting as a potent iron chelator, including intracellular iron, with evidence that this effect of EPAG can reverse the impact of excess heme and elevated reactive oxygen species. Furthermore, in a recent trial of EPAG for moderate aplastic anemia or hypoproliferative unilineage cytopenias, we identified a robust response to EPAG in the one DBA patient enrolled in this clinical trial. This response has been durable over more than three years since study entry but requires continuous EPAG to maintain transfusion independence. From these data, we hypothesize that EPAG may be able to improve production of red blood cells in DBA patients via chelation of iron and subsequent reduction in heme synthesis, resulting in decreased toxicity to bone marrow stem cells and developing erythroid cells.

We will conduct a single-arm, pilot trial in patients with steroid-refractory or steroid-intolerant DBA, treating with a fixed dose of EPAG for 6 months to assess safety and efficacy at improving hematological manifestations of DBA. Responders at 6 months will be able to continue EPAG on the extension part of this protocol for an additional 3 years. We will examine the hematologic, molecular, cytogenetic and clonal responses to EPAG in responders and non-responders alike. Translational studies will examine the mechanism of activity of EPAG in DBA through its effects on iron metabolism, erythroid differentiation, apoptosis, global transcriptome and TPO signaling pathways in patient's hematopoietic stem and progenitor cells (HSPCs).

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Statement of Compliance

This trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent forms, recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. Objectives

The ***primary objective*** is to evaluate safety and efficacy of eltrombopag in subjects with Diamond-Blackfan anemia.

The ***secondary objectives*** include assessments of the impact of eltrombopag upon rates of relapse and survival, clonal evolution, toxicities of extended therapy, the impact of treatment upon health-related quality of life and neurodevelopment in pediatric patients, and the impact of eltrombopag upon stem cell and erythroid cell dynamics and niche interactions including iron homeostasis.

2. Background and Scientific Justification

2.1. Pathophysiology of Diamond-Blackfan Anemia

Diamond-Blackfan anemia (DBA) is a rare, autosomal dominant (rarely X-linked) inherited bone marrow failure syndrome (BMFS) characterized by selective erythroid defects presenting early in life, usually in the perinatal period or first year of life. Mutations in numerous genes can be linked to the pathophysiology of DBA in more than 60% of cases. Among the genes identified are *GATA1* and *FLVCR1*; however, in over three-quarters of cases with an identified mutation, the disease has been conclusively linked to inherited or spontaneous mutations in 19 of the 79 genes coding ribosomal proteins (RP) (1-3). The most common of these, encompassing approximately half of all identified cases, are *RPS19* (25%), *RPL5* (7%), *RPS26* (6.6%), and *RPL11* (5%) (4). Further emphasizing the role of ribosomal biogenesis in DBA, the RPS26 chaperone protein, *TSR2* has also been linked to cases of DBA. DBA is often referred to as a prototypic "ribosomopathy", because of its intimate link to ribosomal biogenesis. That the disease arises from a general loss-of-function disruption of ribosomes is suggested by the fact that RP mutations are rarely duplicated in non-related cases. Indeed, over 80% of DBA cases present with a novel RP mutation. Although 15-20% of cases are familial with high penetrance in the most commonly affected genes, the majority of cases are sporadic heterozygous germline mutations.

As noted above, most cases of DBA, especially those linked to RP, are dominantly-inherited. More specifically, mutation of a single allelic copy is sufficient to produce disease, a condition referred to as haploinsufficiency. Homozygous inactivation of an RP gene would be expected to be embryonically lethal. Instead, the biosynthesis of ribosomes requires such precise balancing of the different components that loss of a single copy of even one gene, at least the 19 genes thus far implicated in DBA, is sufficient to cause disease (3). However, the exact mechanism by which disruption of the stoichiometry of ribosome biogenesis specifically leads to failure of erythroid maturation has remained unclear until recently.

In an emerging model for DBA, it is hypothesized that the imbalance in ribosomal subunits impacts the kinetics of the synthesis of the globin chain of hemoglobin in red blood cells. Under normal

steady-state conditions, the synthesis of the protein components of hemoglobin are exquisitely matched to that of the iron-carrying heme moiety (5). However, the ribosomal defect in the primitive erythroid cells of DBA patients leads to an asynchrony between globin chain and heme biosynthesis (6). An excess of free heme thus develops in these primitive cells, which in turn leads to an accumulation of protein-free iron. The consequence of these imbalances is the production of reactive oxygen species within the sensitive developing erythrocyte, leading to a loss of the erythroid lineage and the clinical presentation of pure red cell aplasia. In addition, a major component of DBA therapy is chronic transfusions (see §2.2), which in time cause whole-body iron loading, and likely exacerbate the iron-mediated oxidative stress in developing erythroid cells, in addition to the other clinical consequences of iron overloading, as discussed below.

Although it is only within the erythrocytes that the subtle imbalance in ribosomal biology leads directly to such a profoundly toxic metabolic disruption, it is unlikely that the ribosomal effects of DBA-associated mutations are restricted to the erythroid lineages of the bone marrow. Likely similar but more subtle disruptions in protein synthesis in other lineages, especially at critical points in embryogenesis and early development, lead to some of the phenotypic changes and dysmorphisms seen in some subtypes of DBA. Furthermore, although the primary presentation is isolated anemia, as improved supportive care has increased the life expectancy of DBA patients, progressive defects in other lineages have now been identified, consistent with a long-term stem cell defect (7). Several mechanisms are likely involved. This may partially be a consequence of the cumulative effects of disordered protein synthesis slowly disrupting other pathways, but with less toxic impact than excess erythroid heme. Additionally, there is growing evidence that the effects of heme accumulation and iron toxicity are not confined intracellularly. Erythrocytes cells develop in erythroblastic islands (EI), aggregates of developing erythroid progenitors surrounding a central macrophage. Within the context of close cellular contact, the oxidative stresses can spread from the affected progenitor, first poisoning adjacent progenitors, the associated macrophage, and eventually other primitive cells within the greater hematopoietic niche.

The role of heme biosynthesis and oxidative stress has been further supported by work on non-ribosomal DBA. *Feline leukemia virus subgroup C receptor-related protein 1* (FLVCR1), is a transport protein that facilitates the export of free cytoplasmic heme. Its mutation has been linked to a non-canonical form of DBA (8). In *Flvcr1* mutated mice, free heme cannot be efficiently cleared from the cells during hemoglobin synthesis, leading to an accumulation reminiscent of the RP-mutated forms of DBA, with similar downstream consequences (9, 10). Furthermore, work with both FLVCR1 and GATA1 have demonstrated a feedback loop between the two proteins, with the assistance of various chaperones that further regulate heme synthesis, heme efflux, and globin translation further underscoring the importance of balancing the components of hemoglobin and providing a model by which ribosome biology, cell signaling, and metabolism intersect to produce disease (11, 12).

2.2. Clinical Features and Current Treatment Options

Typically, DBA presents as an isolated, macrocytic anemia of early childhood. As it is a BMFS, this anemia is accompanied by a lack of marrow precursors and reticulocytes, circulating immature erythrocytes. In the overwhelming majority of patients (>90%), the hematopoietic defects are noted within the first year of life. Indeed, in rare, severe cases, DBA can present as fatal anemia in the neonatal period following early stressors such as the physiologic nadir, or even as hydrops fetalis. These features make up the diagnostic tetrad that defines DBA:

1. Presentation within the first year of life;
2. Macrocytic anemia without other significant cytopenias;
3. Reticulocytopenia; and
4. A paucity of bone marrow erythroid precursors, but with otherwise preserved marrow architecture.

In some cases, onset of signs and symptoms before the first year of life may only be recognized in retrospect in later childhood during disease progression, as the erythroid derangements may be subtle and asymptomatic. In other cases, the hematologic features may be part of a larger spectrum of findings that include congenital malformations, sometimes despite mild or even absent anemia. The diagnosis of DBA requires exclusion of other disorders such as transient erythroblastopenia of childhood (typically normocytic, and affecting older toddlers), dyskeratosis congenita and other telomeropathies, Fanconi anemia, Schwachman-Bodian-Diamond syndrome, infections, toxic exposures, and nutritional defects. Diagnosis may be aided by family history or positive genetic testing of known DBA-associated genes. However, as noted above, less than half of cases are inherited, a causative mutation will not be identified in approximately one-third of cases, and even when found, as many as four-fifths of all mutations will be unique to that family, complicating definitive identification. For this reason, DBA is a clinical diagnosis, supported by hematopathologic investigations.

In addition to the hematologic changes, DBA is often associated with congenital abnormalities. Whereas *RPS19* patients do not demonstrate consistent patterns of developmental defects (13), considerably higher rates of physical malformations are identified in patients with *RPL5* (83%) and *RPL11* (73%) mutations, with *RPL5* tending towards more severe phenotypes (14). These include a five- to six-fold higher rate of cardiac malformations (ranging from septal defects to tetralogy of Fallot). *RPL11* and *RPL5* mutations are strongly associated with hand defects (often thumb) and craniofacial abnormalities (especially cleft lift and palate), respectively, with similar albeit weaker associations of each mutation for the opposite spectrum of abnormalities (14, 15). Although approximately one-half of *RPS19* patients will have an identifiable congenital abnormality, there is no specific genotype-phenotype correlation, they are often milder, and

usually occur in isolation, whereas *RPL5* and *RPL11* patients frequently present with multiple abnormalities.

Although DBA presents early in life, it is a lifetime condition. This is, of course, due in part to the chronicity of the anemia and the developmental defects. However, as supportive care and treatments have improved life expectancy, it has become apparent that DBA is associated with other complications that tend to present later in life. With time, additional, progressive cytopenias may develop (7). The most frequent of these is thrombocytopenia; however, neutropenia has been reported, with some patients progressing to aplastic anemia. Additionally, DBA patients are at increased risk for developing myelodysplastic syndrome (MDS) at a rate over 300-fold higher than expected and acute myeloid leukemia (AML) at an almost 30-fold higher rate than expected, with diagnoses occurring as early as the first or second decades of life for what is typically considered an adult cancer (16, 17). Indeed, when considering hematopoietic stem cell transplant (HSCT) as definitive therapy (see below), all related donors need to be tested for DBA, even if clinical signs and symptoms are absent, as donation from an affected patient carries the risk of engraftment failure, primary graft failure even beyond the peri-transplant period, or subsequent evolution to MDS and/or AML.

DBA is a cancer-predisposition beyond hematologic malignancies. Patients are at risk for carcinomas and sarcomas, including, but not limited to, gastric and colon cancer, lymphoma, osteogenic sarcoma, and breast cancer (16-18). Yet, similar to other BMFS, oncologic treatments are often complicated by prolonged cytopenias and other chemotherapeutic toxicities. Cancer surveillance and optimal treatment modifications for these patients remains uncertain.

Effective treatment of DBA is currently limited to three options:

1. Corticosteroid therapy
2. Chronic transfusions
3. Hematopoietic stem cell transplant (HSCT)

The current standard of care for DBA is systemic corticosteroids. Ideally steroid initiation is delayed until after the end of the first year of life, to avoid developmental effects, especially in a population already at risk for altered growth and development. Initial response to steroid therapy is often within weeks of starting therapy, and is observed in 75-80% of patients (18, 19); however, primary refractory disease is seen in approximately one-fifth of patients. Even in responders, relapse or discontinuation of therapy due to side effects is common, and durable, steroid-independent remission is rare. In long-term longitudinal studies of the Diamond-Blackfan Anemia Registry (DBAR), 40% of patients remain steroid-dependent, and 40% are transitioned to chronic transfusion therapy for either steroid intolerance or relapsed/refractory disease. Only one-fifth of patients maintain remission without steroids or HSCT (18). Long-term steroid therapy carries

significant side-effects and risks including growth retardation, metabolic syndrome with hyperglycemia and diabetes, osteopenia complicated by pathologic fractures and avascular necrosis, hypertension, and cataracts.

Chronic transfusion therapy is frequently initiated for DBA due to intolerance of the long-term consequences of steroids. Although the infectious risk of a chronic transfusion regimen has been significantly minimized in recent decades, such treatments remain burdensome due to their frequency, the risk of alloimmunization (which can complicate subsequent HSCT), and iron loading. It is this iron loading, progressing to clinically significant iron overload (secondary hemochromatosis), that carries some of the greatest long-term risk for DBA patients, especially non-transplant morbidity and mortality.

Secondary hemochromatosis is a systemic complication encountered in many diseases treated via chronic transfusions including sickle cell anemia, thalassemia, and Fanconi anemia. Under normal homeostatic conditions, iron is very efficiently recycled through macrophages within the reticuloendothelial system, and as a result, the overall nutritional iron requirements in a healthy, non-bleeding adult are relatively small (approximately 1 mg daily). Even in the developing child, the nutritional requirements are easily met. However, a single unit of packed erythrocytes can contain 200-250 mg of iron, roughly equivalent to 6-8 months-worth of iron intake. Although acute transfusions are typically undertaken in the setting of blood loss, offsetting this iron infusion, in chronic transfusion, the additive consequence of these transfusions is systemic iron loading. Significant iron loading and subsequent tissue deposition can be seen after just 10 transfusions, and overload (15 mg iron per gram of liver, dry weight) is seen after 20 transfusions, a threshold typically met within the first year of starting transfusions. In the overloaded state, iron is depositing in various tissues. The most concerning organs are the liver and the heart, where dysfunction of either can be life-threatening beginning at tissue iron levels seen by the end of that first year; however, endocrine dysfunction can be significant, especially in the thyroid and pancreas.

Biopsy has historically been used to measure the liver iron concentration (LIC), and as such has served as a measure of total body iron concentration. It is generally considered the “gold standard” for assessing iron load and is more accurate and reliable than serum ferritin (20-22). However, this highly invasive technique, which carries significant risks of complication, is subject to considerable sampling error and bias, and is somewhat limited in the number of times it can be performed (23, 24). Consequently, it has fallen out of favor, especially for routine monitoring of iron loading in asymptomatic patients. For these reasons, annual surveillance with magnetic resonance imaging (MRI) has become the standard of care, especially for any patients on or transitioning to chronic transfusion therapy. The presence of iron alters the signal characteristics of effective T2 (T2*) sequences, where iron concentration leads to an inversely proportional shortening of relaxation. This shortening can be compared to established standards and from this,

LIC can be inferred. This technique has proven to be reliable, reproducible, and correlates well with traditional biopsy results (25, 26). The sequences can be obtained quickly, do not require contrast, and can even include measurements of cardiac and pancreatic iron. Thus, with increasing access to MRI, have replaced biopsy in most clinical settings, even in relatively resource-poor settings (27-29). It also has proven to be a useful tool in the research setting, monitoring the impacts of various treatments upon iron loading (30, 31).

Chelation therapy has proven revolutionary in the management of transfusion-dependent anemias including DBA, greatly reducing morbidity from the disease. Oral chelation agents (deferiprone and deferasirox) have also proven efficacious, while improving quality of life through ease of administration. However, in addition to the difficulties of adding yet another layer to the management of the disease, these agents also carry risks themselves. The most clinically significant complication is the potentially fatal agranulocytosis associated with deferiprone reported in several DBA cases and leading to an FDA black-box label (32, 33). Nonetheless, all of the chelation agents carry risks including sensorineural hearing loss and retinopathy, requiring additional clinical vigilance.

Despite these significant, well-studied complications, alternatives to chronic steroids and transfusion therapy have proven elusive. Therapies such as cytokines or immunomodulation have not proven effective and have significant side effects. Responses with second-line immunomodulatory agents used in other diseases such as aplastic anemia are rare. Ultimately, the only truly curative therapy is hematopoietic stem cell transplant. Although ongoing research into alternative donor transplants, graft-versus-host prophylaxis, and reduced-intensity conditioning have reduced the morbidity and mortality of HSCT while increasing its availability to more patients, it remains a high-risk procedure, especially in the setting of iron overload seen in DBA. Alternative therapeutic modalities for the half of patients without a durable remission are greatly desired.

2.3. Thrombopoietin and Hematopoiesis

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

Several lines of evidence support a pleiotropic role for TPO in hematopoiesis, beyond its role as the primary endogenous factor controlling platelet production from megakaryocytes. TPOR is expressed and functional on primitive HSPCs (34). Animals and patients with genetic defects in either TPO or TPOR have significant reduction in HSPC numbers and activity, along with profound defects in platelet production (35, 36). *In vitro* expansion of functional and phenotypic HSPCs can

be stimulated by TPO, either alone or in combination with other cytokines. Furthermore, TPO has been shown to mobilize a primitive subset of CD34+ cells in cancer patients with normal hematopoiesis (37, 38). The control of TPO levels is complex, and involves TPOR occupancy, with levels generally inversely proportional to megakaryocyte mass. Recently, the Ashwell-Morell receptor has been shown to regulate the hepatic thrombopoietin production by removal of desialylated senile platelets (39).

A slightly modified form of recombinant TPO, termed megakaryocyte growth and development factor (MGDF), was in clinical development by Amgen in the late 1990s. It clearly stimulated platelet production in vivo in healthy control individuals and in patients on chemotherapy, but its development came to a halt when both normal volunteers receiving MDGF prior to donating platelets and cancer patients receiving MGDF following chemotherapy developed neutralizing antibodies after several cycles of MGDF dosing, which reacted not only to MGDF but also to endogenous TPO, causing profound persistent thrombocytopenia (40, 41). It was shown that MGDF was acting as an immunogen due to modification from endogenous TPO, with epitope spreading eventually resulting in autoimmune destruction of endogenous TPO.

As mentioned earlier, proinflammatory cytokines negatively impact HSPC stemness and thus contribute to pancytopenia in bone marrow failure syndromes and potentially FA. Interferon gamma (IFN-γ) reduces proliferation but accelerates differentiation of progenitor cells. However, differentiation is skewed toward myelopoiesis at the expense of erythropoiesis. This effect appears to be associated with upregulation of key hematopoietic transcription factors. Inhibition of TPO signaling (e.g. STAT5) by the suppressor of cytokine signaling (SOCS) results in reduced self-renewal and proliferation of HSPCs in patients with BM failure (42). Interestingly, TPO has also been shown recently to improve the DNA-damage response in HSPC by increasing DNA-PK dependent nonhomologous end-joining efficiency (43, 44).

2.4. Eltrombopag

Eltrombopag (SB-497115-GR, Promacta®), the bis-monoethanolamine salt form, is an orally bioavailable, small molecule 2nd generation thrombopoietin receptor (TPOR) agonist, developed for the treatment of thrombocytopenia by scientists at GlaxoSmithKline (now Novartis) (45). Studies conducted in vitro have shown that eltrombopag is an effective agonist binding to TPOR, the TPO receptor encoded by the *MPL* gene, to stimulate thrombopoiesis. It binds TPOR at a position distinct from the ligand binding site, within the juxtamembrane domain of the receptor, and thus does not compete with TPO for binding to its receptor (46). The differences in binding to the receptor may theoretically result in activation of different signaling pathways from native TPO, however, data indicate similar impact on megakaryocytes and stem cells comparing EPAG to TPO (47).

In vivo, EPAG increased platelet numbers in chimpanzees (the only non-human species which is pharmacologically responsive to eltrombopag) (47). These findings, coupled with supporting clinical efficacy data in humans, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous TPO. Initial clinical trials were carried out in normal volunteers, and then in patients with chronic ITP, based on their inappropriately low or low-normal levels of endogenous TPO (48). The initial phase I/II and randomized, controlled phase 3 registration trials in chronic ITP were very encouraging, with little toxicity and much higher responses in comparison with placebo (49-51), which led to its approval by the Food and Drug Administration (FDA) on November 20, 2008 for patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. Eltrombopag was the first oral TPO-R agonist approved for adult patients with chronic ITP. In November 2012, FDA approval was received for hepatitis C associated thrombocytopenia.

In analogy to TPO, EPAG appears to stimulate HSC proliferation and self-renewal, and was thus utilized by our group in a clinical trial for patients with refractory acquired SAA and shown to result in clinically-meaningful responses in about 50% (52, 53). Furthermore, the fact that the responses were sustained even after EPAG was discontinued supports its direct effect on the stem cell compartment in SAA patients. Based on these results in patients with SAA, EPAG was FDA approved for refractory SAA in August 2014, at an adult dose of 150 mg/day, higher than the doses utilized in ITP (52, 53). TPO levels are already markedly elevated in SAA patients, suggesting additional mechanisms leading to HSC expansion with EPAG treatment. Although EPAG's function appears to be dependent on TPOR, it may activate the receptor differently than endogenous TPO (54-56). The different activation may result in the activation of distinct pathways favoring HSC fitness. Indeed, differences in signaling pathways between TPO and eltrombopag have been observed in platelets (47). In addition, our recent investigations indicate that EPAG can bypass the inhibitory effect of IFN-γ on TPOR signaling (57).

Longer follow-up in the ITP EXTEND trial (NCT00351468) suggests that eltrombopag remains well tolerated. On December 6, 2011 Novartis announced that the FDA agreed to modify eltrombopag's Risk Evaluation and Mitigation Services designed to assure safe use of the novel agent and removed the requirement for healthcare professionals and institutions to enroll in the Promacta Care Program (Promacta® Package Insert, 2011). Monitoring of adverse events for eltrombopag will be continued via post-marketing surveillance programs and ongoing clinical trials, rather than a formal prescriber enrollment program. There has been no suggestion of an increased risk of myelodysplasia or any other clonal bone marrow disorder in patients treated on the EXTEND Trial for ITP.

2.4.1. Rationale for eltrombopag in DBA

In a recent trial of eltrombopag (EPAG) for moderate aplastic anemia or hypoproliferative

unilineage cytopenias (NCT01328587, 11-H-0134), it was with great interest that we identified a patient with a clinical phenotype consistent with DBA and a de novo mutation in *RPS19* who demonstrated a robust response to EPAG despite being refractory to prior therapeutic modalities (58). This response has been stable over three years of treatment but requires continuous EPAG to maintain transfusion independence (Figure 1). Our experience has led us to further examine the mechanism of TPO and TPO-independent pathways in treating DBA.

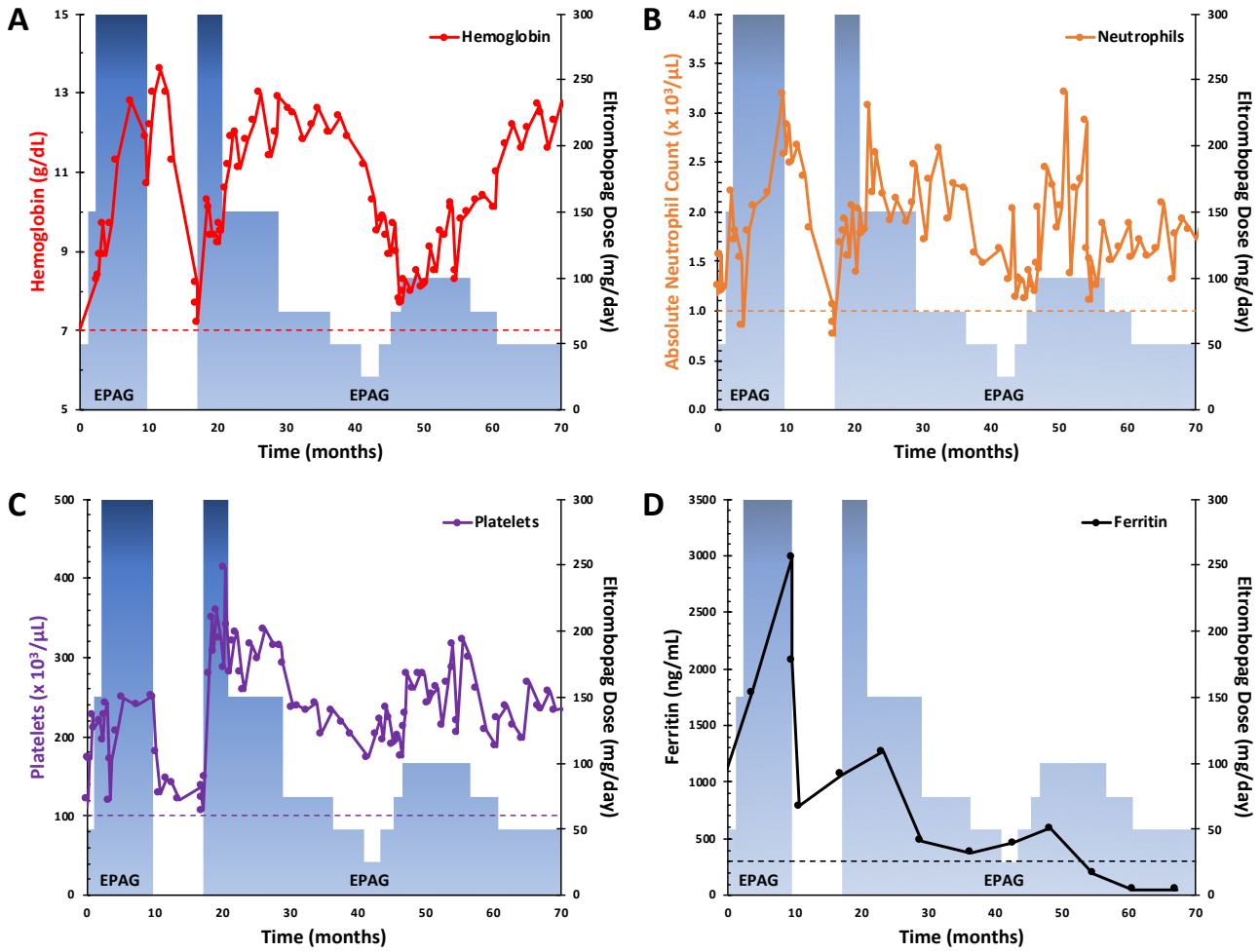


Figure 1: Hematologic response of a DBA patient to eltrombopag therapy. (A) Untransfused hemoglobin (red), **(B)** absolute neutrophil count (orange), **(C)** platelets (purple), and **(D)** ferritin (black) are plotted as a function of time from study enrollment. Periods of treatment with eltrombopag are indicated by the blue shaded areas, with height indicating dose (range 25-300 mg daily). Treatment had been discontinued after 10 months upon reaching endpoint goals, but was restarted at 17 months when counts fell below threshold, and has since been titrated according to hematologic response. **(58)**

2.4.1. Eltrombopag improves trilineage hematopoiesis in a TPOR-independent manner

The trilineage response of patients to EPAG, despite the drug being an agonist directed primary towards the megakaryocytic lineage, suggests that EPAG may have multiple effects *in vivo*. Work by Kao *et al.* is of particular note as they demonstrated that EPAG is capable of stimulating hematopoietic stem cells in mice (59). This is notable because prior work has clearly demonstrated that the mouse receptor does not bind EPAG. Furthermore, romiplostim, an unrelated TPOR agonist, does not have this effect in mice. These results are suggestive of an EPAG-specific mechanism, independent of the TPOR found on megakaryocytes and primitive stem cells. For example, recent work by Guenther *et al.* has suggested that EPAG may improve hematopoiesis by improving the DNA damage response in hematopoietic cells (60). Others have examined the interaction of EPAG with the inflammatory milieu (57).

2.4.2. Eltrombopag has clinically significant iron chelation activity *in vivo*

However, it is the iron-binding capabilities of EPAG that present the most promise for DBA patients, and likely explain the effects seen in our patient. Our work with EPAG has shown that it is capable of mobilizing intracellular iron and acting as a potent chelator, with patients on EPAG long-term dropping levels of storage iron over time (61). Indeed, EPAG has demonstrated a higher affinity for iron than clinically available chelators (62, 63). EPAG can mobilize iron from multiple different cell types at clinically relevant concentrations. Notably, EPAG can mobilize bone marrow iron observed in transfusion-related iron overload (64). Both orally available chelators (deferasirox and deferiprone) are also able to penetrate the cellular membrane due to their smaller size and chemical properties unlike deferoxamine (65-68). As such these agents are believed to have some intracellular chelation activity, especially within the heart, but this activity is highly variable, tissue-dependent, and secondary to their main mechanism of action (69, 70). Indeed, continuous infusion deferoxamine remains the highly effective gold standard in the treatment of cardiac hemochromatosis despite its lack intracellular activity. These different agents are reviewed in (71). Therefore, a more potent intracellular chelator could have significant clinical impact.

Our own experiences with EPAG have confirmed that it is a potent chelator of iron and can mobilize whole body iron stores even in transfusionally iron-overloaded patients (61). Specifically, in patients on EPAG for at least 4 months, whole body iron as measured by serum ferritin was decreased, especially in patients treated on an extended access protocol (Figure 2), including our index DBA patient (Figure 1D). Paradoxically, in patients being treated with EPAG, serum iron levels are actually elevated, reflecting the mobilization of the intracellular iron into the blood stream, from which it is ultimately cleared and lost via hepatic processing of the iron-bound drug. Indeed, in several patients treated on extended doses of EPAG (average 2 years of drug exposure), iron supplementation was required due

to the development of iron deficiency anemia in the face of an otherwise strong response to EPAG. Several investigations into the metabolic effects of EPAG in cells have demonstrated that through the depletion of iron, there is a concomitant reduction in the toxic reactive oxygen species (59, 63, 72, 73).

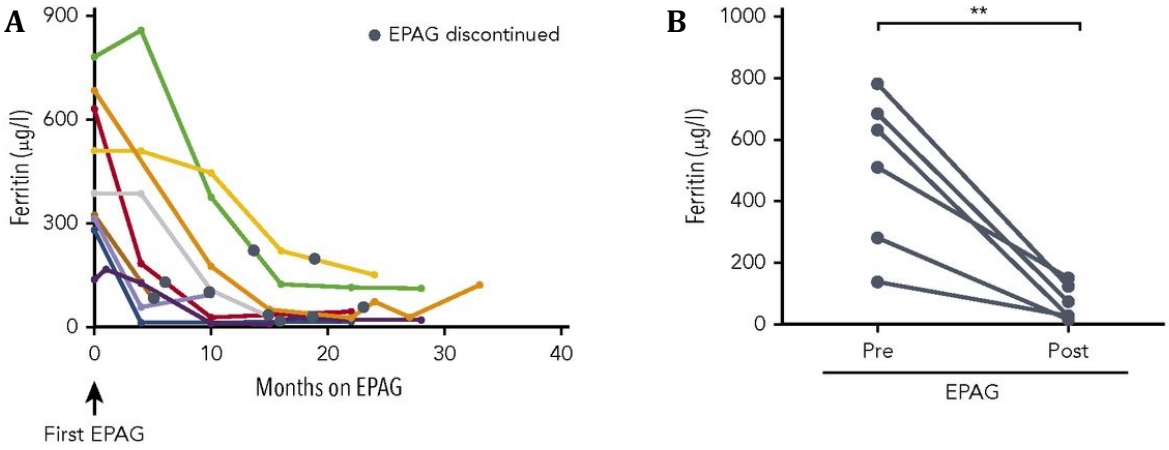


Figure 2: Impact of eltrombopag upon iron stores in patients treated for aplastic anemia. (A) Ferritin levels in patients with EPAG treatment for at least 4 months. (B) Comparison of ferritin levels pretreatment and after EPAG discontinuation in 6 patients with prolonged EPAG treatment and ferritin measurements available at follow-up. Student's *t* test: $^{**}P = .005$ (61)

Given these findings of intracellular iron depletion, improvement in reactive oxygen species levels, and the clinical response we have previously seen in our DBA patient, we hypothesize that through its iron chelating properties, likely in combination with its stimulatory effects upon TPOR, EPAG may be able to offset the toxic effects of aberrant hemoglobin biosynthesis in DBA patients, increasing erythroid production, and ultimately allowing HSC self-renewal, thus improving anemia, preventing the long-term hematologic consequences of DBA, and even avoid the significant toxicities of transplant. By chelating and subsequently removing total body iron effectively, we hypothesize that EPAG will also improve organ function and overall long-term outcomes in DBA patients previously experiencing iron overload as a consequence of prior transfusion therapy.

2.4.3. Rationale for dose selection

Eltrombopag 150 mg once daily in adults or the equivalent in children has been selected as the starting dose for this study because this dose regimen has been safe and effective in increasing platelet counts in our recently-completed non-randomized, off label, phase II study (NCT00922883) of EPAG as a single agent in patients with refractory SAA. Forty-three (43) patients (age range 18-77 years) received 50 mg daily of EPAG with dose escalation every two weeks to a maximum dose of 150 mg daily. Patients were successfully escalated to the 150mg daily dose without dose-limiting toxicities. Hematologic responses

were only observed while patients were receiving the 150mg daily dosing. While it is possible that patients would have responded to lower doses of drug had the dose not been escalated every two weeks, the lack of toxicity at the 150 mg/day dose in the prior study supports our rationale for utilizing this dose throughout the current trial, unless dose modifications based on toxicity or response criteria are required. A starting dose of 75 mg once daily in East Asian and South East Asian patients will be used. Modified dosing for subjects of East Asian and South East Asian heritage (self-reported) has been implemented for the following reasons. In healthy Japanese subjects, plasma eltrombopag $AUC_{(0-\tau)}$ was approximately 80% higher when compared with non-Japanese healthy subjects who were predominantly Caucasian. Similarly, in patients with ITP, plasma EPAG levels were approximately 70% higher in East Asian and South East Asian subjects as compared with non–East Asian subjects who were predominantly Caucasian as higher drug exposure in East Asian and South East Asian subjects has been observed.

The most extensive data on dosing and long-term side effects have been obtained in patients with chronic ITP. An initial randomized phase 2 trial, followed by two randomized phase 3 trials all showed efficacy for EPAG compared with placebo for increasing the platelet count utilizing doses of up to 75 mg per day (49, 50, 74). In ITP subjects, there was a dose response for EPAG 30 mg to 75 mg once daily, with geometric mean $AUC_{(0-\tau)}$ values of 169 μg/mL for the 75 mg once daily regimen. There was no significant difference between the safety profile of ITP subjects receiving 30, 50 or 75 mg of EPAG. A recent update of the EXTEND study reported on 302 patients followed up to 5.5 years on EPAG therapy for chronic ITP, with a median exposure of 121 weeks and median dosing of 51.4 mg/day. Forty-three (43) patients (14%) discontinued therapy due to adverse events, including headaches, thromboembolic events, or hepatobiliary laboratory abnormalities. An independent central pathology review of bone marrow biopsies did not reveal significant increase in reticulin deposition.

Thrombocytosis is a theoretical risk of EPAG treatment when high dosages are administered. However, thrombocytosis has not been observed in the 43 patients with refractory SAA who were treated with 150 mg per day nor in any of our other EPAG protocols (09-H-0199, 11-H-0134, 12-H-0150 and 13-H-0133). Thrombocytosis has been observed in healthy volunteers as well as in subjects with ITP, and there was a suggestion of a higher risk of thrombosis in patients on EPAG compared with placebo in the phase III randomized trials for chronic ITP(50). However, patients with ITP, in contrast to patients with DBA, have hyper-reactive platelets and an increased endogenous risk of thrombosis. In an extensive analysis of ITP patients treated long-term with romiplostim, an alternative TPO mimetic, there was no evidence for increased thrombotic events in the romiplostim-treated patients compared with controls (75). In a meta-analysis of randomized trials using either EPAG or romiplostim, there was a numerically but non-statistically significant trend

to increased occurrence of thromboembolisms compared with controls (76). In the current trial, based on concerns regarding thrombosis, dose reductions of EPAG will be made if the platelet count reaches 600,000/μL or greater.

2.4.4. Rationale for pediatric dose selection

For pediatric subjects, there is a predicted higher weight-adjusted drug clearance than older children and adults based upon studies of several drugs approved for use in children, such as anticonvulsants, proton pump inhibitors, theophylline, and HIV protease inhibitors, have routinely demonstrated that young children have higher weight-adjusted drug clearance than older children and adults (77-81). In the PETIT trial, a phase II pediatric chronic ITP study, subjects between 1 and 5 years received a mean average daily dose of 2.2 mg/kg EPAG (mean max 2.9mg/kg), while subjects between 6 and 11 years of age received an average daily dose of 54.8 mg daily while subjects aged 12-17 year received 57.3mg EPAG. The maximum dose used in the PETIT trial among all age groups is 75 mg daily. Non-Asian patients aged 1-5 years, that were treated in the subsequent randomized, multicenter, placebo-controlled study PETIT2, started with 1.2 mg/kg/day. The median average dose per day was 3.4mg/kg (42.8 mg) in this cohort and 56.9mg/kg in patients aged 6-11 years. Non-East Asian patients aged 12-17 years were treated with a median average dose per day of 69.0 mg. No new pediatric specific safety signal has been identified thus far.

An analysis of combined pharmacokinetic data from the PETIT and PETIT2 trials, encompassing 168 pediatric patients with ITP aged 1 year and older, demonstrated that clearance increases with body weight. Notably, patients in the 1-5 year age group experience approximately 60% higher exposure to drug ($AUC_{(0-\tau)}$ 162 μg·hr/mL 95% CI: 139-187; C_{max} 11.6 μg/mL 95% CI: 10.4-12.9) compared with adults ($AUC_{(0-\tau)}$ 101 μg·hr/mL 95% CI: 91.4-113; C_{max} 7.03 μg/mL 95% CI: 6.44-7.68); whereas teenagers, aged 12 to 17 years, experience exposure ($AUC_{(0-\tau)}$ 103 μg·hr/mL 95% CI: 91.1-116; C_{max} 6.80 μg/mL 95% CI: 6.17-7.50) comparable to adults. Older children and pre-adolescents, aged 6 to 11 years, experience an intermediate exposure ($AUC_{(0-\tau)}$ 153 μg·hr/mL 95% CI: 137-170; C_{max} 10.3 μg/mL 95% CI: 9.42-11.2) that is nonetheless similar to toddlers and young children (82).

The available platelet count, safety, and PK data available for subjects enrolled in the PETIT trial support a starting dose of 2.5 mg/kg once daily for non-East-Asian subjects aged 2-5 years (82-84). Older pediatric patients will start at a fixed total dose; however, as they are expected to experience a higher pharmacologic exposure compared to adults, the starting dose for non-East-Asian subjects, aged 6-11, will be 75 mg daily. Patients 12 years and older will start at the adult dose of 150 mg daily.

2.4.5. Rationale for permitting dose interruption

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

2.4.6. Rationale for treatment duration (primary endpoint 6 month), extended access to EPAG for responding patients and slow tapering of EPAG during this extension part of the study

Eltrombopag is safe and relatively non-toxic with prolonged use. The EXTEND study (NCT00351468), an open label dose modification extension study evaluating the safety and efficacy of extended therapy of EPAG in ITP subjects, has been designed to assess long-term effects of EPAG therapy. At the most recent analysis of 302 patients (presented at EHA, 2016) the median average daily dose was 50.4 mg. The median duration of exposure to EPAG was 2.4 years (range, 2 days to 8.8 years). EPAG was well-tolerated, and both bleeding and clinically-significant bleeding decreased from baseline at all time points. AEs and SAEs occurred in 92% and 32% of patients respectively. SAEs considered possibly drug-related occurred in 8% of patients. AEs leading to withdrawal occurred in 14% of patients. The most frequent AEs leading to withdrawal were increased ALT (*n*=5), increased bilirubin (*n*=4), cataracts (*n*=4), and DVT (*n*=3). Nineteen patients (6%) reported thromboembolic events (TEEs), and 37 reported hepatobiliary laboratory abnormalities. ITP patients have return of their platelet counts to baseline within 1-2 weeks of discontinuation of drug.

In our cohort of 43 patients treated for refractory severe aplastic anemia (SAA), responses only began to be observed at the 3-month time point, and with continued exposure to drug, blood counts of all lineages improved towards the normal range gradually. Maximal response was not reached at 6 months with bone marrow cellularity normalizing by 9-15

months. Eighteen of 43 patients with refractory SAA had drug discontinued for robust response. Only 2 patients showed a decrease in counts clearly related to the discontinuation of EPAG. Re-initiation of EPAG restored hematopoiesis in both patients. However, EPAG was discontinued in one patient because of an intermittently detected cytogenetic abnormality. Since then the counts have remained stable and a repeat bone marrow biopsy showed a normal karyotype. These results suggest that the effect of EPAG on HSPC number or function is long-lasting and prolonged therapy may not be required in SAA.

In patients with DBA the HSPC potential is also reduced but, unlike acquired BMFS, HSPC in subjects with DBA carry inherited mutations impacting hemoglobin synthesis (§2.1). Assuming no increased rates of spontaneous somatic reversion of inherited mutations, a rare but reported event (85-88), resulting in a selective growth advantage of corrected HSCs, hematopoiesis in patients with DBA will continue to rely on HSPC with the underlying genetic defect. Therefore, it is likely that patients with DBA will require continuing or repeated EPAG treatments to improve their anemia. Yet, the lowest dose and shortest exposure able to improve cytopenias into a safe range would be the goal for EPAG therapy in this patient population. Based on the response kinetic of our previous (52, 53) and ongoing EPAG studies in patients with refractory aplastic anemia (13-H-0133, NCT01891994), we hypothesize that 6 months of EPAG treatment will be sufficient to assess for a clinically meaningful response also in DBA patients.

Thus, in this study, to determine the lowest optimal dose of EPAG in patients responding to the drug, we plan a slow taper of EPAG in robust responders or subjects who reach steady state response defined as stable counts for 6 months (see §5.3, Table 3). Toxicity and efficacy data will continue to be collected during that time to help identify the secondary endpoints of duration of response, progression to clonal hematopoiesis, and toxicities with extended duration of therapy.

2.5. Scientific and clinical justification of the protocol

Without treatment, DBA progresses to critical anemia in over four-fifths of all cases. Even with treatment, approximately half of patients will either fail to respond, do not have a durable response, or are unable to continue therapy due to steroid-associated toxicities. For these patients, chronic transfusions with the risk of cardiovascular, hepatic, endocrinologic, and other systemic organ dysfunction and a major impact on quality of life is the only management option with proven benefit. Yet, even with chelation therapy, due to long-term toxicities of chelators and iron loading, coupled with the effects of chronic anemia upon growth and development, cardiovascular health, and neurodevelopment, leads to a reduced life expectancy in DBA. HSCT remains the only definitive treatment for the hematologic complications of DBA. Yet, despite significant advances in

preparative regimens, supportive care, and management of complications, HSCT continues to carry significant morbidity and mortality, especially in the heavily pre-transfused population. In addition, many patients do not have available and sufficiently-matched related or unrelated donors. As a result of these complications of the disease and a paucity of effective alternative treatments, novel treatments for patients with DBA are acutely needed.

As discussed above (§2.1), DBA represents a spectrum of diseases caused by a variety of mutations in at least 21 genes affecting multiple different cellular pathways, but all converging on an excess of iron-containing heme pigment within the erythroid progenitors leading to iron accumulation and subsequently toxic levels of reactive oxygen species. With time, the accumulation of toxic intracellular and extracellular species causes a general bone marrow failure, leading to progressive cytopenias and bone marrow failure. Additionally, the chronic transfusions used to treat the most-affected of these patients further increases the marrow iron loading, exacerbating the cellular damage in addition to the non-hematologic complications of therapy.

Eltrombopag, in addition to stimulating the proliferation and differentiation of hematopoietic lineages, is a potent chelator of intracellular and extracellular iron, with activity demonstrated both *in vitro* and *in vivo* (§2.4.2). We hypothesize that this iron-depleting activity reduces the intracellular iron burden and thus the oxidative stresses upon the progenitor cells, thus leading to the clinical response we have seen in our single previously-treated DBA patient (§2.4.1). Therefore, based upon safety evidence provided by prior clinical trials and efficacy suggested by pre-clinical data and our clinical experience, we propose this study to demonstrate that by targeting the common pathway of DBA, this effect should be generalizable to all DBA patients and thus provide a novel treatment paradigm for DBA that will reduce or eliminate the transfusion burden and steroid side effects that plague DBA patients.

DBA, as noted above, is a cancer-predisposition syndrome (§2.2) including both solid tumors and hematologic malignancies. Although the biology of this predisposition is poorly understood, it likely represents genotoxic stress from the same toxic processes that lead to erythroid aplasia. In the marrow, the combined hematopoietic stresses of ineffective hematopoiesis, ongoing cellular damage leading to cytopenias and likely driving clonal progression, oxidative stress of free heme, and increased total body iron burden, especially within the marrow compartment, predisposes to dysplasia and malignant transformation. Although beyond the immediate scope of this trial, we hypothesize that by reducing these combined stresses, EPAG treatment will reduce the risk of hematopoietic and even non-hematologic malignancies in DBA. Furthermore, by reducing the need for HSCT, EPAG may have the secondary effect of reducing the risk of treatment-related morbidity, malignancies, and mortality.

The primary objective of this trial will be to evaluate the safety and efficacy of eltrombopag as a single agent in subjects with Diamond-Blackfan anemia, especially patients with relapsed disease,

refractory disease, or patients who cannot tolerate ongoing standard therapies. The secondary objectives of this trial will include assessments of the impact of eltrombopag upon rates of relapse and survival, clonal evolution, toxicities of extended therapy, the impact of treatment upon health-related quality of life and neurodevelopment in pediatric patients, and the impact of eltrombopag upon stem cell and erythroid cell dynamics and niche interactions including iron homeostasis.

3. Study Design

The study is designed as a single-site, non-randomized, single-arm pilot study of the oral TPOR agonist eltrombopag (EPAG) in Diamond-Blackfan anemia (Figure 3) to test the hypothesis: ***eltrombopag improves the production of red blood cells in Diamond-Blackfan anemia patients.*** The primary endpoint (see §8.2) is measured at 6 months (24 weeks ± 14 days). Subjects who cannot tolerate the medication or fail to respond by the primary endpoint will be taken off study drug. Subjects who ***respond*** at the primary endpoint (hemoglobin increase of 1.5 g/dL or more and/or transfusion independence for the previous 8 weeks, see §8.2.1) will be able to continue EPAG on the extension part of this protocol for an additional 3 years. During the 3-year extension phase, subjects will remain on the same dose of EPAG until they reach a ***robust response*** (defined as hemoglobin ≥10 g/dL in the absence of transfusions for more than 8 weeks, see §8.2.1). Drug dose will then be tapered slowly to the lowest dosage that maintains a stable hemoglobin (≥ 10 g/dL) and eventually discontinued if possible (per §5.3), until they meet off study criteria (see §8.6), or the study is closed. Drug will be restarted during extension if robust response is lost (*i.e.* hemoglobin falls below 9 g/dL).

Up to 30 subjects (18 for Stage 1 & 12 for Stage 2) will be screened to accrue 25 eligible subjects for this study.

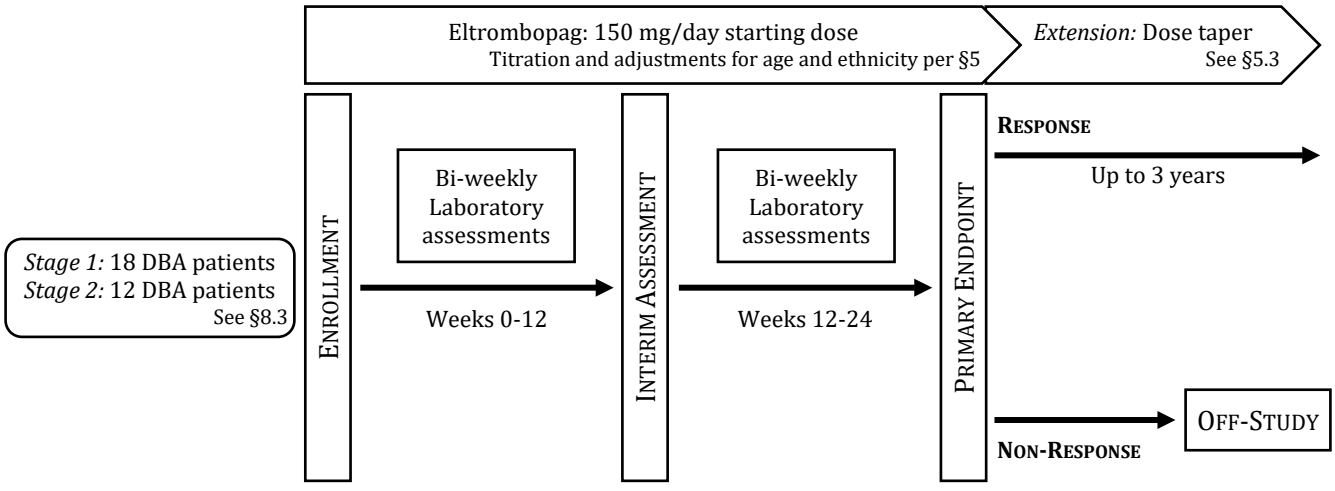


Figure 3: Study enrollment and treatment schema

4. Eligibility Assessment

4.1. Inclusion criteria

In order to be participate in this study, individuals must meet all of the following criteria:

4.1.1. Diamond-Blackfan anemia defined as anemia presenting on or before the third year of life with reticulocytopenia and greatly reduced or absent bone marrow erythroid precursors, supported by, but not requiring either:

- familial history
- gene mutation testing demonstrating a known disease-causing mutation or a mutation of disease-associated gene in combination with clinical characteristics of DBA

Patients with late-onset DBA (diagnosed after the third year of life) may also be included if gene mutation testing confirms a disease-causing mutation as above.

4.1.2. Clinically-significant anemia as defined as either:

- hemoglobin less than 9.0 g/dL
- red cell transfusion of at least 2 units PRBC for adults or 30 cc/kg for children (whichever is less) in the eight weeks prior to study enrollment

4.1.3. Relapsed and/or steroid-refractory or intolerant of systemic corticosteroids

4.1.4. Age ≥ 2 years

4.1.5. Weight ≥ 12 kilograms

4.1.6. Residence within the United States of America or territories, or able to reside within the US or its territories while on drug during trial participation

4.2. Exclusion criteria

Individuals meeting any of the following criteria will not be eligible for participation in this study. However, having met exclusion criteria in the past does not preclude study participation if the criteria are no longer met, unless otherwise specified (*i.e.* patients with modifiable factors such as laboratory abnormalities or acute health problems may be re-screened). For laboratory assessments, this requires no less than two weeks from the previous exclusionary finding. The intervals for health problems that must elapse prior to re-screening are specified below.

4.2.1. Platelet count > 400,000 / μL

4.2.2. Stage 4 or greater kidney disease as defined by creatinine > 2.5 mg/ dL or GFR < 30 mL/min/1.73 m^2

4.2.2.1. For pediatric patients 17-years-old or younger, GFR shall be used. This can be estimated using the bedside Schwartz equation, the Counahan-Barratt method, or a similar methodology. Direct measurement including, but not limited to, 24-hour urine creatinine clearance or radiographic methods is recommended for patients with stage 3 disease (GFR ≤ 45 mL/min/1.73 m^2).

4.2.3. Direct bilirubin > 2.0 mg/ dL, including congenital abnormalities in the bilirubin level

4.2.4. SGOT (AST) or SGPT (ALT) > 5 times the upper limit of normal

4.2.5. Treatment with androgens (danazol or oxymetholone) or corticosteroids less than 4 weeks prior to initiating eltrombopag.

4.2.5.1. Physiologic steroid replacement for adrenal insufficiency or other similar conditions is not exclusive of trial participation

4.2.6. Treatment with any medications that may interfere with the metabolism of eltrombopag (e.g., CYP1A2 and CYP2C8 modulators) or whose own altered metabolism by eltrombopag cannot be adjusted for (see §5.8, 5.9, and 10.3.1)

4.2.7. Hypersensitivity to eltrombopag or its components

4.2.8. Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy, or that death within 7-10 days is likely

4.2.9. Life expectancy of less than 3 months for any cause

4.2.10. Subjects with known liver cirrhosis in severity that would preclude tolerability of eltrombopag as evidenced by albumin < 3.5g/dL

4.2.11. History or current diagnosis of cardiac disease indicating significant risk of safety for patients participating in the study such as uncontrolled or significant cardiac disease, including any of the following:

- Recent myocardial infarction (within last 6 months),
- Uncontrolled congestive heart failure,

- Unstable angina (within last 6 months),
- Clinically significant (symptomatic) cardiac arrhythmias (e.g., sustained ventricular tachycardia, and clinically significant second or third-degree AV block without a pacemaker.)
- Long QT syndrome, family history of idiopathic sudden death, congenital long QT syndrome or additional risk factors for cardiac repolarization abnormality, as determined by the investigator
- Impaired cardiac function such as corrected QTc>450msec using Fridericia correction on the screening EKG, other clinically significant cardio-vascular disease (e.g. uncontrolled hypertension, history of labile hypertension), history of known structural abnormalities (e.g. cardiomyopathy).

4.2.12. Known active or uncontrolled infections not adequately responding to appropriate therapy.

4.2.12.1. HIV infection is not exclusive of trial participation if the infection is effectively controlled with medications not known to interfere with eltrombopag metabolism or be metabolized by pathways known to be altered by eltrombopag (as per §4.2.6). HIV RNA viral load must be undetectable at the time of enrollment, and CD4 cell count must be ≥ 200/μL. Patients must remain on antiretroviral therapy throughout study participation and must be periodically monitored for suppression of viral load and CD4 cell count. If drug-drug interactions between antiretroviral medications and eltrombopag are suspected, these must be addressed by a qualified clinical pharmacist or pharmacologist, and any changes to antiretroviral therapy need to be approved in consultation with an Infectious Disease and/or HIV specialist prior to enrollment.

4.2.13. Active malignancy or likelihood of recurrence of malignancies within 12 months

4.2.14. Evidence for MDS or AML as defined by WHO criteria.

4.2.15. Patients who have received chemotherapeutic treatment or other specific antineoplastic drugs or radiation therapy within 6 months of study entry

4.2.16. Female subjects who are nursing or pregnant (positive serum or urine β-human chorionic gonadotrophin (b-hCG) pregnancy test) at screening or pre-dose on Day 1.

4.2.17. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of

contraception during dosing and for 30 days after the last dose of eltrompobag. Highly effective contraception methods include:

- Total abstinence (when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
- Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male sterilization (at least 6 months prior to screening). For female patients on the study the vasectomized male partner should be the sole partner for that patient.
- Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS), or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception.
- In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment.
- Women are considered post-menopausal and not of child bearing potential if they have had over 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile age appropriate (e.g. generally 40-59 years), history of vasomotor symptoms (e.g. hot flushes) in the absence of other medical justification or have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment should she be considered not of childbearing potential.
    - Sexually active males unless they use a condom during intercourse while taking the study treatment and for 30 days after stopping study treatment and should not father a child in this period. A condom is required to be used also by vasectomized men as well as during intercourse with a male partner in order to prevent delivery of the drug via seminal fluid.

4.2.18. History of thromboembolic events other than catheter-related thromboses

4.2.19. Active alcohol/drug abuse

4.2.20. Unable to understand the investigational nature of the study or give informed consent and does not have a legally authorized representative or surrogate that can provide informed consent

4.2.21. Unable to take investigational drug

4.2.22. Concurrent participation in an investigational study within 30 days prior to enrollment or within 5-half-lives of the investigational product, whichever is longer. Note: parallel enrollment in a disease registry is permitted.

5. Treatment Plan

5.1. Administration of study drug (eltrombopag)

Subjects will initiate EPAG at a starting daily dose as detailed below, according to age and ethnicity. Subjects 12 years of age and above will receive the adult dose of 150 mg. Those between 6 and 11 will start at 75 mg, and children 2 and 5 years of age will start at 2.5 mg/kg, not to exceed 75 mg (Table 1).

To adjust for the higher expected exposure in subjects of East Asian and South East Asian ancestry, the starting dose for East Asian and South East Asian subjects 12 years of age and above will be 75 mg once daily. For East Asian and South East Asian subjects between 6 and 11 years of age, the starting dose will be 37.5 mg once daily, and for children between 2 and 5, the starting dose will be 1.25 mg/kg (Table 1).

Table 1: Starting daily dose of eltrombopag according to age and ethnicity

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

Eltrombopag dose may be interrupted when clinically indicated at the discretion of the investigator. If EPAG dosing is delayed or interrupted for more than 1 week (7 days) and the interruption is not the result of a severe adverse event related to EPAG, then the subject will receive EPAG for additional time in order to receive a total of 24 weeks as originally planned. Interruptions shall be recorded in the medical record. Whether interruptions are reported (e.g. as deviations or adverse events) shall be determined as per Policy 801.

Medication dosing errors, dose delays or dosing interruptions:

Interruptions such as delays in request for medication refills or medication errors by subjects, unless they result in a serious adverse event or impact the integrity of the research data, will not be considered as deviations, but will be recorded in the medical record.

5.2. Dose adjustments of eltrombopag

Table 2: Criteria for the adjustment of the daily dose of eltrombopag

Platelet Count	Dose Adjustment or Response
≥400,000/μL (untransfused) at any time on study	Decrease dosage by 25 mg/day (or 12.5 mg in children under 12) every 2 weeks (± 3 days) to highest dosage that maintains hemoglobin ≥9 g/dL without increasing platelet count ≥400,000/μL. For subsequent elevations over 400,000/μL, the dose may be reduced by up to 50%.
≥600,000/μL (untransfused) at any time on study	Discontinue eltrombopag for one week (± 3 days); when platelets fall to <400,000/μL, restart at dosage decreased by at least 25 mg/day (or 12.5 mg in children under 12) or up to 50%.

5.3. Taper of eltrombopag in the extension protocol

Once a robust blood count recovery occurs in the extension part (*i.e.* hemoglobin >10 g/dL in the absence of transfusions for more than 8 weeks), EPAG dose will be tapered according to Table 3. If hemoglobin falls to <9 g/dL or the patient again requires RBC transfusions then EPAG will be either re-initiated or the dose will be increased to the last effective strength.

Table 3: Eltrombopag taper in the extension protocol

Age group	Starting dose	Step 1	Step 2	Step3	Step 4
Non-Asian					
⩾12	150 mg	75	37.5	25	stop
6-11	75 mg	37.5	25	12.5	stop
2-5	2.5 mg/kg	1.25mg/kg	stop		
East Asian, South East Asian					
⩾12	75 mg	37.5	25	12.5	stop
6-11	37.5 mg	25	12.5	stop	
2-5	1.25 mg/kg	stop			

Each step is 8 weeks

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 EPAG 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

Recommended dose modifications for isolated ALT or AST elevation are listed in Table 4.

Table 4: Eltrombopag dose modifications for isolated ALT or AST elevation

Elevation	Dose modification and Follow-up
>ULN - 3.0 x ULN	Maintain dose level.
>3.0 – 5.0 x ULN	Maintain dose level. Repeat liver function tests as soon as possible, preferably within 48-72 hours, from awareness of the abnormal results. Monitor liver function tests weekly or more frequently if clinically indicated until resolved to ≤ **3.0 x ULN**.
>5.0 – 10.0 x ULN	Interrupt dose. Repeat liver function tests as soon as possible; preferably within 48-72 hours from awareness of the abnormal results. Monitor liver function tests weekly, or more frequently if clinically indicated until resolved to ≤**3.0 x ULN** then: If resolved in ≤**14 days**, maintain dose level. If resolved **>14 days**, restart eltrombopag at a dose level 25mg/day (12.5mg/day for children under 12) lower than the prior dose.
>10.0 – 20.0 x ULN	Interrupt dose. Repeat liver function tests as soon as possible, preferably within 48-72 hours from awareness of the abnormal results. Monitor liver function tests weekly, or more frequently if clinically indicated until resolved to **baseline**. If resolved, restart eltrombopag at a dose level 25mg/day (12.5mg/day for children under 12) lower than the prior dose.
>20.0 x ULN	Discontinue subject from study drug treatment. Repeat liver function tests as soon as possible, preferably within 48-72 hours from awareness of the abnormal results. Monitor liver function tests weekly or more frequently if clinically indicated until resolved to **baseline or stabilization** over **4 weeks**.

5.5. Dose delays, modifications or discontinuation for hematologic side effects

5.5.1. Thrombosis/Embolism

Subjects who experience a deep venous thrombosis or a pulmonary embolus, transient ischemic attack or stroke, or a myocardial infarction at any time while on EPAG will discontinue drug and go off study. They will be treated for the thrombotic event as clinically-indicated.

5.5.2. Peripheral blood smear shows new morphological abnormalities

The presence of persistent morphologic abnormalities (red cell teardrop forms or immature blasts) or the development of significant sudden worsening of platelet or neutrophil counts while on study will require discontinuation of EPAG and performance of a bone marrow examination to assess for development of abnormal fibrosis or progression to MDS or AML.

5.6. Extended access to study drug

Subjects who demonstrate response by protocol criteria at 24 weeks (± 10 days) may be consented to continue EPAG on study. This part of the study will be referred to as extended access phase. Per dosing criteria given in §5.1, patients may remain on the extended access phase as long as they maintain a response. While on extended access, participants may have drug tapered, discontinued, and/or re-started as per §5.2, 5.3 and 5.4.

5.7. Permitted supportive care

- Transfusion supportive care (e.g., blood and platelets) as clinically indicated.
- Iron chelation therapy (e.g. deferasirox, deferoxamine, deferiprone) ongoing at the time of enrollment will be continued, titrating and/or discontinuing according to established standards of care.
- Estrogens or combination oral contraceptives as indicated for uterine bleeding.
- Prophylactic antibiotics and antivirals as clinically indicated.
- The PI should be informed about all medications initiated after starting treatment with EPAG.

5.8. Non-permitted medications

- Romiplostim (N-Plate)
- IL-11 (Neumega)
- Androgens (danazol and oxymetholone) within 4 weeks prior to starting EPAG.
- Corticosteroids within 4 weeks prior to starting EPAG, except at physiologic replacement dosing for adrenal insufficiency

- Investigational or not marketed drugs without a well-known safety profile within 7 days or 5-half-lives (whichever is longer) prior to the first dose of study treatment and until eltrombopag is discontinued unless, in the opinion of the Investigator and Sponsor, the medication will not interfere with the study.
- Herbal supplements within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5-half-lives (whichever is longer) prior to the first dose of study treatment until eltrombopag is discontinued, unless in opinion of the Investigator and Sponsor, the medication will not interfere with the study treatment.

5.9. Concurrent medications

OATP and BCRP substrates: In vitro studies demonstrated that eltrombopag is an inhibitor of the transporters OATP1B1 (Organic Anion Transporter Polypeptide-1B1) and BRCP (Breast Cancer Resistance Protein). Administration of eltrombopag 75 mg once daily for 5 days with a single 10 mg dose of rosuvastatin, an OATP1B1 and BCRP substrate, increased plasma rosuvastatin C_{max} by 2-fold and AUC_{∞} by 55% on average. Interactions are also expected with other HMGCoA reductase inhibitors.

When co-administered with eltrombopag, a reduced dose of HMG-CoA reductase inhibitors should be considered, and careful monitoring should be undertaken. Concomitant administration of eltrombopag and other OATP1B1 and BCRP substrates should be undertaken with caution.

> **Examples of OATP substrates:** aliskiren, ambrisentan, anacetrapib, atenolol, atrasentan, atorvastatin, bosentan, bromociptine, caspofungin, cerivastatin, celiprolol, danoprevir, empangliflozin, ezetimibe, fimasartan, fexofenadine, fluvastatin, glyburide, maraviroc, SN-38 (irinotecan metabolite), rosuvastatin, simvastatin acid, pitavastatin, pravastatin, repaglinide, rifampin, valsartan, olmesartan, telmisartan, montelukast, ticlopidine.
>
> **Examples of BCRP substrates:** atorvastatin daunorubicin, doxorubicin, hematoporphyrin, imatinib, methotrexate, mitoxantrone, pitavastatin, rosuvastatin, SN-38, ethinyl estradiol, simvastatin, sulfasalazine, sofosbuvir, topotecan, sulfasalazine.

Inhibitors of cytochrome p450: *In vitro* studies demonstrate that CYP1A2 and CYP2C8 are involved in the oxidative metabolism of EPAG. Trimethoprim, gemfibrozil, ciprofloxacin, fluvoxamine and other moderate or strong inhibitors of CYPs may therefore theoretically result in enhanced activity of EPAG, 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 patients with bone marrow failures 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 EPAG activity and toxicity.

HMG-CoA Reductase Inhibitors (statins): Patients will be permitted to use HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA) inhibitors during the study, but these drugs should be used with caution and a 50% dose reduction of the HMG-CoA reductase inhibitor is recommended, with close monitoring for safety, such as liver chemistry and signs and symptoms of myolysis, and efficacy, such as cholesterol and triglycerides (refer to individual product information for monitoring recommendations).

5.10. Instructions to patients

Timing in relation to food: Subjects will be advised to take EPAG on an empty stomach *i.e.* 1 hour before or 2 hours after a dairy-free meal containing no drugs, antacids, or products with polyvalent cations (*i.e.* aluminum, calcium, magnesium, iron, selenium and zinc) such as mineral supplements. For meals containing dairy products, patients will be advised to take EPAG at least 4 hours before or after meals.

Timing in relation to antacids and other products containing polyvalent cations: Because co-administration of EPAG with antacids decreased plasma AUC of EPAG by 70%, patients will be advised to take EPAG at least 4 hours before or after from antacids and other products containing polyvalent cations such as mineral supplements and dairy products.

5.11. Health Related Quality of Life (HRQL) Questionnaires

The relevant dimensions of HRQL being assessed in this study include (1) PROMIS Global Health, Sleep Disturbance, Applied Cognition-Abilities, Anxiety and Depression and (2) FACT- Anemia, Thrombocytopenia and Neutropenia. Patient-Reported Outcomes Measurement Information System (PROMIS®), is an initiative based on an NIH grant to establish and provide the public a free, reliable and validated commonly used measures of patient-reported outcomes. The FACT instruments are a health assessment instrument designed to measure multi-dimensional quality of life in chronic illness and its associated therapy. The different subscales selected for this study are specific for patients with diseases or treatments with hematological effects.

6. Clinical Evaluations

Samples will be ordered and tracked through CRIS. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. For laboratory assessments, the following limits will be observed:

Adult Patients: The amount of blood that may be drawn from adult patients and volunteers (*i.e.* those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. The amount of blood to be drawn from

volunteers and the frequency of collection shall be specified in the clinical research protocol, and exceptions to the 10.5 mL/kg or 550 mL limitations shall be approved by the IRB.

Pediatric Patients: For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period.

6.1. Pre-study evaluation

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

- Medical history and physical examination
- Concurrent medication review
- Complete blood count with differential
- Peripheral blood smear
- Reticulocyte count
- Hemoglobin F quantification by hemoglobin electrophoresis
- Erythrocyte adenosine deaminase
- DAT (direct antiglobulin test)
- Chromosomal breakage analysis (if results not available from outside/referring institute)
- Genetic testing (if results not available from outside/referring institute)
    - Targeted sequencing if a family history of DBA exists and the mutation is known
    - Inherited bone marrow failure panel (NeoGenomics "Inherited bone marrow failure panel", University of Chicago "Hereditary Myeloid Malignancy and Inherited Bone Marrow Failure Panel", Cincinnati Children's Hospital "Bone Marrow Failure Syndromes Panel by next-generation sequencing", or a comparable test encompassing known DBA genes)
- Acute care panel (Na, K, Cl, CO_2, Creatinine, Glucose, and Urea Nitrogen)
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Total Protein, CK, Uric Acid, LDH
- Mineral (Phosphorus, Magnesium, Albumin, and Calcium)

- Coagulation and thrombosis screens (PT, PTT)
- Viral serologies for hepatitis A, B, C, HIV, HSV, EBV and CMV (positive serologies will have viral nucleic acid testing performed)
- Folate level
- B12 level
- Copper and zinc levels
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- Pregnancy test (blood or urine hCG in women of child bearing potential)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, iron, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
- Bone marrow chromosomal analysis via standard cytogenetic techniques
- Pediatric consult for patients under 18 years of age
- Electrocardiogram (EKG)
- Review of red blood cell transfusion records for the 8 weeks prior to study entry

6.2. Baseline labs

Laboratory evaluations performed as part of screening may be used if obtained within 7 days prior to consent. Screening results (§6.1) may be used as baseline studies if performed within 12 weeks or as indicted below.

- Complete blood count with differential (7 days or less prior to signing consent)
- Reticulocyte count (7 days or less prior to signing consent)
- Acute care panel (Na, K, Cl, CO_2, Creatinine, Glucose, and Urea Nitrogen) (7 days or less prior to signing consent)
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin) (7 days or less prior to signing consent)
- Pregnancy test (blood or urine hCG in women of childbearing potential) (7 days or less prior to signing consent)

The below baseline testing will be obtained once patient is consented onto study:

- MRI T2* imaging to assess liver iron concentration
- Neurodevelopmental testing for patients less than 18 years of age (recommended, but at PI's discretion)
- 24-hour urine collection to determine the total iron (at PI's discretion)
- HLA typing

- HRQL survey administration
- Immunoglobulins
- Thyroid function panel (T3, T4, and TSH)
- Research blood as detailed in §7

6.3. On-Study evaluations (through 6 months ± 10 days)

Subjects may be followed by their home physician or at the Clinical Center. Lab tests not done at the NIH will be faxed or securely e-mailed to the Research Nurse. The PI will review outside test results and these will be uploaded into CRIS, and all lab data will be recorded. Any progress notes from local assessments will be faxed or securely e-mailed to the research nurse and reviewed by the principal investigator.

- Complete blood counts with differential (every 2 weeks ± 10 days)
- ALT, AST, Total Bilirubin or Direct Bilirubin (every 2 weeks ± 10 days)
- Ferritin (every 2 weeks ± 10 days)
- Full iron panel (transferrin, % saturation, and total serum iron) in addition to ferritin preferred but not required

6.4. Landmark 3-month and 6-month evaluations

Subjects must be evaluated at the 3- and 6-month (± 14 days) time points. The 6-month time point shall be at the NIH Clinical Center. The 3-month assessment may be either at a home institution or at the NIH.

- Review of interval history and events
- Physical exam (may be omitted for 3-month evaluation if visit is not at the NIH)
- Complete blood counts with differential
- Acute care panel (Na, K, Cl, CO_2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Other (Total Protein, CK, Uric Acid, and LDH) panel
- Reticulocyte count
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- Blood or urine pregnancy test (woman of childbearing potential only)
- Review of transfusion records (if available)

Additional assessments to be obtained at the NIH Clinical Center at the 6-month evaluation:

- Neurodevelopmental assessment for patients less than 18 years of age (if previously performed)

- Peripheral blood smear
- Hemoglobin F quantification by hemoglobin electrophoresis
- Erythrocyte adenosine deaminase
- Copper and zinc levels
- MRI T2* imaging to assess liver iron concentration
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies
- Blood for pharmacokinetic evaluation of EPAG levels at PI's discretion. Samples drawn pre-dose, and at 2-, 4-, 6- and 8-hour post-dose timepoints, with an optional final sample at 24 hours after dose
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
- Bone marrow chromosomal analysis via standard cytogenetic techniques
- HRQL survey administration
- Research blood as detailed in §7

6.5. Long term follow-up for responding patients enrolled in extended access phase

Patients fulfilling response criteria (per §8.2) at the 6-month visit will be offered participation in the extended access phase and after signing consent will be permitted to continue on EPAG for an additional 3 years. Subjects taking EPAG must be evaluated at the Clinical Center every 6 months (± 30 days) while they remain on extended access and continue periodic laboratory monitoring listed below in their home physician's office or the NIH every 4 weeks (± 10 days). Progress notes and lab tests not done in NIH will be faxed or securely e-mailed to the Research Nurse. The PI will review outside test results and these will be uploaded into CRIS and filed in the research charts, and all lab data will be recorded. The periodic laboratory monitoring will include:

- Complete blood counts with differential (every 4 weeks (± 10 days).
- Reticulocyte count every 4 weeks (± 10 days).
- ALT, AST, Total Bilirubin or Direct Bilirubin every 4 weeks (± 10 days).
- Ferritin every 4 weeks (± 10 days).

6.5.1. Testing required for patients on EPAG while on extended access EPAG (up to 3 years after the 6-month visit):

- Complete blood counts with differential (monthly ± 10 days)
- Peripheral blood smear (every 6 months ± 30 days)
- ALT, AST, Total Bilirubin or Direct Bilirubin) (monthly ± 10 days)

- Hepatic panel (Alkaline phosphatase, ALT, AST, Total Bilirubin and Direct Bilirubin) (every 6 months ± 30 days)
- Reticulocyte count (monthly ± 10 days)
- Hemoglobin F quantification by hemoglobin electrophoresis (every 6 months ± 30 days)
- Erythrocyte adenosine deaminase (every 12 months ± 30 days)
- Copper and zinc levels (every 12 months ± 30 days)
- Ferritin (monthly ± 10 days); full iron panel (ferritin, transferrin, % saturation, and total serum iron) preferred but not required
- Pregnancy test (blood or urine HCG in women of child bearing potential) (every 6 months ± 30 days)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities. Iron stains will be performed at the PI's discretion as indicated by clinical status or laboratory testing. (every 6 months ± 30 days)
- Bone marrow chromosomal analysis via standard cytogenetic techniques (every 6 months ± 30 days)
- HRQL survey administration (every 6 months ± 30 days)
- Iron panel (ferritin, transferrin, % saturation, total serum iron; every 6 months ± 30 days)
- MRI T2* imaging to assess liver iron concentration (every 6 months ± 30 days during the first year of extended phase, and then if deemed necessary by the PI every 12 months ±30 days thereafter)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 6 months ± 30 days)
- Document Drug Accountability
- Review of interval history and events, and physical examination (every 6 months ± 30 days)
- Review of transfusion records (if available, every 6 months ± 30 days)
- Pediatric consult (every 6 months ± 30 days) at PI's discretion
- Research blood as detailed in §7 (every 6 months ± 30 days)

6.5.2. Subjects who have EPAG discontinued after tapering for a robust response during the extended access phase (see Table 3) will be followed in the following manner (up to 3 years after the 6-month visit):

- Complete blood counts with differential (monthly ± 10 days for 6 months off eltrombopag, then every 3 months ± 30 days until they reach off study criteria as per §8.6 or the study is closed)
- Ferritin (monthly ± 10 days for 6 months off eltrombopag, then every 3 months ± 30 days until they reach off study criteria as per §8.6 or the study is closed)
- Iron panel (ferritin, transferrin, % saturation, total serum iron; every 12 months ± 60 days)
- Hemoglobin F quantification by hemoglobin electrophoresis (every 12 months ± 60 days)
- MRI T2* imaging to assess liver iron concentration (every 12 months ± 60 days if the PI deems necessary, if drug is discontinued during first year of extended phase, also obtain imaging at the 6-month visit as above in §6.5.1)
- Erythrocyte adenosine deaminase (every 12 months ± 60 days)
- Peripheral blood smear (every 12 months ± 60 days for three years)
- Bone marrow aspiration and core biopsy, to be stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities. (every 12 months ± 60 days for three years)
- Bone marrow chromosomal analysis via standard cytogenetic techniques (every 12 months ± 60 days for three years)
- HRQL survey administration (every 12 months ± 60 days for three years)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 12 months ± 60 days)
- Research blood as detailed in §7 (every 12 months ± 60 days)
- Review of interval history and events, and physical examination (every 12 months ± 60 days)
- Review of transfusion records (if available)

Patients who discontinue EPAG according to the response criteria who need to have EPAG reinitiated due to counts falling (see §5.3) will go back onto the evaluation schedule for patients on EPAG in the extended access phase.

6.6. Off study assessment

We will offer patients a follow-up evaluation at the NIH 6 months following being taken off EPAG treatment either due to lack of response or for the other reasons listed in §8.6, but this visit will

not be required. If they do not return to the NIH, we will contact their primary hematologist for information on their current hematologic status 6 months following their final dose of EPAG, and then take them off study.

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

- Review of interval history and events, and physical examination
- Neurodevelopmental assessment (if previously performed)
- CBC with differential
- Hemoglobin F quantification by hemoglobin electrophoresis
- Erythrocyte adenosine deaminase
- Acute care panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- Other (Total Protein, CK, Uric Acid, and LDH)
- Reticulocyte count
- Peripheral blood smear
- Bone marrow biopsy with reticulin and collagen staining and aspiration with cytogenetics
- Research blood as detailed in §7
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- MRI T2* imaging to assess liver iron concentration, if being followed per above
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron
- Review of transfusion records (if available)

6.7. MRI Imaging to Assess Iron Loading

Liver iron concentration should be assessed using MRI-based techniques. Hepatic effective T2 (T2*) magnetic resonance imaging sequences will be used, comparing observed signal relaxation to expected and experimentally derived controls to determine iron concentration. Native T1 mapping and fat-water separation sequences may also be obtained to better characterize the liver. Contrast is not required for these studies, and contrast allergy is therefore not considered exclusionary. The hepatic imaging will be targeted toward assessment of iron deposition of the liver and will not include a comprehensive abdominal assessment. Therefore, any additional questions of liver and abdominal pathology would require a separate clinical MRI scan. Although the sequences chosen should focus upon the liver, note will be made, where possible, of signal within the heart. Additional, cardiac-specific sequences are not required unless indicated for good clinical care. Likewise, imaging of other organs such as the brain, are not required, unless indicated clinically. Although it is expected that these imaging studies will be performed utilizing one of the

1.5 T scanners at the NIH Clinical Center for reasons of standardization, existing protocols, and scheduling constraints, the choice of device is not prescribed by this protocol and will be selected by the responsible imaging physicians(s). However, once selected, the same scanner will be preferentially used for all of the studies performed on a given patient to ensure comparability, or should the scanner be unavailable or be changed over the course of the study, at a minimum, a scanner with the same field strength will be used.

Imaging should be performed during the initial enrollment evaluation, and at the 6-month primary endpoint evaluation. For patients continuing treatment into the extended phase, it should be performed at the 6-month evaluation, and then annually (*i.e.* 12-month evaluation, 24-month evaluation, *etc.*) thereafter while on eltrombopag if the PI deems necessary. If eltrombopag is discontinued less than 3 months prior to a planned MRI evaluation, that evaluation should still be performed as was previously planned.

Inability to perform MRI imaging due to patient age, weight, or intolerance (e.g., claustrophobia), will not be reported to the IRB as a deviation, but will be noted within the medical record. If imaging is not obtained at a given timepoint, it should nonetheless be attempted at subsequent timepoints according to the above schedules and level of necessity.

6.8. Genetic analysis

Patients will undergo genetic testing as part of their initial enrollment assessments, if results are not available from a previous, outside assessment (see §6.1). Functional testing (e.g., ribosomal assembly, adenosine deaminase, *etc.*) will be considered for purposes of supporting a diagnosis of DBA but are not sufficient to substitute for genetic testing.

Intended use: The purpose of this analysis will be to identify, if possible, a disease-defining mutation and to rule-out alternative diagnoses; however, it is understood that approximately half of all patients so screened will not have an identifiable mutation (see §2.1). The presence of a disease-defining mutation is not required for study inclusion, nor is its absence considered exclusionary (see §4.1.1). This testing will also be important to rule-out the presence of MDS-defining mutations that would be considered exclusionary (§4.2.14). This testing will take one of two forms:

> ***Patients with a familial history of a known mutation:*** For patients where a specific mutation known to cause DBA has been identified in a first-degree, biological relative (*i.e.* parent, sibling, or child), the appropriate gene-specific testing (e.g., direct sequencing, fluorescence-in-situ hybridization, *etc.*) will be performed by a CLIA-certified clinical laboratory.

Patients without a familial history: For patients without a familial history of DBA, or for which a specific, disease-causing mutation has not previously been identified, germline somatic mutation analysis will be performed using the NeoGenomics "Inherited bone marrow failure panel". If this specific test cannot be performed, a comparable CLIA-certified test encompassing known DBA genes is acceptable (e.g., University of Chicago "Hereditary Myeloid Malignancy and Inherited Bone Marrow Failure Panel", Cincinnati Children's Hospital "Bone Marrow Failure Syndromes Panel by next-generation sequencing", *etc.*). Unbiased whole-genome or whole-exome sequencing from outside, CLIA-certified sources provided by the patient or referring institution may be accepted at the PI's discretion.

Privacy and Confidentiality: The results of all genetic testing will be kept confidential. DBA-specific results (*i.e.* identification of a disease-causing mutation) will be linked to the anonymized, study-specific identifier assigned to patient at the time of enrollment to allow subgrouping of patient data the data analysis phase and for subgrouping of any banked biospecimens. This data will be stored on secure, NIH-maintained servers, accessible only to study personnel, and only for purposes of research. After review, the results of all genetic testing, including additional, clinically actionable variants will be scanned into the medical record (CRIS).

Clinically Actionable Results: Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. Subjects will be contacted at this time and will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

7. Laboratory Research Studies

Intended use: Blood and bone marrow will be collected during the initial phase of this study at the baseline and 6-month landmark timepoints, and during the extended phase of this study every 6 months (for patients continuing on eltrombopag) or every 12 months (for patients for whom eltrombopag was discontinued during the extended phase). The volume to be collected shall be 60 mL of blood, or the lesser of either 3 mL/kg or 60 mL for pediatric subjects (less than 18 years of age), and 10 mL of aspirated bone marrow. These samples will be for use in the correlative laboratory research studies listed below.

Baseline samples may be obtained under 04-H-0012. Additionally, in selected patients we will collect urine for 24 hours at each of the landmark visits. These studies are not used in assessing the primary endpoint but are undertaken as descriptive or exploratory ancillary studies. Except for the urine studies all test will be performed on each subject's material.

- Extended peripheral blood and bone marrow flow cytometric phenotyping for cell surface or intracellular proteins (e.g. six color flow cytometry to measure primitive HSCs) and assessment of reactive oxygen species
- Hematopoietic progenitor colony, long term-culture-initiating cell, and in vitro erythrocyte differentiation assays for primitive cell content and function
- Studies to investigate pathways that can improve erythroid differentiation in DBA
- Studies to examine ribosome function and biology
- Evaluation of clonal dynamics (single/bulk cell whole exome/genome sequencing)
- mRNA expression studies (RNA seq) on blood or bone marrow cells
- Whole genome/exome/epigenome sequencing or targeted sequencing may be performed in this study to assess the presence of somatic mutations in genes known to be recurrently mutated in MDS/AML or novel recurrently mutated genes. The data obtained will be handled confidentially. The digital data will be password protected and available to only the relevant PI and co-investigators. The specimens will be used only to study the genetics of the rare disease. DNA will not be used for unrelated investigations unless permission is first granted by the NHLBI IRB as well as the subject. We do not intend to report mutations in genes associated with disease risk that are incidental to our research aims, commonly referred to as "incidental findings."
- Serum cytokine, chemokines and soluble receptor levels including but not limited to IFN-γ, TNF-α and TPO levels.
- Pharmacokinetic studies of EPAG kinetics (selected patients)
- Should there be any remaining sample, it will be stored with the subject's permission for other exploratory laboratory research studies reviewed and approved by the IRB and listed in APPENDIX A: Schedule of Activities.

Storage: Research samples will be stored coded in the secure laboratory of Cynthia Dunbar, M.D.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside the NIH without an executed MTA.

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

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

8. Biostatistical Considerations

8.1. Objectives

The ***primary objective*** is to evaluate safety and efficacy of 6 months of therapy with eltrombopag in subjects with Diamond-Blackfan anemia.

The ***secondary objectives*** include assessments of the impact of eltrombopag upon rates of relapse and survival, clonal evolution, toxicities of extended therapy, the impact of treatment upon health-related quality of life and neurodevelopment in pediatric patients, and the impact of eltrombopag upon stem cell and erythroid cell dynamics and niche interactions including iron homeostasis.

8.2. Endpoints

8.2.1. Primary endpoint

Primary efficacy endpoint will be the proportion of drug responders at 6 months. Best response (robust or not) will be noted, regardless of when it is achieved; however, for protocol purposes, primary response will be assessed according to patient status at the time of the 6-month primary endpoint assessment. Time-to-response will also be measured according to the time from eltrombopag initiation to the first time the patient met criteria for response.

Response to treatment will be defined by one or more of the following:

- Erythroid response for subjects with a pretreatment hemoglobin less than 9 G/dL will be defined as an increase in hemoglobin by >1.5 G/dL from enrollment baseline, *and/or*
- a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment - compared with the pretreatment transfusion number in the previous 8 weeks.

Robust response to treatment will be defined both:

- Stable hemoglobin (two consecutive measurements at least 2 weeks apart) greater than 10 g/dL, *and*

- Transfusion independence for the previous 8 weeks

For sub-analyses, ***transient response*** to treatment (robust or otherwise) is defined as any response meeting the respective criteria above within the first 6 months that is subsequently lost and not regained prior to the 6-month primary endpoint assessment. While note will be made of such patients, for the purposes of primary response analysis, these patients will be considered ***non-responders*** along with patients who fail to meet the above response criteria.

Primary safety endpoint will be the toxicity profile assessed at 6 months using CTCAE criteria.

Transfusion Units: In pediatric patients, the administration of red blood cell transfusions is typically weight based, typically 10-15 mL/kg, reaching a maximum of one bag of packed erythrocytes (300-350 mL). For protocol purposes, a "transfusion unit" for pediatric patients is 15 mL/kg of packed erythrocytes. For adults and pediatric patients weighing approximately 20 kg or greater, "units" will be measured in bags. Where ambiguity exists, the clinical record shall be reviewed to determine the intent of the ordering physician as to whether dosing was per-bag or on a weight basis. It will be the responsibility of the PI to adjudicate any remaining ambiguity. Transfusions administered for non-disease indications (e.g., trauma, acute blood loss, perioperative hemostasis, *etc.*) will be noted, but not included in statistical and response considerations.

8.2.2. Secondary endpoints

Secondary endpoints will include:

- Hematological responses at 3 months (as defined above)
- Time to response
- Best response, regardless of response status at primary endpoint
- Impact on platelet count and neutrophil count
- Relapse during the extension phase
- Clonal evolution, according to cytogenetic, mutational or flow cytometric markers
- Toxicities with extended duration of therapy
- The impact of treatment and treatment response on quality of life.
- Evaluation of neurodevelopmental changes at baseline and 6-month evaluations.
- Iron loading as measured by ferritin trends at each evaluation, and by iron staining of bone marrow biopsies at baseline and 6-month evaluations (and during extension at the PI's discretion)
- Evaluation of reactive oxygen species HSPCs (ROS flow cytometry assay at baseline and 6 months), and at interval evaluations during extended access at the PI's discretion

- Evaluation of global transcriptome in HSPCs (single cell RNA seq at baseline and 6 months)
- The impact of eltrombopag on reconstitution of the stem cell compartment (multicolor flow cytometry of bone marrow cells) and erythroid compartment (multicolor flow cytometry and CFU-E/BFU-E assays) at baseline and 6-month evaluations, and at interval evaluations during extended access at the PI's discretion

8.3. Sample size

Because the efficacy of eltrombopag in this patient population is unknown, we would like to reject the treatment as quickly as possible with a small number of patients if the treatment is not effective. We will use the Two-Stage Minimax Design outlined in Table 1 of Simon (89) 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 H_0: $p \leq 10\%$ versus the alternative H_1: $p \geq 30\%$ at a significance level of 0.05 and a power of 0.8. At the first stage, we will accrue 15 subjects, and the null hypothesis will be accepted if no more than 1 subject responds to the treatment at 6 months. If 2 or more subjects respond to the treatment at 6 months at the first stage, then we will accrue an additional 10 subjects, 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 at 6 months is 5 or less. These efficacy thresholds were selected as being clinically relevant for the avoidance of alternative therapies such as hematopoietic stem cell transplant. Given an observed screening fail rate of 16.7%, we will recruit 30 patients (18 in the first phase, 12 in the second phase) to ensure that the target of 25 eligible patients is reached.

With an incidence of approximately 1 in 100,000-200,000 live births (18), and an expectation of approximately 25-35 new diagnoses a year in the USA, of which 15-20 will likely prove refractory to treatment, and in conjunction with recruitment through patient networks and ongoing natural history studies at the NIH (see §10.1), accrual is projected to take 2.5-3 years.

8.4. Statistical methods

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

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

without the event of interest as a competing risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. Graphical tools will be used to display the appropriate estimates (*i.e.* estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their corresponding 95% confidence intervals. Exploratory subgroup analysis will be used to compare the possible different response rates among male, female and different age categories.

8.5. Stopping rules

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

1. Death considered to be probably or definitely related to eltrombopag
2. Any Grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities
3. Grade IV thrombosis/embolism

We anticipate the rate of these specified TRSAEs within the 24-week study period to be 20% or less. Following Geller *et al.* (90), our stopping rule is determined by a Bayesian approach. The stopping boundary for an experiment is reached if the Bayesian posterior probability that the true probability of developing one or more of the specified TRSAE's exceeds this benchmark rate of 20% is at least 90%. We take our prior distribution to be a beta distribution with parameters $(\alpha, \beta) = (1, 4)$. The parameters are chosen so that the mean $\alpha / (\alpha + \beta) = 0.2$ as the expected proportion of specified TRSAE's and the sum $\alpha + \beta = 5$ as the "worth" we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is 5/25=20% 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. All participants will be included in monitoring and evaluation for safety. Table 5 summarizes the threshold numbers for stopping the study.

Table 5: Threshold numbers for stopping the study

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 5	3
≤ 9	4
≤ 12	5
≤ 16	6
≤ 20	7
≤ 24	8

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
25	9

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 25 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. Table 6 summarizes the proportion of stopped studies under a number of scenarios for *p*:

Table 6: Predicted rates of stopping under various scenarios

Probability of TRSAE = *p*	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
Proportion of stopped studies	1.8%	7%	18.2%	35.7%	55.5%	73.2%	86.4%	94.1%
Average number of subjects	24.7	23.9	22.4	20.1	17.3	14.5	11.8	9.7
Average number TRSAEs	2.5	3.6	4.5	5	5.2	5.1	4.7	4.3

These simulation results suggest that our stopping rule has a low probability stopping a study when the proportion of TRSAE within the 24-week is below 20%, and the probability of stopping a study is high when the true proportion of TRSAE within the 24-week exceeds 20%. Therefore, we believe that our Bayesian stopping rule for TRSAE has satisfactory statistical properties.

8.6. Off study criteria

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

Per principal investigator decision: Should any of the following events occur during the study period, or in the extension treatment arm in responders, EPAG will be discontinued. The subject will be followed until resolution of the event. For study purposes labs relevant to the adverse event will be monitored for 30 days after discontinuation of EPAG. We will offer patients a follow-up evaluation at the NIH 6 months following being taken off EPAG treatment either due to lack of response or because they met other criteria for coming off study, but this visit will not be required. If they do not return to the NIH, we will contact their primary hematologist for information on their current hematologic status 6 months following their final dose of eltrombopag, and then take

them off study. If a patient initiates an alternative disease directed therapy at any time, the subject's participation on this study will be considered complete and the subject will go off study.

- Intolerance of EPAG 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 §10.3.1
- New or worsening morphological abnormalities or cytopenia(s) indicative for transformation to MDS or AML
- No treatment response by week 24 (+/- 7 days)
- Any Grade IV toxicity considered related to the study medication excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities (see §9.2.1, 10.3.1)
- Significant progression of disease such as development of a solid tumor while on EPAG, 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 Diamond-Blackfan anemia
- Subject non-compliance
- Lost to follow-up
    - Multiple, good-faith efforts will be made according to good standards of clinical research to re-establish contact with any subjects prior to declaring them lost to follow-up and removed from study
- Study completion

Completion of protocol participation: Three years after a responding patient agrees to continue the drug on the extension phase, protocol participation will be deemed complete. During the initial consenting process, the patient will be informed that eltrombopag cannot be provided after study completion. For responding patients wishing to continue eltrombopag, we will assist the referring physician with helping the patient obtain eltromobopag, including via the Novartis Patient Assistance Program. Once off study, subjects will be referred back to the referring physician or consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) or evaluated for eligibility for another Branch protocol, depending on the wishes of the subject and availability of appropriate additional clinical trials at the NIH.

Death: Death of subject

9. Data and Safety Monitoring

9.1. Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, David J Young, M.D., Ph.D.

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

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

Monitoring: As per ICH-GCP 5.18 and 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be:

1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject;
2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;
3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information);
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 NIH IRB, the site monitors, and the NHLBI staff for confirmation of the study data.

FDA and IND 104877: An annual progress report, protocol amendments, and any information amendments (e.g., change in the status of the protocol) will be forwarded to FDA Project Manager (designee):

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
phone: (301) 796-0683

Novartis: Progress reports, any amendments to the protocol, and any change in the status of the protocol will be forwarded to

Dram Laine, Sr. Clinical Research Scientist
Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080
phone: (862) 778-7281
dram.laine@novartis.com

9.2. Event Characterization and Reporting

Events include ***adverse events*** (AE), ***serious adverse events*** (SAE), ***protocol deviations*** (PD), ***unanticipated problems*** (UP), and ***non-compliance***.

The principal investigator will review all events (AEs, SAEs, PDs, UPs and non-compliance) to determine the seriousness, expectedness, and reportability of the event. As required and/or needed, the principal investigator will review the events with the Sponsor to make the final determination of seriousness and reportability.

9.2.1. Definitions

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

9.2.2. Adverse events Management

AEs will be graded by severity utilizing CTCAE version 5.0 (summarized in Table 7) and attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease (Table 8). The current CTCAE grading criteria can be downloaded from the CTEP homepage at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Table 7: Grading of adverse events

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[1]
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2]
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

[1]**Instrumental ADL** refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[2]**Self-care ADL** refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Table 8: Attribution of adverse events

Relationship	Attribution	Description
Unrelated to investigational agent/intervention[1]	Unrelated	The AE is clearly NOT related to the intervention
	Unlikely	The AE is doubtfully related to the intervention
Related to investigational agent/intervention[1]	Possibly	The AE may be related to the intervention
	Probably	The AE is likely related to the intervention
	Definitely	The AE is clearly related to the intervention

[1]**NOTE:** AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A).

In view of the underlying disease (Diamond-Blackfan anemia), all patients will enter the study with abnormally low blood counts that would meet criteria for grade 2 and more commonly higher toxicity. Also, platelet and red blood cell counts will be impacted by frequent transfusions. Therefore, AEs regarding low hematologic lab values, including thrombocytopenia or platelet-transfusion dependence, anemia or red cell transfusion dependence, neutropenia, lymphopenia, or leukopenia are not evaluable and will not be recorded.

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will include any change from laboratory assessments done prior to first dose of study medication that result in a progression to grade 3 or 4 laboratory toxicity or are characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Adverse Events that meet the criteria of CTCAE v 5.0 grade 2 and above plus laboratory abnormalities that meet the definition of an AE will be captured at each study visit on the appropriate case report form (CRF) regardless of relationship. AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study drug and study procedure (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. These AEs will be followed to adequate resolution.

Abnormal non-hematologic laboratory values recorded in the database will be recorded at the highest grade and resolved in the database when the value is a grade 2 or lower. However, with the exception of ALT, AST and direct bilirubin any laboratory abnormalities higher than grade 2 will automatically result in discontinuation of the study drug.

Hypertension CTCAE v5 Grade 1 is not an applicable category for this study and therefore only hypertension greater than grade 2, that require medical intervention, will be documented.

Unscheduled laboratory results or patient reports that have been sent in addition to the protocol required ones will be reviewed for AEs and will be recorded on secure network drives or in approved alternative sites that comply with NIH information security standards and not in the research database.

Thirty days after the last dose of study drug, adverse event collecting and reporting will be limited to serious adverse events considered possibly, probably, or definitely related to study drug.

9.2.3. Serious Adverse Events Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely

(doubtfully related to the research) and unrelated (clearly not related to the research) (Table 8).

Treatment related SAEs (TRSAEs) are those attributed as definitely, probably, or possibly related (Table 8) that will be monitored and considered for early stopping of the study according to statistically determined criteria. These include death and any grade IV toxicity considered to be probably or definitely related to study medication.

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

9.2.4. Reporting events

All events will be reported to the Principal Investigator of this study:

David J. Young, M.D., Ph.D., TSCBB, NHLBI, NIH, Clinical Center
10 Center Dr. Building 10, Room CRC 5-3150
Bethesda, MD 20892-1452
Tel: (301) 827-7823
Fax: (301) 827-0915
E-mail: david.young2@nih.gov

9.2.4.1. NIH Intramural IRB and NHLBI Clinical Director (CD) Reporting

Expedited Reporting:

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

Reports to the NIH Intramural IRB at the time of Continuing Review (CR):

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

Reports to the CD:

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

9.2.4.2. NHLBI DSMB Reporting

Reports of serious adverse events that are unexpected and thought to be related (possible, probably or definitely) to the experimental drug will also be forwarded no later than seven days in the case of death or life-threatening serious adverse

events or within fifteen days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). All SAEs will be included in the DSMB reports for review by the DSMB at the time of scheduled DSMB meetings (DSMB meetings held 2 times a calendar year and DSMB determines the frequency of protocol review per DSMB minutes).

9.2.4.3. Sponsor and FDA Event Reporting

IND Sponsor: NHLBI OCD

IND Sponsor Representative: Cynthia E. Dunbar, MD, TSCBB, NHLBI

The PI will report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The Sponsor (or designee) will determine the reportability of the event to the FDA and IND safety report will be submitted to the FDA as required as either an IND Safety Report or Annual report.

IND Annual Report

A summary of all SAEs, non-serious AEs, and other events will be recorded and submitted to the Sponsor and FDA in annual progress reports (21 CFR 312.64(b)). Annual progress reports will be submitted within 60 days after the anniversary date of the IND.

IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The sponsor must notify the FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. The sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

15-day reporting

The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group

The sponsor must submit each IND safety report on a MedWatch form (Form FDA 3500A) or in a narrative form to the Project Manager.

9.3. Reporting Serious Adverse Events to Novartis

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Novartis within 24 hours of learning of its occurrence. Information about all SAEs is collected and recorded on a Serious Adverse Event Report Form. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and **send the completed, signed form along with the Novartis provided fax cover sheet to the Novartis Oncology Drug Safety and Epidemiology (DS&E) department by fax (fax: 877-778-9739) or via email (email: clinicalsafetyop.phuseh@novartis.com) within 24 hours.**

Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Any SAEs experienced after the 30-day safety evaluation follow-up period (or 5 half-lives, if half-life is established, whichever is longer) should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment.

Follow-up information is submitted in the same way as the original SAE Report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the

event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology Novartis Drug Safety and Epidemiology (DS&E) department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

9.4. Reporting of pregnancy

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be reported by the investigator to the Novartis Oncology Drug Safety and Epidemiology Department (DS&E) by fax **(fax: 877-778-9739)** or via email **(email: clinicalsafetyop.phuseh@novartis.com)**. In the case of adverse pregnancy outcomes, follow up should include an assessment of the possible relationship to the investigational drug. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded and reported to Novartis as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported to Novartis. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported to Novartis. While the investigator is not obligated to actively seek this information in former study participants, he/she may learn of an SAE through spontaneous reporting.

Further advice on the length of post-natal follow up can be sought from the Novartis Pediatric Advisory Group and should be driven by the type of congenital abnormality expected.

9.5. Data management

The principal investigator, associate investigators, research nurses and/or a data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as intake forms and the case report forms. Laboratory data from NIH will be reviewed

using CRIS. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from the subjects' home physician.

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. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable.

Research data will be prospectively collected by authorized Investigator personnel and entered into the NHLBI approved Database. The data base is, 21 CFR 11 compliant and will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

The database will maintain complete data records on each research subject. Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes. These protocol notes will serve as the primary source material from which data will be collected in NHLBI approved database. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards. Neither individual personal identifiers nor the key linking coded data to individuals will be released without prior IRB approval and an executed CDA or MTA. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA.

Data with subject personal identifiers may be sent to associate investigators and collaborators outside of the NIH only after an executed reliance agreement with NIH's IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

Storage: All samples will be stored in the laboratory of Dr. Cynthia Dunbar under the supervision of Dr. David Young. Collected samples will be de-identified prior to storage in the laboratory of the principal investigator following current NHLBI DIR BSI Policy. Efforts to ensure protection of patient information include:

- Each sample is assigned a unique number
- Vials holding patient samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information
- An electronic database is used to store patient information related to the coded samples
- The laboratory is located in a controlled access building and laboratory doors are kept locked. Visitors to the laboratory are required to be accompanied by laboratory staff at all times
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.

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 an executed MTA or CTA. Identifiable samples will not be sent without IRB approval.

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. At the completion of the protocol (termination), samples and data will be maintained in a repository for future research.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

Privacy and Confidentiality: All efforts, within reason, will be made to keep subjects' private identifiable information (PII) private. Using or sharing ("disclosure") such data must follow federal privacy rules. Under certain circumstances, the United States Office of Human Research Protections (OHRP), The US Food and Drug Administration (FDA), and the NIH Institutional Review Board (IRB), will be able to inspect and copy confidential study-related records which identify participants by name. Therefore, absolute confidentiality cannot be guaranteed.

Data sharing and future use of data

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII. Future research use of identifiable data not defined in the research protocol may occur only after IRB review and approval. Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

This study will be conducted in accordance with the following publication and data sharing policies and regulations: NIH Public Access Policy, which ensures that the public has access to the

published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the 2023 NIH Data Management and Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov.

9.6. Future use of biospecimens

Following analyses of biospecimens for primary research purposes, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB or OHSRP approval, as applicable. Biospecimens may be destroyed only when permitted by the clinical director.

Any future research use of biospecimens not defined in the protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. , and an executed transfer agreement.

10. Human Subject Protection

10.1. Rationale for subject selection

With an incidence of about seven cases per million live births each year in the US and with a 50-60% expected rate of refractory disease and/or failed first-line therapy, DBA constitutes a rare disease (18). The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol, or have a guardian authorized to provide consent on their behalf. No subjects will be excluded from participation based on gender, race or ethnicity.

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

For subjects with renal impairment: The pharmacokinetics of EPAG has been studied in adult patients with renal impairment. Following administration of a single 50 mg dose, there was a trend for reduced plasma EPAG exposure in patients with renal impairment, but there was substantial variability and significant overlap in exposures between patients with renal impairment and

healthy volunteers. Therefore, patients with impaired renal function will be included and given the protocol-defined dosages, but participation will be monitored closely.

For subjects with hepatic impairment: Pharmacokinetics of EPAG have been studied in adult patients with hepatic impairment. Following the administration of a single 50 mg dose, the $AUC_{0-\infty}$ of EPAG was increased by 41% in subjects with mild hepatic impairment and by 80% to 90% in subjects with moderate or severe hepatic impairment compared with healthy volunteers. Therefore, patients with minimally impaired hepatic function will be included but participation will be monitored closely. Patients with baseline moderate to severe hepatic impairment will be excluded from the study.

Recruitment efforts: The study may use the following strategies of recruitment including:

- ClinicalTrials.gov website
- Clinical Center Research Studies ("Search the Studies") website
- Diamond-Blackfan Anemia Foundation, Inc. (dbafoundation.org) website
- National Heart, Lung and Blood Institute (NHLBI) patient recruitment website
- Twitter messages and chats with study investigators
- Facebook Posts
- Google Ad Words
- In collaboration with the National Cancer Institute (NCI) Inherited Bone Marrow Failure Syndrome Study (IBMFS), a natural history Cohort Study
- Use of Clinical Center Office of Patient Recruitment services including creation and distribution of study flyers and information through pre-existing recruitment avenues such as the NIH recruitment listserv.

The DIR Patient Recruitment Office (PRO) will work with study investigators to ensure accrual goals are being met. Biannual reviews will be performed by investigators and the PRO. If recruitment goals are lagging, an enhanced plan of recruitment will be triggered, which may include a communication blitz to known and newly contacted hematologists and oncologists throughout the country; additional Twitter feeds and chats, Facebook posts and recirculation of updated and/or new patient recruitment flyers. All recruitment materials and tools will use IRB-approved language and information to include standard recruitment contacts.

Recruitment language to be used:

> ***Do you know someone with Diamond-Blackfan anemia (DBA)?***
>
> *Researchers at the National Institutes of Health (NIH) in Bethesda, Maryland are conducting an investigational treatment study with eltrombopag to help patients with Diamond-Blackfan anemia (DBA). Eligible volunteers must be diagnosed with DBA, especially DBA that has not responded to or recurred despite standard therapies. DBA*

is an inherited disease that can lead to anemia and ultimately bone marrow failure, with increased risks of cancer. Eltrombopag is a drug that can help patients with aplastic anemia, an acquired bone marrow failure syndrome. Whether it can also help patients with different, inherited bone marrow failures is a point of ongoing research at the NIH. This study will attempt to determine whether eltrombopag is safe and effective for patients with DBA.

Though considered primarily a blood disease, DBA may affect all systems of the body, especially as the treatments involved (chronic transfusions and/or steroids) have wide-ranging effects. Eltrombopag has been shown to help reduce the iron that can build up in DBA patients, and consequently may help reduce some of these side effects. In addition to investigating whether eltrombopag can improve blood cell counts in DBA patients, this study will help answer this question. Travel, food and lodging may be provided consistent with guidelines of the National Heart, Lung and Blood Institute policies.

Study Design:

- *1-2 visits to the NIH Clinical Center in Bethesda, Maryland before starting drug and then every 6 months while on drug*
- *Patients will take eltrombopag over a 6-month period*
- *Patients who exhibit improvements after 6 months of taking eltrombopag may be eligible to continue taking eltrombopag for up to 3 additional years*

Who Can Participate:

- *Patients 2 years or older diagnosed with Diamond-Blackfan anemia (DBA)*
- *Patients diagnosed with DBA who have not responded to therapy, have relapsed, and/or are unable to tolerate current therapy*
- *Patients willing to take eltrombopag for a minimum of 6 months*
- *Patients willing to have their blood drawn every two weeks by your home physician*

The NIH Clinical Center, America's Research Hospital, is located in Bethesda, Maryland, on the Metro red line (Medical Center stop).

For more information:

NIH Clinical Center Office of Patient Recruitment
866-444-1132
TTY users call via MD Relay 7-1-1
Se habla español
Email: PRPL@cc.nih.gov
NIH Study: 20-H-0021

For shorter recruitment materials such as Twitter or Facebook one of the following shall be used:

Option 1 (Post):

Do you or someone you know have Diamond-Blackfan anemia (DBA)? Researchers at the National Institutes of Health (NIH) in Bethesda, Maryland are conducting an investigational treatment study with eltrombopag to help patients with DBA. Treatment and research procedures are provided at no cost. Contact us at 1-800-411-1222, PRPL@cc.nih.gov. Refer to study # 20-H-0021

Option 2 (Post):

NHLBI researchers are looking for patients who have been diagnosed with Diamond-Blackfan anemia (DBA). Eligible volunteers must be diagnosed with DBA, especially DBA that has not responded to or recurred despite standard therapies. To learn more about this study, call the Office of Patient Recruitment at 1-800-411-1222 (refer to study # 20-H-0021)

Option 3 (Post):

Though considered primarily a blood disease, Diamond-Blackfan anemia (DBA) may affect all systems of the body, especially as the treatments involved (chronic transfusions and/or steroids) have wide-ranging effects. Researchers at the National Heart, Lung, and Blood Institute are conducting an investigational treatment study with eltrombopag to help patients with DBA. Eltrombopag has been shown to help reduce the iron that can build up in DBA patients, and consequently may help reduce some of these side effects. To learn more about this study, call the Office of Patient Recruitment at 1-800-411-1222 (refer to study # 20H0021) [URL shorten version]

Option 4 (Tweet):

Researchers at the @NHLBI need patients who have been diagnosed with Diamond-Blackfan anemia (DBA). NIH Clinical Center Office of Patient Recruitment at 1-800-

411-1222. Refer to study # 20-H-0021

Option 5 (Tweet):

Researchers from @TheBethesdaLabs and @NIHClinicalCntr are seeking volunteers diagnosed with Diamond-Blackfan anemia, especially DBA that has not responded to or recurred despite standard therapies. #ResearchMatters

Option 6 (Tweet):

Do you know someone with #DiamondBlackfanAnemia (DBA)? @TheBethesdaLabs is seeking volunteers for a study with eltrombopag to help #patients with #DBA. #ResearchMatters

Option 7 (Tweet):

#HCPs: A new #clinicaltrial from @TheBethesdaLabs will determine whether #eltrombopag is a safe and effective treatment for patients with #DiamondBlackfanAnemia. #DBA #ResearchMatters

Option 8 (Tweet):

#Patients diagnosed with #DiamondBlackfanAnemia may be eligible for a new #clinicaltrial at the @NIHClinicalCntr. Learn more: #DBA #ResearchMatters

Option 9 (Tweet):

A new #NHLBI study may provide an alternative treatment option for #patients with #DBA. Learn more: #ResearchMatters @NIHClinicalCntr

Reimbursement for protocol participation, travel, food, and lodging will be consistent with

NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.

Payment for participation: $0 – Subjects will not be compensated for their participation in this study.

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

10.2. Participation of Pediatric patients

As outlined in §2.2. the anemia from red cell aplasia that is the hallmark of Diamond-Blackfan anemia usually manifests in the first few years of life. We are currently treating pediatric patients with EPAG on 4 different protocols: in the combination with immunosuppression (12-H-0150), headed by Dr. Neal Young, protocols 11-H-0134 for patients with moderate aplastic anemia and 13-H-0133 for patients with refractory aplastic anemia, led by Dr. David Young and Dr. Cynthia Dunbar, and protocol 17-H-0121 for patients with Fanconi anemia, headed by Dr. Andre Larochelle. Based on this experience we have now established a dosing schedule that is associated with very limited toxicities. However higher weight-adjusted drug clearance is possible, particularly in younger children. Pharmacokinetic studies (PK) in pediatric patients with Diamond-Blackfan anemia treated with EPAG have not been performed, although similar studies in patients with Fanconi anemia are ongoing (protocol 17-H-0121). Therefore, PK studies will be performed. If PK studies indicate significant faster drug clearance potentially affecting its efficacy, we will amend the protocol and adjust the pediatric dosing accordingly. Given the early presentation of the disease, and the importance of early intervention, we propose enrolling patients 2 years of age or older. As EPAG is a potent iron chelator, and given the role of iron in neurodevelopment, we will perform baseline and landmark neurodevelopmental testing on pediatric patients where possible.

10.3. Risks and Discomforts

10.3.1. Related to Promacta® (Eltrombopag)

Potential Serious Adverse Effects:

WARNING: RISK FOR HEPATIC DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C RISK OF HEPATOTOXICITY See full prescribing information for complete boxed warning In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation. PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Monitor hepatic function and discontinue dosing as recommended.

Warnings and Precautions with eltrombopag:

Hepatic Decompensation in Patients with Chronic Hepatitis C

In patients with chronic hepatitis C, PROMACTA in combination with interferon and

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

Hepatotoxicity

PROMACTA may increase the risk of severe and potentially life-threatening hepatotoxicity. Measure serum ALT, AST, and bilirubin prior to initiation of PROMACTA, every 2 weeks during the dose adjustment phase, and monthly following establishment of a stable dose. PROMACTA inhibits UDP-glucuronosyltransferase-1A1 (UGT1A1) and organic anion-transporting polypeptide-1B1 (OATP1B1), which may lead to indirect hyperbilirubinemia. If bilirubin is elevated, perform fractionation. Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until resolved or stabilized. Discontinue PROMACTA if ALT levels increase to greater than or equal to 3 x ULN in patients with normal liver function or greater than or equal to 3 x baseline (or greater than 5 x ULN, whichever is the lower) in patients with pre-treatment elevations in transaminases and are:

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

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

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

Thrombotic/Thromboembolic Complications

Thrombotic/thromboembolic complications may result from increases in platelet counts

with PROMACTA. Reported thrombotic/thromboembolic complications included both venous and arterial events and were observed at low and at normal platelet counts.

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

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

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

Cataracts

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

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

Clinical Experience:

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

Severe Aplastic Anemia: In the single-arm, open-label trial, 43 patients with severe aplastic anemia received PROMACTA. Eleven patients (26%) were treated for greater than 6 months and 7 patients (16%) were treated for greater than 1 year. The most common adverse reactions (greater than or equal to 20%) were nausea, fatigue, cough, diarrhea, and headache (see Table 9).

Table 9: Adverse reactions (≥10%) from one open-label trial in adults with severe aplastic anemia

Adverse Reaction	**Eltrombopag (*n* = 43) (%)**
Nausea	33
Fatigue	28
Cough	23
Diarrhea	21
Headache	21
Pain in extremity	19
Dyspnea	14
Pyrexia	14
Dizziness	14
Oropharyngeal pain	14
Febrile neutropenia	14
Abdominal pain	12
Ecchymosis	12
Muscle spasms	12
Transaminases increased	12
Arthralgia	12
Rhinorrhea	12

Rash was reported in 7% of patients.

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

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category C

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

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

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

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

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

Nursing Mothers

It is not known whether eltrombopag is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in

nursing infants from PROMACTA, a decision should be made whether to discontinue nursing or to discontinue PROMACTA taking into account the importance of PROMACTA to the mother.

Pediatric Use

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

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

Severe cutaneous reaction

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

The safety of eltrombopag has also been studied in healthy volunteers who received the drug for 5 days at doses as high as 200mg daily with safety profiles similar to placebo (91).

Investigator Brochure, Version 13, dated 4/13/2016 - "Adverse Events considered to be Expected for Reporting Purposes"

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

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

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

Table 10: Adverse events considered to be expected for reporting purposes in chronic ITP adults

Infections and infestations	
Common:	Pharyngitis
	Urinary tract infection
Gastrointestinal disorders	
Very Common:	Nausea
	Diarrhea
Common:	Dry mouth
	Vomiting
Hepatobiliary disorders	
Common:	Increased aspartate aminotransferase
	Increased alanine aminotransferase
	Blood bilirubin unconjugated increased
Uncommon:	Drug-induced liver injury
Skin and subcutaneous tissue disorders	
Common:	Alopecia
	Rash
Musculoskeletal and connective tissue disorders	
Common:	Back pain
	Musculoskeletal chest pain
	Musculoskeletal pain
	Myalgia
Vascular disorders	
Rare: post-marketing cases of Thrombotic microangiopathy with acute renal failure reported spontaneously	

Table 11: Additional adverse events considered to be expected for reporting purposes in chronic ITP pediatric patients (aged 1 to 17 years) in addition to those seen in chronic ITP in adults

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

Table 12: Adverse events considered to be expected for reporting purposes in severe aplastic anemia

Blood and lymphatic system disorders	
Very common:	Anemia

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

Table 13: Adverse events considered to be expected for reporting purposes in MDS/AML

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

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

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

Additional Eltrombopag risks

Although the risk is considerably lower than with other forms of inherited bone marrow failure syndromes such as Fanconi anemia or Dyskeratosis Congenita, patients with

Diamond-Blackfan anemia may be at risk of developing chromosomal abnormalities and progressing to another form of bone marrow failure syndrome called myelodysplasia. As noted in the scientific justification above (§2.4.1), prior evidence indicates that eltrombopag acts as a chelator of iron, reducing intracellular reactive oxygen species stress, improving hematopoiesis and reducing the intrinsic genotoxic stresses upon erythroid precursors and hematopoietic stem cells. Additionally, previous work has suggested that eltrombopag may improve DNA repair mechanisms in hematopoietic stem cells *in vivo*. Combined, these effects may counteract or delay any naturally occurring malignant transformation in Diamond-Blackfan patients. However, eltrombopag stimulates stem cells and potentially also pre-malignant stem cells. Therefore, it is hypothetical that eltrombopag accelerates malignant transformation, although this has not been borne out by prior studies in patients with existing pre-malignant conditions.

Nonclinical Pharmacology

Studies conducted in vitro have shown that EPAG is an effective agonist binding to TPOR, the thrombopoietin (TPO) receptor encoded by *MPL*, to stimulate thrombopoiesis. It binds TPOR at a position distinct from the ligand binding site, and thus does not compete with TPO for binding to its receptor. 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 TPOR agonist that functions in a similar manner to endogenous TPO. Additionally, in vitro electrophysiology studies have been performed and in vivo safety pharmacology studies assessed the effects of eltrombopag on cardiovascular, respiratory and central nervous systems.

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

Comprehensive nonclinical pharmacokinetic, distribution, metabolism and excretion studies were conducted in the mouse, rat and dog with EPAG. Absorption of EPAG 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.

Nonclinical toxicology

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

The toxicity profile of eltrombopag has been defined in a single dose study in dogs and repeat dose toxicity studies of up to 13 weeks in mice, 28 weeks in rats and 52 weeks in dogs. In addition, repeat dose toxicity was assessed in 2-year carcinogenicity studies in mice and rats.

The principal nonclinical toxicology findings associated with eltrombopag treatment include:

Cataracts (mice and rats)

In vitro phototoxicity (3T3 and CHO cells) was observed. In mice and rats, the development of cataracts was dose- and time-dependent and the rapidly developing lenses of young mice were shown to be more susceptible. Cataract development was not associated with drug accumulation in ocular tissues. No treatment-related ocular abnormalities were evident in dogs given the maximum tolerated dose of 30 mg/kg/day (418 µg/mL) for 52 weeks based on detailed ophthalmologic and histologic examinations. There was no evidence of acute photo-ocular toxicity in albino or pigmented rats. An apparent delay in onset and a lower incidence of cataracts in albino mice housed in subdued versus ambient light was observed suggesting that light may contribute to cataract development in young mice. However, there was no evidence of ocular phototoxicity in young albino or pigmented mice given

eltrombopag and exposed to repeated doses of solar-simulated ultraviolet radiation (UVR). B6C3F1 mice (a pigmented strain) given eltrombopag with or without UVR exposure appeared to be more susceptible than albino mice to eltrombopag-induced cataractogenesis. However, given that eltrombopag has not been shown to be selectively retained in melanin-containing tissues, this likely represents a strain difference in sensitivity to cataract induction.

Renal toxicity (mice and rats).

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

Hepatotoxicity (mice, rats and dogs)

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

Genotoxicity

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

Carcinogenicity

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

Teratogenicity

Eltrombopag was not teratogenic in rats or rabbits and did not affect fertility in male rats or fertility, early embryonic development, embryofetal development, maternal

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

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

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 EPAG with a polyvalent cation-containing antacid (1,524 mg aluminum hydroxide, 1,425 mg magnesium carbonate, and sodium alginate) decreased plasma EPAG 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 EPAG 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 EPAG.

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

Race: Based on both non-compartment analysis and population pharmacokinetic analysis, plasma eltrombopag exposure was approximately 70% higher in some Asian subjects (East and South East Asian) with ITP as compared to non-Asian subjects who were predominantly Caucasian in these trials. In addition, the pharmacodynamic (PD) response to eltrombopag was qualitatively similar in the Asian subjects, but the absolute PD response was somewhat greater. An approximately 40% higher systemic EPAG 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 EPAG clearance than females, after adjustment for the body weight difference.

Hepatic Impairment: Plasma EPAG 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 EPAG. The degree of hepatic impairment was based on Child-Pugh score. Plasma EPAG 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.

10.3.2. 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.

10.3.3. 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.

10.3.4. Related to neurodevelopmental testing

The only anticipated adverse consequences associated with neurodevelopmental testing will be the time required for the participants to complete the assessment.

10.3.5. Related to HRQL

The only anticipated adverse consequences associated with the HRQL will be the time required for the participants to complete the questionnaire.

10.3.6. Related to MRI imaging

MRI imaging may cause discomfort to patients, especially patients with claustrophobia. Anxiolytics will be provided to patients to relieve this discomfort. For pediatric patients and severely affected adults, it may be necessary to provide sedation. There is no risk of allergic reaction in patients with a positive history as contrast (intravenous or oral) is not planned for these studies. There is a risk of burns or injury if metal is present in the scanner; patients will be pre-screened prior to all scans.

10.3.7. Risks related to genetic testing

Accidental disclosure of genetic information derived from research testing will be unlikely as all samples and research data will be coded and kept separately from the patient demographic information. Individually identifiable genetic data directly related to this study will not be released to participants. We do not intend to report mutations in genes associated with disease risk that are incidental to our research aims, commonly referred to as "incidental findings." With increasing technology, it is possible that a person may be identifiable from their sequence data submitted as part of a publication of this material. We will attempt to limit this, by again using only coded specimens.

10.4. Risks in Relation to Benefit

10.4.1. For adult subjects

The benefits to the patients could be improvement of cytopenias (increased blood cell numbers) and/or reduction or even abolition of 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.

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.

10.4.3. For Cognitively Impaired subjects

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

10.5. Informed Consent Processes and Procedures

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent prior to entry onto the study. Consent may be obtained with required signatures on the

hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study will use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both will view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic).

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

Subjects who are cognitively impaired and who lack capacity to provide consent: The consent process for these subjects will follow the requirements of Policy 403.

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

Justification for inclusion: This research provides the prospect of direct benefit; therefore, inclusion is justified. There are no plans to include institutionalized participants.

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

Minor Participants: If the subject is a minor, the parent who signs the consent for the minor must be a legally authorized parent or guardian. For minors that are age 7 and up, 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. Age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. Verbal assent will be obtained as appropriate for children less than 12 years of age, exclusive. Children under the age of 18, but who are 12 years of age or older will be asked to sign an age-appropriate assent form. Children under the age of 7 years, exclusive, will not be required to provide assent as they typically do not have the ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide assent (verbal versus written) as applicable. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. All children will be reconsented upon reaching the age of 12 years, as described below. For pediatric patients with cognitive disabilities, the requirement for assent, written and/or verbal, will be adjusted as deemed appropriate by the clinician and the child's parent(s) and/or guardian(s).

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

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

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

The consent process for cognitively impaired minor subjects who reach the age of majority will follow the requirements of Policy 403.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (f)(3), each of which must be addressed in relation to the protocol:

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

10.6. Conflict of Interest

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

10.7. Collaborative Agreements

Cooperative Research and Development Agreement (CRADA)

This protocol has an associated CRADA with Novartis.

Material Transfer Agreements (MTA)

Between NHLBI (David Young, MD, Ph.D.) and Dr. Jan Abkowitz at University of Washington. Upon execution of the MTA, coded peripheral blood or marrow samples from 20H0021 may be sent to the University of Washington. The goal of this collaboration is to identify the defect in red blood cell development for each patient, matching these results with disease-causing mutation (if known) and drug response, the final goal of which is to understand the underlying cause of

response or lack thereof. The NIH research team will receive research results that can be linked to identifiers as part of this research collaboration.

11. Pharmaceuticals

11.1. Eltrombopag (Promacta®)

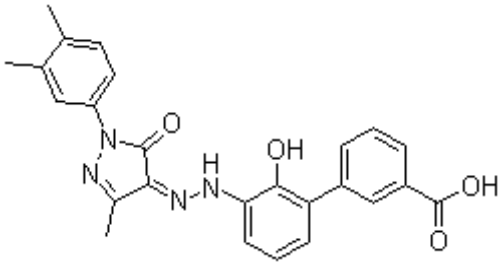
Supply: Novartis

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

Molecular formula: $C_{25}H_{22}N_4O_4.2(C_2H_7NO)$.

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

Chemical and structural formula:



Physical form: red/brown solid.

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

Supply: The drug Novartis is providing for this study may be either investigational or commercial material, based on their supply. It is available as tablets of 12.5, 25, 50 and 75mg. It is also available as a powder for oral suspension (PfOS) that will be available in 12.5 mg or 25 mg strength.

- **Tablets:**

  White, round, film-coated tablets without debossing are provided, containing eltrombopag olamine equivalent to 12.5 mg, 25 mg, 50 mg, or 75 mg of eltrombopag free acid. Placebos to match the active tablets are available. Tablets

are packaged in white HDPE bottles with white plastic, induction-seal, child-resistant caps.

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

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

- **Powder for Oral Suspension:**

  Doses for children between the ages 4 to 5 (2.5mg/kg non-East/South Asian participants, and 1.25mg/kg East/South Asian participants) will be provided in the form of sachets (when available). The powder for oral suspension is a reddish brown to yellow powder in a sachet. Two PfOS strengths are available, containing eltrombopag olamine equivalent to 12.5 mg and 25 mg of eltrombopag free acid. Reconstitution of eltrombopag PfOS will follow instructions outlined in the Investigator's Brochure and Package Insert.

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

Shipping: The NIH Investigational Drug Management and Research Section (IMDRS) will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for Novartis supplied investigational agent is:

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

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

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13. Appendices

APPENDIX A: Schedule of Activities

Table 14: Schedule of Activities (Pre-enrollment through first 6 months of treatment)

Visit/Time Point	Baseline[1]	Week (± 10 days[2])											Month 6[3]	Month 12 Off-study[5]
		2	4	6	8	10	12[3,4]	14	16	18	20	22		
Clinical Assessments														
Consent	X													
Medical history / Interval History	X						X						X	X
Physical examination	X						X[4]						X	X
Pediatric consult	X													
Neurodevelopmental testing	X[9]						X[6]						X	
Concurrent medication review	X[7]													
Transfusion records review	X						X						X	X
Laboratory Evaluations														
Complete blood count with differential	X[7]	X	X	X	X	X	X	X	X	X	X	X	X	X
Reticulocyte count	X[7]						X						X	X
Peripheral blood smear	X												X	X
Hemoglobin F quantification	X						X[6]						X	X
Erythrocyte adenosine deaminase	X						X[6]						X	X
DAT (direct antiglobulin test)	X													
Chromosomal Breakage Analysis	X[8]													
Genetic testing	X[8]													
Acute care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)	X[7]						X						X	X
Mineral (Phosphorus, Magnesium, Albumin, and Calcium)	X						X						X	X
Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)	X[7]						X						X	X

Visit/Time Point	Baseline[1]	Week (± 10 days[2])											Month 6[3]	Month 12 Off-study[5]
		2	4	6	8	10	12[3,4]	14	16	18	20	22		
Laboratory Evaluations (continued)														
Hepatic (ALT, AST, Total Bilirubin, OR Direct Bilirubin)		X	X	X	X	X		X	X	X	X	X		
Total Protein	X						X						X	X
CK	X						X						X	X
Uric Acid	X						X						X	X
LDH	X						X						X	X
Coagulation and thrombosis screens (PT, PTT)	X													
Viral serologies: HepA, HepB (HBsAg, HBsAb, DNA PCR), HCV, HIV, HSV, EBV and CMV	X													
Folate level	X													
B12 level	X													
Metals: copper and zinc level	X												X	X
Iron panel: ferritin, transferrin, % saturation, total serum iron	X	X[0]	X[0]	X[0]	X[0]	X[0]	X	X[0]	X[0]	X[0]	X[0]	X[0]	X	X
Ferritin		X	X	X	X	X		X	X	X	X	X		
24-hour urine collection to determine the total iron	X[9]												X[9]	X[9]
HLA typing (if not performed & available)	X													
Pregnancy test (blood or urine HCG in women of child bearing potential)	X[7]												X	
Immunoglobulins	X													
Thyroid function panel (T3, T4, and TSH)	X													

Visit/Time Point	Baseline[1]	Week (± 10 days[2])											Month 6[3]	Month 12 Off-study[5]
		2	4	6	8	10	12[3,4]	14	16	18	20	22		
Procedures														
Bone marrow aspiration and core biopsy[10]	X												X	X
Bone marrow chromosomal analysis via standard cytogenetic techniques	X												X	X
Electrocardiogram (EKG)	X													
MRI (T2* liver iron concentration)[11]	X												X	X
Research Testing and Assessments														
Research Blood	X												X	X
PK													X	
HRQL survey administration	X[12]												X[12]	
Document Drug Accountability													X	

[1] may use screening results if obtained within 7 days of consent, unless indicated otherwise
[2] all timepoints must be completed within a range of ± 10 days *except* for the 12-week, 6-month and optional 12-month timepoints
[3] 12-week and 6-month evaluations must be completed within a range of ± 14 days
[4] 12-week evaluation may be completed at either home institution or at the NIH Clinical Center; if performed at home institution, a physical exam is optional
[5] optional 12-month off-study evaluation may be completed within a range of ± 30 days
[6] Neurodevelopmental testing for pediatric patients (if previously performed at baseline) and specialized laboratory testing should be performed at the 3-month landmark if the 12-week evaluations are performed at the NIH Clinical Center
[7] May use screening results if obtained within 7 days of consent
[8] not required if already performed elsewhere and records available
[9] at PI's discretion if indicated
[10] stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess iron, reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
[11] MRI will be omitted if unable to be performed due to patient age, size, or intolerance; this will not be reported as a deviation, but will be noted in the medical record
[12] only adult subjects 18 years and older who read English or Spanish will complete the survey
[0] recommended, but not required

Table 15: Schedule of Activities (Extended phase, beginning after 6 months of treatment)

Visit/ Time Point	Monthly (±10 days)	Every 6 Months (±30 days)	Every 12 months (±60 days)	Off study (optional) (±30 days)
Clinical Assessments				
Interval medical history		X[1]	X	X
Physical examination		X[1]	X	X
Neurodevelopmental assessment			X[2]	
Pediatric consult		X[2,3]	X[2,3]	
Laboratory Evaluations				
Complete blood count with differential	X[4]	X[4]	X[4]	X
Peripheral blood smear		X[1]	X[1]	X
Reticulocyte count	X[3]	X[3]	X[3]	X
Hemoglobin F quantification		X	X	X
Erythrocyte adenosine deaminase			X	X
ALT, AST, Total Bilirubin, or Direct Bilirubin	X[3]			
Hepatic panel: Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin		X	X	X
Mineral panel: Phosphorus, Magnesium, Albumin, and Calcium				X
Acute care panel: Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen		X	X	X
Total Protein				X
CK				X
Uric acid				X
LDH				X
Pregnancy test		X[3]	X[3]	
Ferritin	X[4]	X[1]	X[1]	
Iron panel: ferritin, transferrin, % saturation, total serum iron	X[0]	X[1]	X[1]	X
Metals: copper and zinc levels			X	X
Research Blood		X[1]	X[1]	X
24-hour urine collection to determine the total iron		X[1,2]	X[1,2]	X[2]

Procedures				
Bone marrow aspiration and core biopsy[5]		X[1]	X[1]	X
Bone marrow chromosomal analysis via standard cytogenetic techniques		X[1]	X[1]	X
MRI (T2* liver iron concentration)[6]		X[7]	X[7]	
Research Testing and Assessments				
HRQL survey administration		X[1]	X[1]	
Document Drug Accountability		X	X	

[1] per section 6.5.2, only every 12 months ± 60 days for robust responders who discontinue eltrombopag
[2] at PI's discretion
[3] per section 6.5.2, not required for robust responders who discontinue eltrombopag
[4] per section 6.5.2, monthly (± 10 days) for 6 months and then every 3 months (± 30 days) for robust responders who discontinue eltrombopag
[5] stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess iron, reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
[6] MRI will be omitted if unable to be performed due to patient age, size, or intolerance; this will not be reported as a deviation, but will be noted in the medical record
[7] imaging performed at first 6-month follow-up during extended phase, and then annually thereafter while on eltrombopag (see section 6.5) if deemed necessary by the PI
[0] recommended, but not required

APPENDIX B: Collection of samples for PK Assessments

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

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

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

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

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

Shipping Instructions
Samples should be shipped **only on Monday, Tuesday, or Wednesday,** not less often than every 2 months. Samples must be shipped on dry ice via overnight courier to:
LiMajor Pittman PPD
2246 Dabney Road
Richmond VA, 23230, USA
Tel: (804) 977-8017
e-mail: limajor.pittman@ppdi.com