Protocol with Statistical Analysis Plan Cover Page:

Official Title:	Eltrombopag for Patients with Fanconi Anemia
NCT number:	NCT03206086
Document Type:	Protocol with Statistical Analysis Plan (SAP)
Document Date:	July 7, 2026

CLINICAL RESEARCH PROJECT

Protocol # 17-H-0121
IND # 104877
IND Sponsor: NHLBI OCD

Date: July 7, 2026

Title: Eltrombopag for Patients with Fanconi Anemia

Short Title: Eltrombopag for Fanconi Anemia

Other Identifying Words: Fanconi anemia, DNA repair, inflammation, hematopoiesis, CD34, pancytopenia, anemia, thrombocytopenia, bone marrow, Promacta® (eltrombopag)

Principal Investigator:
André Larochelle, M.D., Ph. D., CMTB, NHLBI (E) 301-451-7139 Bldg. 10, CRC 5-5132

Subjects in study:	Number	Gender	Age range
	25 eligible subjects	M/F	6 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

PRÉCIS

Fanconi anemia (FA) is a rare genetic disease that often presents as a bone marrow failure (BMF) syndrome but also can affect any other organ. Etiologically, loss of function mutations in more than 21 different gene members of the FA core complex (i.e. FANCA-FANCV) have been associated with FA. The FA core complex is involved in interstrand cross-link DNA damage repair during cell division. Impaired DNA repair causes genomic instability which consequently can cause apoptosis of the cell or malignant transformation. In addition to impaired DNA repair mechanisms, FA cells exhibit increased sensitivity to pro-inflammatory cytokines (e.g. IFN-γ, TNF-α) and elevated levels of these cytokines have been associated with bone marrow failure in subjects with FA and other inherited bone marrow failure syndromes.

Patients with FA may present with congenital anomalies, such as microcephaly or short stature. However, the failure of the hematopoietic stem cell (HSC) compartment to produce sufficient numbers of peripheral blood cells, and progression to myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) are the greatest risk factors for morbidity and mortality, particular in young patients with FA. In a few reported cases, spontaneous somatic reversion of inherited mutations has resulted in a selective growth advantage of corrected HSCs that subsequently restored hematopoiesis. However, therapeutic options are limited in FA. Although HSC transplantation outcomes have significantly improved over the past two decades, donor availability, graft failure, and FA-specific transplant toxicities are still significant hurdles towards a curative treatment of FA-associated BMF. Moreover, attempts at genetic correction of FA are not yet ready for patient care.

The thrombopoietin (TPO) mimetic eltrombopag (EPAG) has recently been shown to be effective in restoring tri-lineage hematopoiesis in patients with treatment refractory acquired severe aplastic anemia (SAA). Of particular interest for patients with FA is the observation that EPAG also improves the repair of double strand DNA breaks, a mechanism that is impaired in patients with FA. Additionally, our pre-clinical studies indicate that EPAG evades INF-γ blockade of signal transduction from the TPO receptor (cMPL) resulting in improved survival and proliferation of HSCs. Based on these clinical and pre-clinical studies, we hypothesize that EPAG will improve peripheral blood cell counts in patients with FA and thus reduce morbidity and mortality.

This phase II clinical trial proposes to treat patients with FA for 6 months with EPAG to assess safety and efficacy at improving hematological manifestations of FA. Responders at 6 months will be able to continue EPAG on the extension part of this protocol for an additional 3 years. During this time frame we anticipate further improvement of peripheral blood cells counts that will eventually result in the discontinuation of EPAG after a tapering period. Translational studies will explore EPAG effects on DNA repair activity, apoptosis, global transcriptome and TPO signaling pathways in patient's hematopoietic stem and progenitor cells (HSPCs).

TABLE OF CONTENTS

1. OBJECTIVES 5
2. BACKGROUND AND SCIENTIFIC JUSTIFICATION 5
2.1 Pathophysiology of Fanconi Anemia 5
2.2 Clinical Features and Treatment 6
2.3 Thrombopoietin and Hematopoiesis 7
2.4 Eltrombopag 8
2.5 Scientific and Clinical Justification of the Protocol 22
3. STUDY DESIGN 25
4. ELIGIBILITY ASSESSMENT 26
4.1 Inclusion Criteria 26
4.2 Exclusion Criteria 26
5. TREATMENT PLAN 30
5.1 Administration of Study Drug (eltrombopag) 30
5.2 Dose Delays, Adjustments or Discontinuation of eltrombopag for Hematologic Side Effects 32
5.3 Dose Delays, Adjustments or Discontinuation for Non-Hematologic Side Effects 33
5.4 Taper of eltrombopag in the Extension Protocol 36
5.5 Extended Access to Study Drug 36
5.6 Permitted Supportive Care 37
5.7 Non-Permitted Medications 37
5.8 Concurrent Medications: 37
5.9 Instructions to Patients 38
5.10 Health Related Quality of Life (HRQL) Questionnaires 38
6. CLINICAL MONITORING 39
6.1 Screening and Baseline Evaluation within 16 weeks prior to Starting EPAG (Table 10): 39
6.2 Baseline Evaluation within 7 days prior to Starting EPAG (Table 10): 40
6.3 On Study Monitoring (through 6 months) 41
6.4 Landmark 3-month and 6-month Monitoring 42
6.5 Long-Term follow up for Responding Patients Enrolled in Extended Access Phase 43
6.6 Off Study Assessment 45
6.7 Genetic Analysis 46
7. EXPLORATORY RESEARCH STUDIES 47
8. BIOSTATISTICAL CONSIDERATIONS 49
8.1 Objectives 49
8.2 Endpoints 49
8.3 Sample Size 51
8.4 Statistical Methods 51

8.5 Stopping Rules 52
8.6 Off Study Criteria 53
9. DATA AND SAFETY MONITORING 55
9.1 Safety Monitoring 55
9.2 Event Characterization and Reporting 56
9.3 Reporting Serious Adverse Events to Novartis 61
9.4 Reporting of Pregnancy 61
9.5 Data Management 62
10.1 Rationale for Subject Selection 64
10.2 Participation of Pediatric Patients 66
10.3 Risks and Discomforts 66
10.4 Risks in Relation to Benefit 79
10.5 Informed Consent Processes and Procedures 80
10.6 Conflict of Interest 83
10.7 Collaborator(s) 83
11. PHARMACEUTICALS 83
11.1 Eltrombopag (Promacta®): 83
12. REFERENCES 86
13. APPENDICES 93
APPENDIX A: SCHEDULE OF EVENTS 93
APPENDIX B: PHARMACOKINETIC STUDIES 101

1. OBJECTIVES

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

Secondary objectives include assessment of relapse, clonal evolution, toxicities with extended duration of therapy, the impact of treatment and treatment response on quality of life, and the impact of EPAG on organ systems commonly involved in FA (e.g. skin lesions, endocrine dysfunction, and incidence of new head/neck, oropharyngeal, gastrointestinal, anogenital or skin cancers).

Exploratory laboratory objectives include HSPC immunophenotyping, progenitor and reconstitution assays, mutational/clonal dynamics, transcriptomic studies, cytokine/chemokine/soluble receptor profiling, pharmacokinetics and pharmacogenomics on selected patients, and evaluation of DNA repair activity in HSPCs.

2. BACKGROUND AND SCIENTIFIC JUSTIFICATION

2.1 Pathophysiology of Fanconi Anemia

Fanconi anemia (FA) is a rare autosomal recessive (rarely X-linked or dominant) inherited BMFS linked to mutations in at least 21 genes (FANCA to FANCV) encoding proteins involved in the DNA repair complex. Patients with FA may also present with congenital anomalies, such as microcephaly, short stature, abnormal thumbs and radii, and renal anomalies. Most patients develop BMF during the first decade of life, with a high risk of progression to myelodysplastic syndrome and acute myelogenous leukemia. The relative risk of other malignancies, particularly squamous cell carcinoma of the head and neck but also solid tumors of liver, brain or vulva, is dramatically increased in Patients with FA compared to the general population (1).

The FA core complex is involved in interstrand crosslink DNA repair during cell division. Recruitment of the complex to cross-linked DNA initiates ubiquitination of the FANCD2-FANCI (I-D2) complex. Ubiquitinated I-D2 complex activates downstream nucleases to cut either side of crosslinked DNA (unhooking), allowing polymerase to repair one duplex, which then serves as a template for homologous recombination of the other strand (2). Additionally, FA proteins are involved in other DNA maintenance pathways like homologous recombination and non-homologous end joining (NHEJ) (2). Genomic instability due to dysregulated DNA repair/maintenance is thought to be responsible for transformation and high incidence of malignancies in patients with FA. The exact mechanism causing bone marrow failure in patients with FA is unclear. Replicative stress in the context of impaired DNA repair machinery has been shown to cause hyperactivation of the p53/p21 axis responsible for a G0/G1 cell cycle arrest in hematopoietic stem and progenitor cells from patients with FA. Consequently, impaired self-

renewal or proliferation could cause a progressive reduction of the HSPC compartment, eventually manifesting with pancytopenia (3-5).

Extrinsic factors like dysregulated cytokine profiles and hypersensitivity to these humoral factors may further contribute to the impaired function of the FA HSPC compartment. Increased serum levels of TNFα have been reported in patients with FA (6). Furthermore, in vitro and in vivo studies suggest that hematopoietic cells from patients with FA are hypersensitive to IFN-γ induced apoptosis (7-10). However, higher TNFα levels and elevated IFN-γ expression have also been associated with acquired aplastic anemia (AA) (11-14) suggesting a more general and potentially secondary effect in the pathogenesis of bone marrow failure syndromes. Either way, correcting dysregulated proinflammatory cytokines could be promising therapeutic intervention to improve severely impaired hematopoiesis in FA.

2.2 Clinical Features and Treatment

FA is a heterogeneous disease with manifestations in almost every organ system. However, most patients will develop BMF during the first decades of life, and this complication accounts for significant morbidity. Progression to myelodysplastic syndrome and acute myelogenous leukemia usually occurs during teenage years, and solid tumors occur most frequently in the third decade of life. However, hematological and malignant manifestations are quite variable, and a clear genotype-phenotype correlation is not yet established. Registry studies showed that about 86% of patients with FA often develop some degree of BMF at a median age of 7 years. The cumulative BMF incidence of abnormal blood counts at the age of 40 years is 50-90% (15, 16).

Spontaneous somatic mosaicism resulting in a correction of the FA genotype have been reported. Consequently, clonal selection of naturally corrected HSPCs may result in normalization of peripheral cytopenias (17, 18). Despite these rare cases of spontaneous remission, hematopoietic stem cell (HSC) transplantation (HSCT) remains the only long-term treatment option for hematologic manifestations of FA. The general hypersensitivity and poor tolerance of chemotherapy and radiation therapy remains the greatest challenge for the design and utilization of transplantation regimens for patients with FA. Reduced intensity conditioning regimens with the avoidance of alkylating agents and marked reduction of total body irradiation doses improved the rate of immediate toxicities and reduced graft failures. However, donor availability and long term risk for secondary malignancies still remain significant problems for HSCT as a curative treatment of FA-associated BMF (19, 20). Oxymetholone, danazol or other androgens have been successfully used to stabilize or even improve FA associated cytopenias. About half to two third of patients with FA will at least respond transiently to androgens, but side effects, particularly in young patients, often prevent prolonged therapy (21-25). Large prospective studies on the efficacy and safety have not been performed. More novel HSC gene therapy approaches designed to cure the hematological manifestations of FA by correction of the

genetic defect have failed to provide long-term clinical benefits for patients with FA, primarily due to the major challenge of collecting sufficient target HSPCs to correct.

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 the receptor cMPL. 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. The cMPL receptor is expressed and functional on primitive HSPCs (26). Animals and patients with genetic defects in either TPO or cMPL have significant reduction in HSPC numbers and activity, along with profound defects in platelet production (27, 28). *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 (29, 30). The control of TPO levels is complex, and involves cMPL receptor 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 (31).

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 (32, 33). 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 (34). Interestingly, TPO has also been shown recently to improve the DNA-damage response in HSPC by increasing DNA-PK

dependent nonhomologous end-joining efficiency (35, 36).

2.4 Eltrombopag

Eltrombopag (SB-497115-GR, Promacta®), the bis-monoethanolamine salt form, is an orally bioavailable, small molecule 2nd generation thrombopoietin receptor (TPO-R) agonist, developed for the treatment of thrombocytopenia by scientists at GlaxoSmithKline (now Novartis) (37). Studies conducted in vitro have shown that eltrombopag is an effective agonist binding to cMPL, the TPO-R, to stimulate thrombopoiesis. It binds cMPL 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 (38). 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 (39).

In vivo, EPAG increased platelet numbers in chimpanzees (the only non-human species which is pharmacologically responsive to eltrombopag) (39). 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 (40). 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 (41-43), 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% (44, 45). 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 (44, 45). 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 the cMPL receptor, it has been shown to activate the receptor differently than endogenous TPO (46-48). 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 (39). Our own laboratory research indicates that EPAG may also decrease FA associated genomic instability and improve

stemness by overcoming negative regulatory effects of inflammatory cytokines (see section 2.4.1).

Longer follow-up in the ITP EXTEND trial suggests that eltrombopag remains well tolerated (EXTEND Trial, NCT00351468). 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 FA

A significant proportion of subjects with FA may experience bone marrow failure due to an impaired DNA damage response and chronic exposure to elevated levels of proinflammatory cytokines. EPAG may overcome both mechanisms of marrow failure in FA. We have conducted pre-clinical studies to investigate the DNA repair activity of EPAG and its potential to evade the inhibitory impact of inflammatory cytokines on HSPC.

2.4.1.1 Eltrombopag has DNA Repair Activity in HSPC

Recent studies have uncovered a specific function of TPO in the regulation of HSPC DNA damage response. To investigate whether EPAG also has a role in HSPC DNA repair, G-CSF mobilized human CD34+ cells from 5 independent healthy donors were cultured in the presence of SCF and Flt3-L (SF), SF and TPO (SFT), or SF and EPAG (SFE) for 24 hours before exposure to 2Gy γ-irradiation, and then cultured for an additional 5 to 24 hours. DNA damage was quantified by flow cytometric determination of γH2AX expression, a marker of irradiation-induced DNA double-strand breaks (DSB), and CD34+ cell survival was measured by flow cytometry using Annexin V and a viability dye.

We found significantly fewer γH2AX+ cells 5 hours post-irradiation when the culture included TPO or Eltrombopag than with SF alone. Five hours post-irradiation, cultures containing TPO or Eltrombopag had significantly increased percentages of live cells, as well as decreased percentages of cells undergoing apoptosis compared to cultures with SF alone. Twenty-four hours post-irradiation, cells cultured with TPO or EPAG had significantly increased percentages of live cells, and decreased percentages of dead cells compared to cells cultured with SF alone. Progenitor cell survival was assessed using the CFU assay. The number of colony-forming cells was 5.9 (± 0.4) and 3.6 (± 0.2) fold higher

when cultured with TPO or EPAG, respectively, before γ-irradiation than when cultured with SF alone (p=0.005 and 0.006, respectively, n=4). Survival of long-term repopulating HSCs was assessed by quantifying human CD45+ cell engraftment at least 2 months after intravenous injection of immunodeficient NSG mice with irradiated human CD34+CD38- cells pre-cultured for 24 hours with SF, SFT or SFE. Engraftment of cells cultured with TPO or EPAG was significantly higher than engraftment obtained after injection of cells cultured with SF alone.

We conclude that, analogous to TPO, EPAG favors DNA double-strand break repair and, consequently, survival of both hematopoietic stem and progenitor cells after γ-irradiation. These pre-clinical data indicate that Eltrombopag may be of benefit in the treatment of bone marrow failure in patients with FA by favoring DNA repair in HSPC (49).

2.4.1.2 Eltrombopag Evades Interferon-γ blockade of Signal Transduction from cMPL

TPO is the main regulator of HSPC self-renewal and survival. Upon binding to its receptor, cMPL, TPO activates cell signaling, through JAK-STAT and other pathways, which is tightly balanced by negative regulatory signaling processes. Recent studies indicate that chronic exposure of HSPCs to IFNγ, as exemplified in subjects with SAA and FA, impairs self-renewal by perturbing TPO signaling pathways. Despite elevated levels of TPO in subjects with SAA, the TPO receptor agonist EPAG improves trilineage hematopoiesis in these patients, suggesting that it may activate signaling within HSPC in a way that is distinct from TPO under inflammatory conditions.

To address the paradox of EPAG efficacy despite high endogenous TPO levels in bone marrow failure, G-CSF mobilized human CD34+ cells from 6 healthy donors were cultured in the presence of SCF, FLT3 and either TPO or EPAG, with or without IFNγ 100 ng/ml. After 7 days in culture, cells were characterized via flow cytometry, CFU assay and transplantation in immunodeficient (NSG) mice. The percentages and absolute numbers of CD34+ cells in cultures containing TPO or EPAG alone were similar, but were better preserved with EPAG than TPO in the presence of IFN-γ. The adjusted numbers of CFUs after 7 days in the presence of IFN-γ similarly decreased 2.7-fold with TPO but remained unchanged with EPAG compared with cultures without IFNγ. Importantly, when the 7-day expanded progeny of an equal starting number of CD34+ cells was transplanted into NSG mice, human cell engraftment was superior with EPAG (12.3 ± 0.7% human CD45+ cells) than with TPO (2.7 ± 0.8% human CD45+ cells, $p<0.05$) cultures in the presence of IFN-γ, suggesting an impact of EPAG on the most primitive long-term repopulating HSPCs.

To investigate potential mechanisms by which EPAG positively affects maintenance of HSPCs under inflammatory conditions, we examined cell signaling pathways induced

upon binding of TPO, and EPAG to cMPL in human CD34+ cells. At a concentration of 5ng/mL, TPO induced a rapid (peak < 1 hour) and high potency rise in STAT5 phosphorylation followed by a rapid (< 2 hours) decay in signal. In contrast, EPAG induced a slow (peak 4 hours) low potency rise in STAT5 phosphorylation, and the signal persisted for at least 10 hours. We next investigated the impact of IFN-γ on TPO and EPAG-induced STAT5 phosphorylation at the signal peak (<1 and 4 hours, respectively). As previously shown in murine HSPCs, IFN-γ impaired TPO signaling in human HSPCs. Strikingly, EPAG-induced STAT5 phosphorylation was preserved or increased in the presence of IFN-γ. Activation of IFN-γ receptor by its ligand induces phosphorylation of STAT1 and subsequent expression of suppressor of cytokine signaling-1 (SOCS-1), a negative regulator of both IFN-γ and cMPL receptors. Our findings show that SOCS genes, both at the RNA and protein level, are expressed at a similar level in CD34+ cells when exposed to TPO or EPAG in the presence of IFN-γ. Furthermore, we investigated a potential interaction between TPO and IFN-γ that would prevent efficient binding of TPO to cMPL, thus explaining the deficit in STAT5 signaling. By using molecular docking and microscale thermophoresis we showed that TPO and IFN-γ seem to have a strong and specific interaction. In addition, we showed that TPO-dependent cMPL internalization is sizably altered in the presence of IFN-γ, whereas EPAG-dependent internalization is unaltered.

These data indicate that EPAG evades the IFN-γ blockade of a critical pathway of growth factor cell signaling. This may explain its efficacy in improving hematopoiesis in SAA despite high TPO levels. Importantly, these data also indicate that eltrombopag may favor HSPC survival and proliferation under inflammatory conditions in FA subjects (50).

2.4.2 Rationale for Adult Dose Selection prior to February 2024:

Eltrombopag 150 mg once daily has been selected as the starting dose for this study because this regimen has been safe and effective in increasing platelet counts in our recently completed non-randomized, off label, phase II study (NCT00922883) of 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 150 mg daily dosing. While it is possible that patients would have responded to lower doses of drug had the dose not been escalated every two weeks, the lack of toxicity at the 150 mg/day dose in the prior study supports our rationale for utilizing this dose throughout the current trial, unless dose modifications based on toxicity or response criteria are required. A starting dose of 75 mg once daily in East

Asian and South East Asian patients will be used. Modified dosing for subjects of East Asian and South East Asian heritage (self-reported) has been implemented for the following reasons. In healthy Japanese subjects, plasma eltrombopag $AUC_{(0-\tau)}$ was approximately 80% higher when compared 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 (41, 42, 51). 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. Six of 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(42). However, patients with ITP, in contrast to patients with FA, 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 (52). 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 (53). In the current trial, based on concerns regarding thrombosis, dose reductions of EPAG will be made if the platelet count reaches 400,000 per uL or greater.

2.4.3 Rationale for Pediatric Dose Selection prior to February 2024

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 (54-58). 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-τ) 162 μg·hr/mL 95% CI: 139-187; Cmax 11.6 μg/mL 95% CI: 10.4-12.9) compared with adults (AUC(0-τ) 101 μg·hr/mL 95% CI: 91.4-113; Cmax 7.03 μg/mL 95% CI: 6.44-7.68); whereas teenagers, aged 12 to 17 years, experience exposure (AUC(0-τ) 103 μg·hr/mL 95% CI: 91.1-116; Cmax 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-τ) 153 μg·hr/mL 95% CI: 137-170; Cmax 10.3 μg/mL 95% CI: 9.42-11.2) that is nonetheless similar to toddlers and young children (Novartis Pharmaceuticals, Corp., Package Insert-Revised July 2018).

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 (59, 60). 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.

2.4.4 Justification for adjusting eltrombopag doses in adult and pediatric Fanconi anemia patients in the extension phase after February 2024

Justification based on fundamental insights into HSC biology.

The proposed adjustment of EPAG doses for patients with FA is grounded in robust scientific evidence, emphasizing the latest breakthroughs in HSC biology. Pioneering research, such as Wilkinson et al.'s study (61), has been instrumental in establishing the synergistic effects of elevated thrombopoietin (TPO) levels (100 ng/ml) alongside other hematopoietic cytokines in HSC expansion in vitro. This study illuminates how increased TPO levels significantly enhance HSC maintenance and expansion. Given EPAG's role in activating the TPO receptor (cMPL), an increased dose could replicate this beneficial effect, potentially ameliorating hematopoietic outcomes for FA patients.

Further supporting this hypothesis, Cui et al.'s research (62) on surrogate cMPL ligands illustrates that modulating cMPL signaling markedly affects HSC differentiation and proliferation. They discovered that modified agonists not only retained stem-like properties of HSCs but also inhibited oncogenic signaling pathways. This pivotal finding highlights the necessity of precisely regulating cMPL signaling to optimize HSC functionality. Consequently, increasing the dosage of EPAG could effectively fine-tune this signaling pathway, enhancing both the maintenance and functional capacity of HSCs in FA patients.

Moreover, recent insights from Tsutsumi et al. (63) into the TPO-cMPL signaling complex structure further reinforce this rationale. Their research demonstrated how modified TPO variants could decouple platelet production from significant HSC expansion by manipulating cMPL dimerization and signaling pathways. Understanding these structural intricacies also suggests that adjusting EPAG dosages could alter receptor signaling dynamics, potentially achieving a more desirable equilibrium between promoting HSC maintenance and preventing excessive proliferation or differentiation.

In conclusion, considering the genetic underpinnings of FA and its profound effects on HSC integrity, a strategic approach involving increased EPAG doses may be advantageous. This adjustment could markedly enhance therapeutic efficacy, offering a promising avenue for improving clinical outcomes in FA patients. The collective body of research presented forms a robust scientific foundation for the proposed escalation of dosage. As summarized below, this approach is further substantiated by our clinical observations in Fanconi Anemia and corroborated by clinical evidence observed in other hematological disorders.

Enhanced hematological outcomes following eltrombopag dose escalation from 75 mg to 150 mg in our ongoing clinical trial.

As of February 2024, our clinical study has enrolled 23 participants, with 16 meeting the inclusion criteria and completing a 6-month evaluation period after receiving eltrombopag at the previously established dosage for adults and children for an average duration of 26 months (ranging from 7 to 42 months). Our findings indicate a significant bone marrow response in the majority of evaluable patients (87%) after 6 months of treatment. However, peripheral blood (PB) responses have been less pronounced, often limited to a single lineage, predominantly the erythroid lineage. Specifically, 9 out of 15 participants (60%) exhibited a PB response in one (n=5), two (n=3), or three (n=1) lineages at the 6-month mark. No participants receiving an eltrombopag dose equal or lower than 75mg experienced a meaningful increase in platelet counts above 40-50 K/uL or a clinically significant improvement in absolute neutrophil counts. Similarly, although there was a common improvement in hemoglobin levels of more than 1.5 g/dL from baseline, all participants remained anemic.

An important observation involves two pediatric participants who were initially receiving 75 mg daily but had their dose increased to 150 mg daily upon turning 12 years old, as per our study protocol. In both cases, we observed a marked increase in PB counts coinciding with the higher eltrombopag dosage, suggesting that a higher dose might facilitate a more substantial PB response. Despite the normalization of hemoglobin levels in these participants, increases in absolute neutrophil and platelet counts remained below normal ranges, even with the maximum daily dose of 150 mg. Consequently, these participants continue to face heightened risks of infectious and bleeding complications, as depicted in Figure 1.

Globally, these data underscore the potential benefits of dose escalation in eltrombopag treatment for Fanconi anemia, particularly in enhancing PB responses.

Favorable safety profile in FA patients at the previously approved doses.

All participants were able to tolerate the maximum approved dose for their respective age groups. Among the adverse events reported, iron deficiency was the only one definitively linked to the study drug. This outcome is consistent with the known iron chelating properties of eltrombopag, as highlighted in the studies by Vlachodimitropoulou et al. (64), Kao et al. (65), and Zhao et al. (66). Participants who entered the study without transfusional iron overload exhibited a progressive depletion of iron stores, even at a relatively low dose of 50 mg. To address this, iron supplementation was administered, which was well-tolerated, and iron stores were successfully re-established within one month of initiating these supplements.

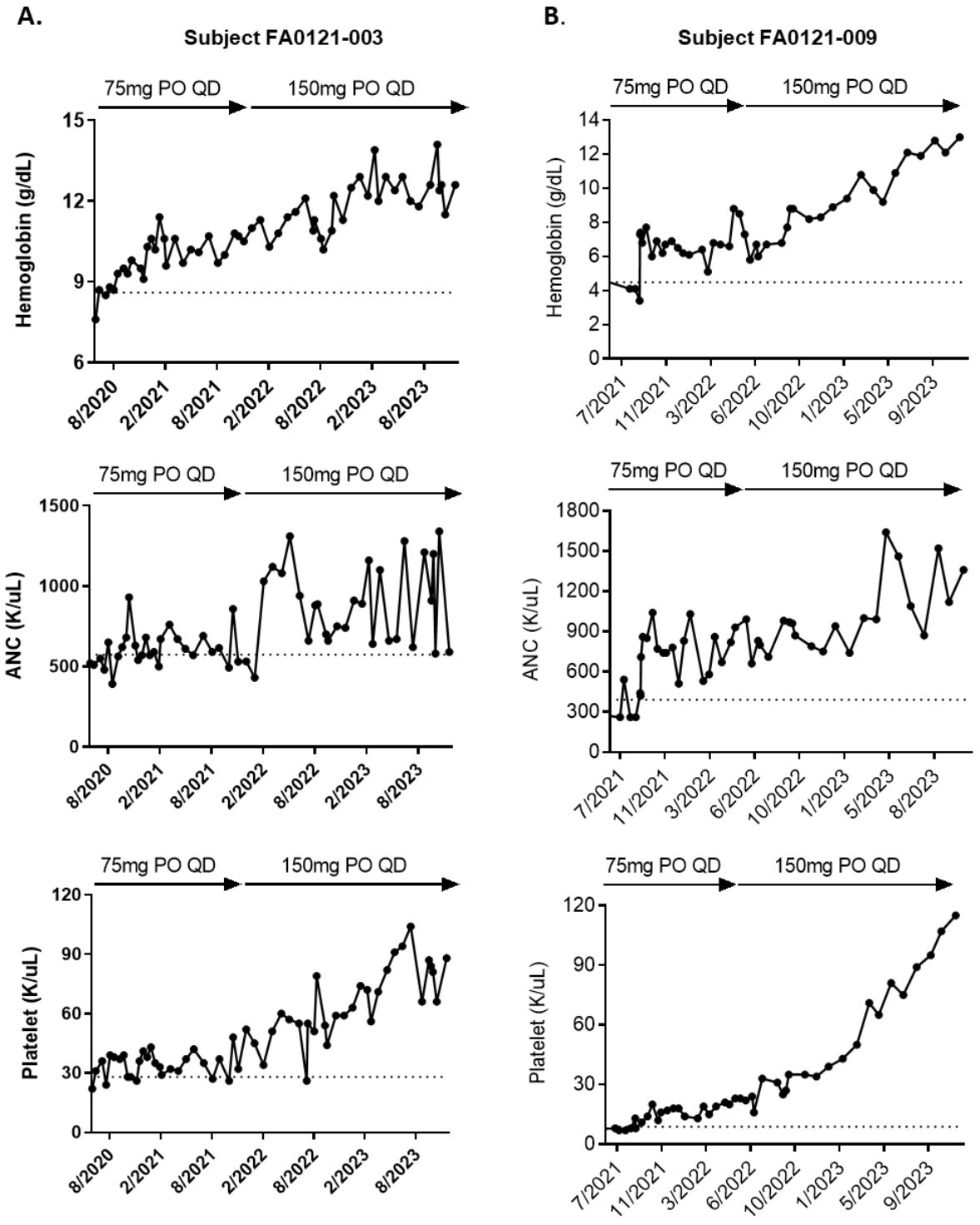


Figure 1. Longitudinal Hematologic Response to Eltrombopag Dosage Escalation. This figure presents time-course hematologic data for two subjects, FA0121-003 (Panel A) and FA0121-009 (Panel B), undergoing eltrombopag therapy. It includes the hemoglobin concentration (upper panel), absolute neutrophil count (ANC, middle panel), and platelet count (lower panel). Initially, both subjects received 75mg of eltrombopag daily while aged less than 12 years, followed by an increase to 150mg daily upon reaching 12 years of age. The baseline for each hematological parameter, established prior to the commencement of eltrombopag, is depicted by the dashed lines in each respective graph.

Two participants, identified as FA0121-003 and FA012-013, experienced transient transaminitis during treatment, necessitating a temporary suspension of EPAG. However, transient elevations in liver function tests are common in patients with Fanconi anemia, though the underlying mechanisms remain unclear. As depicted in Figure 2, similar elevations in liver enzymes were recorded in both cases before the initiation of EPAG treatment, suggesting that these increases might not be directly related to the medication.

In the current study, we have not observed any instances of marrow fibrosis, solid malignancies, or MDS among the participants. However, the development of AML was observed in one subject following treatment with EPAG at a dosage of 50 to 75 mg daily for a duration of 12 months. As of February 2024, this case represents an incidence rate of 6.25% in our study cohort. A thorough review of the cancer risk in FA patients reveals that out of 2190 individuals with FA studied from 1927 to 2012, 188 cases of leukemia were documented, with 84% of these being AML (1). This data translates to an AML incidence rate of approximately 7.2% in the FA population, a figure notably similar to the rate observed in our study. Another participant also treated with EPAG for 12 months at a dose ranging from 50 to 75 mg daily, developed monosomy 7, as identified through standard metaphase analysis of morphologically normal marrow. Monosomy 7 is also a frequent occurrence in untreated FA patients, as documented in studies by Nowell (67), Thurston (68), and Alter (69). Following the detection of monosomy 7, the patient underwent allogeneic stem cell transplantation and is currently in good health. Interestingly, no other participants in the study exhibited monosomy 7 after a median follow-up period of 26 months (ranging from 7 to 42 months). It is also worth noting that one of the 23 candidates assessed for the study was excluded during the screening phase, prior to the initiation of EPAG, due to monosomy 7 with AML transformation. This highlights the inherent genomic instability in Fanconi anemia and the prevalence of such cytogenetic abnormalities and malignant transformation independent of EPAG treatment.

Consequently, although it cannot be categorically excluded that eltrombopag contributed to the observed leukemic transformation and cytogenetic abnormality, the likelihood of its involvement in these serious adverse events appears to be low.

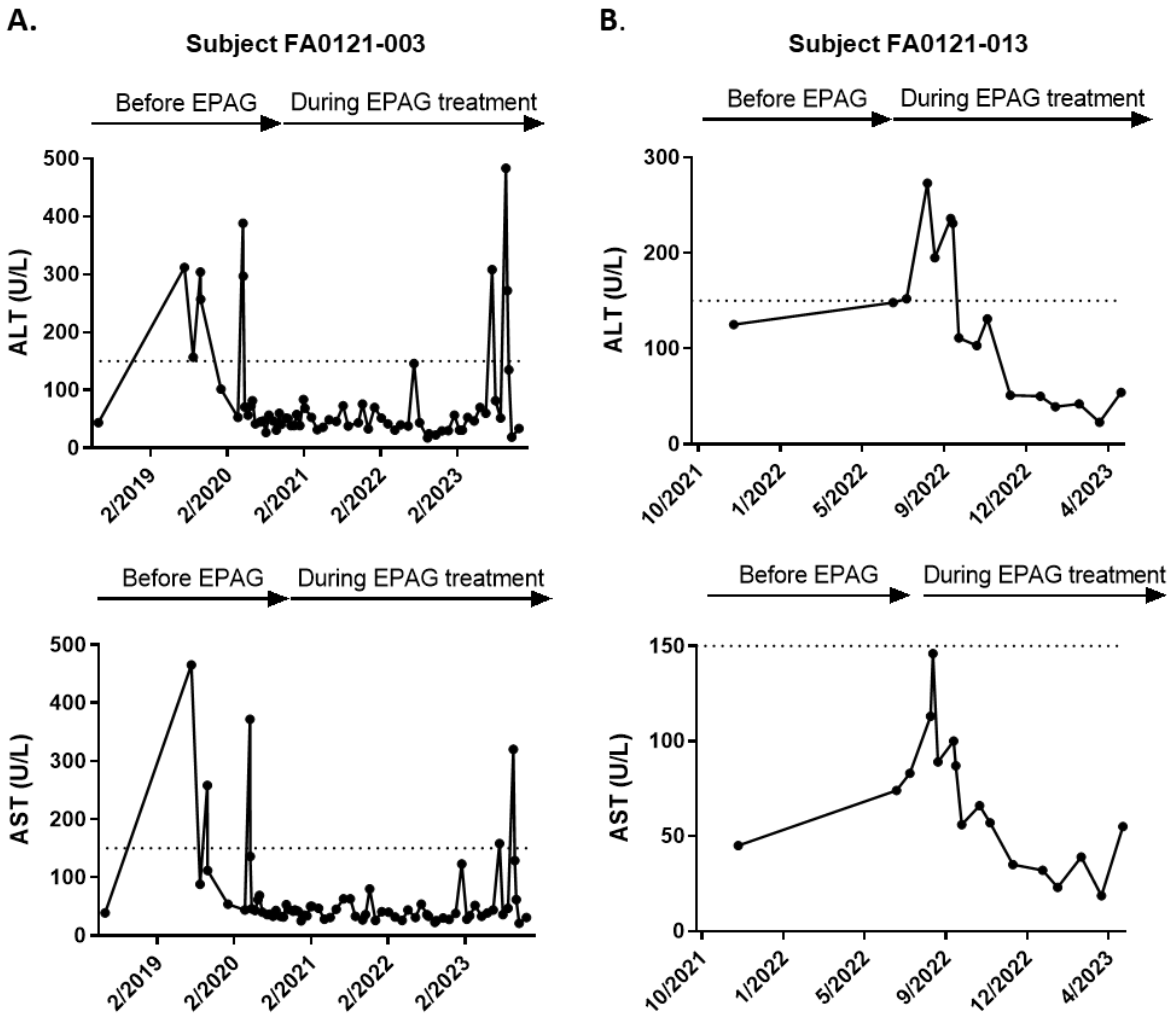


Figure 2. Longitudinal Evaluation of Liver Function During Eltrombopag Therapy. This figure tracks the liver function tests (LFTs) for subjects FA0121-003 (Panel A) and FA0121-013 (Panel B) before and while receiving eltrombopag (EPAG), charting levels of alanine transaminase (ALT, upper panels), and aspartate transaminase (AST, lower panels). Fluctuations in LFTs were recorded both before and after the initiation of EPAG, suggesting that these variations may be attributed to the underlying disease pathology rather than eltrombopag itself. As of December 2023, the remaining 13 evaluable subjects showed no eltrombopag-related alterations in LFTs. The dashed lines in each graph denote the upper limits for LFTs beyond which a temporary suspension of EPAG treatment is deemed necessary according to our study protocol.

Favorable safety profile of eltrombopag at a dosage of 300 mg daily in other hematological disorders.

In a phase 2 study led by Fan et al. (NCT01328587) and published in Blood Advances in 2020 (70), patients with moderate aplastic anemia (MAA) and unilineage cytopenia (UC) were enrolled to evaluate the effectiveness of EPAG in alleviating cytopenias. The study administered escalating doses of EPAG, up to 300 mg daily for participants aged 12 and above, and up to 150 mg for children between 6-11 years. The dose escalation was stopped if the patient's platelet count exceeded 400 x 10^9/L, hemoglobin levels rose above 15 mg/dL, or if non-hematologic toxicities occurred. Out of 34 patients, 32 tolerated EPAG well. However, one patient discontinued the study due to grade 2 nausea and vomiting at 10 weeks, and another halted dose escalation because of grade 2 nausea. Two adult patients with MAA, both of whom responded to EPAG in the extension trial, developed bone marrow cytogenetic abnormalities during treatment, although there was no dysplasia or increase in blasts observed in the bone marrow. The incidence of adverse events in this study did not exceed that observed in previous studies involving bone marrow failure patients treated with a maximum of 150 mg daily of EPAG, except possibly for the occurrence of nausea.

Another study (NCT00903422), discussed in Lancet Haematology (71), involved adult patients with advanced MDS, secondary AML following MDS, or de-novo AML. These patients received daily doses of EPAG up to 300 mg (n=64) or a matching placebo (n=34). The study found the most common adverse events possibly linked to EPAG were nausea (31% for EPAG vs 21% for placebo), diarrhea (30% vs 18%), vomiting (19% vs 3%), abdominal pain (13% vs 6%), constipation (17% vs 12%), headache (14% vs 9%), and dizziness (14% vs 3%). The authors concluded that EPAG doses up to 300 mg were generally well-tolerated in these patient groups. Additionally, the study did not find any dose-dependent relationship with toxicity, nor were there significant differences in hepatobiliary laboratory abnormalities or thromboembolic events at doses up to 300 mg compared with other EPAG trials.

In summary, these studies suggest that EPAG, even at doses as high as 300 mg daily, maintains an acceptable safety profile in treating conditions like moderate aplastic anemia and various forms of hematological malignancies, with adverse events comparable to those observed at lower dosages.

Antiproliferative effects of eltrombopag at high dose.

The observation that EPAG, particularly at high doses, exhibit notable antiproliferative effects is expressly relevant for patients with Fanconi anemia who are at increased risk of malignant transformations, such as myelodysplastic syndrome, acute myeloid leukemia, and squamous cell carcinoma.

Contrary to concerns that EPAG might stimulate the proliferation of malignant cells, preclinical studies have demonstrated no evidence of such an effect in cell lines or primary cells from patients with MDS or AML. In fact, several studies have reported antiproliferative effects associated with EPAG, particularly at higher concentrations achieved with doses above 100 mg in vitro. Notable references include the work of Will B. et al. (72), Erickson-Miller CL et al. (73), Kalota et al. (74), Mavroudi I. et al. (75), Roth M. (76), and Sugita et al. (77). These studies have collectively indicated that eltrombopag does not promote, but rather inhibits, the growth of malignant cells.

Therefore, high doses of EPAG may offer dual benefits for patients, particularly those with conditions like Fanconi anemia. Not only can these doses potentially improve hematological responses, but they may also reduce the proliferative potential of malignant cells. This dual action positions EPAG as a valuable therapeutic agent in managing conditions with heightened risk of malignant transformation.

Optimized dose selection strategy for eltrombopag in Fanconi anemia

The accumulated evidence based on empirical data and clinical experiences underscores the potential efficacy of escalated doses of eltrombopag to enhance HSC functionality within the bone marrow, improve peripheral blood counts, exert an antiproliferative influence, and retain a favorable safety profile in patients with Fanconi anemia. Accordingly, adjustments of eltrombopag dosage are outlined in section 5.1.

2.4.5 Rationale for Permitting Dose Interruption

The effect of dose interruption is unknown in the FA 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 FA or SAA, and EPAG is being utilized in that disorder to overproduce platelets in the bone marrow to compensate for increased antibody-mediated platelet destruction. In FA, 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 will be hospitalized for other disease-related issues such as fever and neutropenia 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 (TRA105325), 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 (HBLAs. 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 FA the number of HSPC is also reduced but, unlike acquired BMFS, HSPC in subjects with FA carry inherited mutations impacting their DNA repair function and sensitivity to inflammatory cytokines. Assuming no increased rate of spontaneous

somatic reversion of inherited mutations resulting in a selective growth advantage of corrected HSCs, hematopoiesis in patients with FA will most likely still rely on HSPC with hypomorphic or null mutations in the FANC genes. Therefore, it is possible that patients with FA will require continuing or repeated EPAG treatments to maintain their peripheral blood counts. 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 (44, 45) and ongoing EPAG studies (13-H-0133) in patients with refractory aplastic anemia, we hypothesize, that 6 months of EPAG treatment will be sufficient to observe a clinically meaningful response also in FA 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 section 5.4, table 6). 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

Bone marrow failure and progression to MDS or leukemia are the greatest risk factors for morbidity and mortality, particularly among young patients with FA (19). To date HSCT remains the only curative treatment for hematological presentations of FA. Improved conditioning regimens and alternative HSPC source, i.e. umbilical cord blood, improved the outcome considerably over the past 20 years. Despite this progress, HSCT is still associated with a substantial risk for treatment related morbidity and mortality, including an accelerated risk for solid tumors as late complications compared with non-transplanted patients (78-80). Oxymetholone, Danazol or other androgens have been successfully used to stabilize or even improve FA associated cytopenias. About two third of the patients will at least respond transiently but side effects, particularly in young patients, often prevent prolonged therapy (21-25). G-CSF can be considered in neutropenic patients (ANC<500/µl) with or without recurrent infections (81). Thus, alternative treatment options are direly needed to improve hematological manifestations of FA.

In this clinical trial, we propose the use of EPAG as a novel therapeutic modality for patients with FA, based on safety and efficacy evidence provided by pre-clinical and clinical investigations. As previously discussed in detail in section 2.4.1, our *in vitro* and *in vivo* pre-clinical studies indicate that EPAG evades blockade of signal transduction from cMPL induced by inflammatory cytokines that may contribute to bone marrow failure in patients with FA. Additionally, we found that EPAG, in analogy to its natural counterpart TPO, enhances DNA repair activity in human HSPC. Thus, EPAG may positively influence two of the main known mechanisms leading to bone

marrow failure in Fanconi anemia.

Our experience from clinical studies using EPAG for the treatment of various bone marrow failure states has demonstrated safety and efficacy in these patient populations. In our first clinical trial using EPAG, trilineage improvement in peripheral blood cell counts in heavily pretreated patients with refractory severe aplastic anemia provided the first evidence of a direct stimulus of the stem cell compartment by EPAG. Furthermore, significantly higher hematological response rates were observed when EPAG was added to standard immunosuppression for the treatment of newly diagnosed SAA (82). In responders, bone marrow cellularity (Figure 3A), percentages of CD34+ cells in the bone marrow (Figure 3B), and enumeration of phenotypically defined HSCs (CD34+CD38-CD45RA-CD90+CD49f+Rhodamine^{low}, Figure 3C) significantly improved after treatment with hATG/CSA and EPAG.

Patients with FA are at risk of progression to clonal marrow dysfunction, including MDS and leukemia. Although not proven, there is a theoretical concern that chronic hyperstimulation with hematopoietic cytokines could accelerate or promote clonal transformation. A retrospective analysis of 167 Japanese children with severe aplastic anemia showed that among the 50 children treated with G-CSF and immunosuppression 10 evolved to MDS/AML. There were no evolutions in children treated with immunosuppression alone (n=24) or only G-CSF (n=20)(83). Another retrospective study by the European Group for Blood and Marrow Transplantation in 2006 showed a significantly higher hazard ratio of developing MDS/AML with the use of G-CSF in patients treated with immunosuppression for severe aplastic anemia (84). However large prospective multi-institutional studies did not support this increased risk (85, 86). In patients

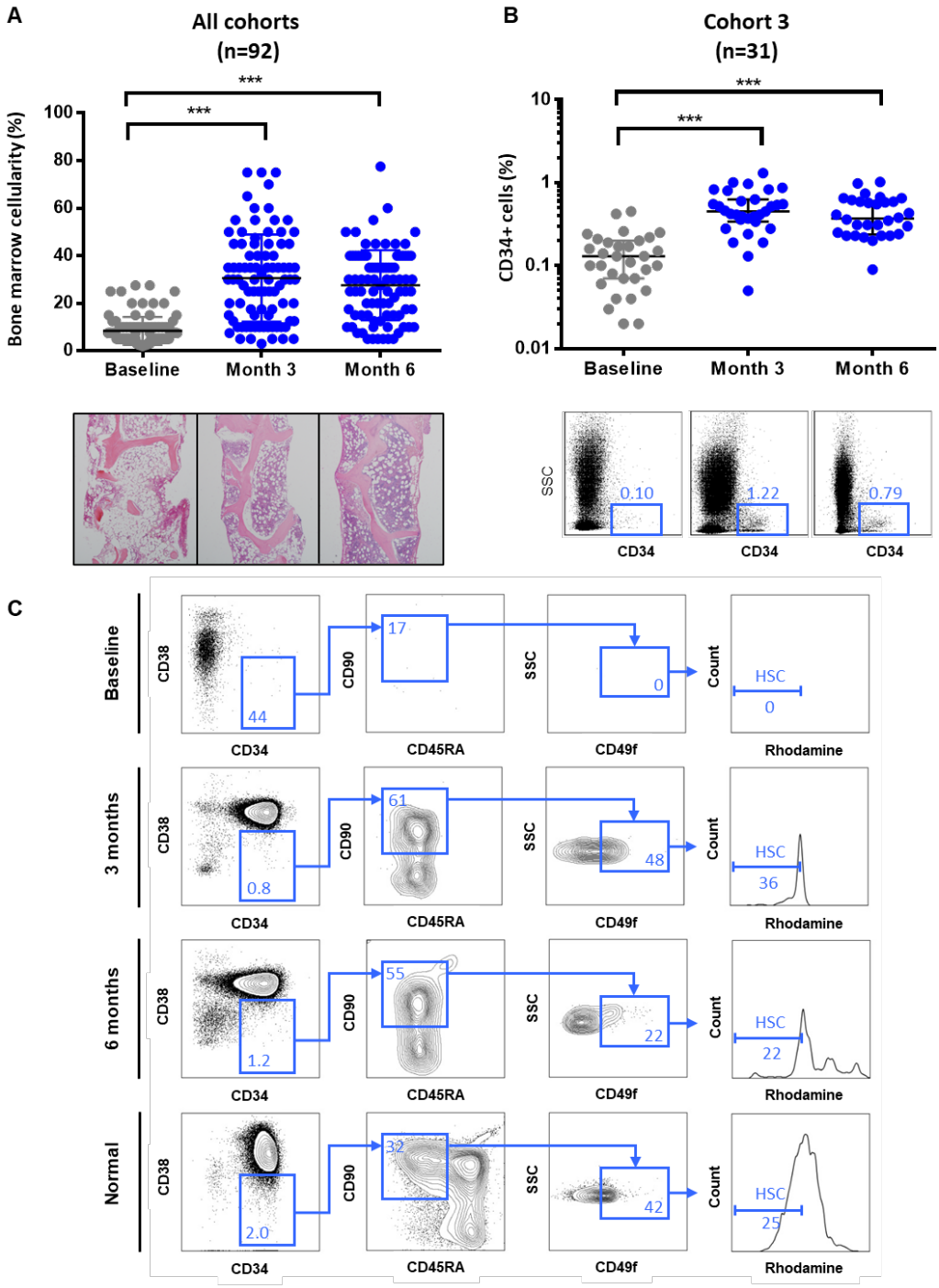


Figure 3. Eltrombopag's Influence on Primitive Hematopoietic Stem and Progenitor Cells in Severe Aplastic Anemia. (A) Compiles the bone marrow cellularity data across all participants (n=92), showcasing representative bone marrow core biopsy images of patient #7 at baseline, 3 months, and 6 months post-treatment initiation. **(B)** Depicts the quantified changes in the CD34+ cell population over time, with a baseline median (grey), and measurements at 3 and 6 months post-treatment (blue). There was a five-fold median increase in CD34+ cells at 3 months compared to baseline (from 0.13% to 0.45%), which slightly decreased to 0.37% at 6 months. **(C)** Displays serial flow cytometry analyses of phenotypically defined hematopoietic stem cells (HSCs) (CD34+CD38-CD45RA-CD90+CD49f+Rhodaminelow) in the bone marrow of patient #29 compared to a healthy control (normal). Post-enrichment of nucleated bone marrow cells for CD34+ cells via MACS separation, these cells were further analyzed by flow cytometry to identify HSC markers. Percentages in the flow cytometry plots indicate the proportion of cells within the specified fractions. Statistical significance was confirmed using one-way ANOVA, with $p < 0.001$ indicating a highly significant difference.

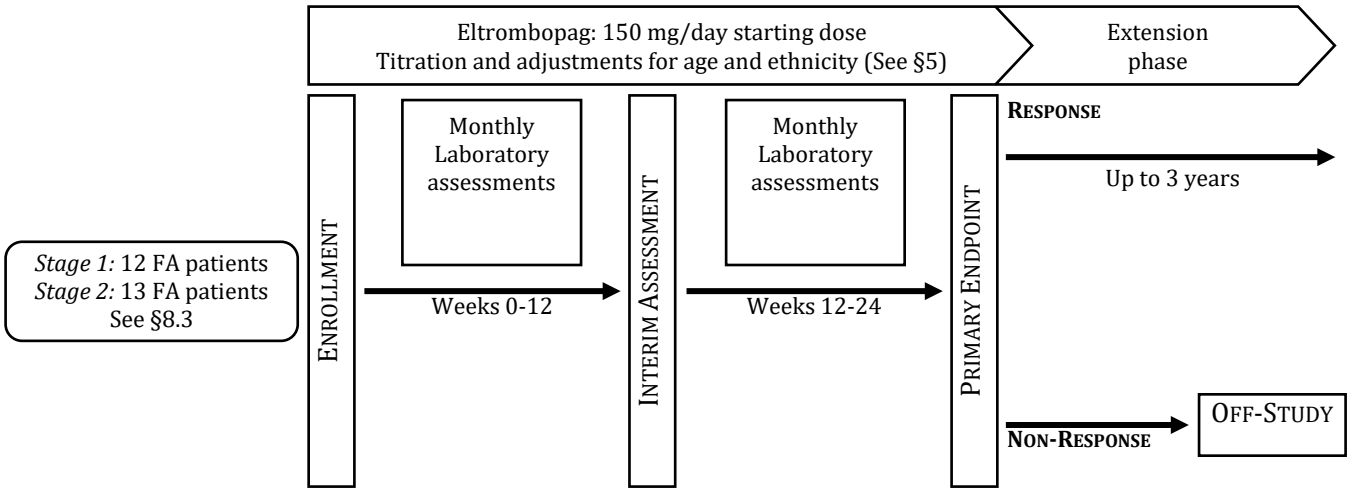
with FA, G-CSF usage has been reported safe but clonal evolution as evidenced by cytogenetic abnormalities and progression to leukemia has been reported (87). Eltrombopag is being investigated as therapy for SAA, MDS and for acute myeloid leukemia, based on experimental laboratory data suggesting that normal HSCs are stimulated relative to leukemic cells by this drug (72). Multiple ongoing trials have shown efficacy in improving platelet counts in some patients with MDS, and to date no evidence for an increased rate of progression to AML has been reported. In our initial trial for refractory SAA, 8/44 patients including 6 non-responders and 2 responders, developed new cytogenetic abnormalities. Cytogenetic abnormalities included partial or complete loss of chromosome 7 in 5 non-responding patients. Bone marrow cells of two responding patients were found with deletion of chromosome 13 and another non-responder was diagnosed with trisomy 8 (44). In our ongoing treatment protocol for refractory aplastic anemia, cytogenetic abnormalities were detected in 6/38 patients. Although the overall rate of cytogenetic evolution appears to be similar to historical data, these observations require longer follow-up and completion of a randomized controlled trial comparing initial SAA therapy with and without EPAG added to IST in order to establish whether there may be increased risk of progression to clonal hematopoiesis in SAA patients treated with EPAG. In the proposed study, in order to detect cytogenetic abnormalities, we will perform bone marrow biopsies after 3 months and during the response assessment after 6 months of treatment. Subsequent bone marrow biopsies will be performed every 6 months while on drug and yearly after discontinuation of EPAG until off study criteria are fulfilled.

The primary objective of this trial is therefore to assess safety and efficacy of EPAG for patients with Fanconi anemia. Secondary objectives include assessment of relapse, clonal evolution, toxicities with extended duration of therapy, the impact of treatment and treatment response on quality of life, evaluation of DNA repair activity in HSPCs, assessment of mutational landscape, the impact of EPAG on the inhibitory effects on proinflammatory cytokines in hematopoietic stem cells, the impact of EPAG on reconstitution of the stem cell compartment, and the impact of EPAG on other organ systems commonly involved in FA (e.g. skin lesions, endocrine dysfunction, and head/neck, oropharyngeal, gastrointestinal, anogenital or skin cancers).

3. STUDY DESIGN

The study is designed as a non-randomized, Phase II study of the oral TPO-R agonist eltrombopag in Fanconi anemia. The primary endpoint is measured at 6 months (± 14 days). Subjects who cannot tolerate the medication or fail to respond by 6 months (± 14 days) will be taken off study drug. Subjects who respond at 6 months will be able to continue EPAG on the extension part of this protocol for an additional 3 years. The dosing protocol for patients enrolled in the extension phase of the study will include an optional dose escalation strategy (section 5.1). Alternatively, subjects may remain on the same dose of EPAG until they reach robust count criteria (defined as platelets >50,000/μL, Hb>10 g/dL in the absence of transfusions, and

neutrophils > 1,000 for more than 8 weeks) or until they reach steady state response (defined as stable counts for 6 months). Drug dose may then be tapered slowly to the lowest dosage that maintains a stable platelet count and eventually discontinued as per table 6, until they meet off study criteria (section 8.6) or the study is closed.



4. ELIGIBILITY ASSESSMENT

4.1 Inclusion Criteria

4.1.1 Confirmed diagnosis of Fanconi anemia. Diagnosis is confirmed by a biallelic mutation in a known FANC gene and/or by positive chromosome breakage analysis in lymphocytes and/or skin fibroblasts (for mosaicism).

4.1.2 One or more of the following three clinically-significant cytopenias: One or more of the following three clinically-significant cytopenias: platelet count ≤ 50,000/μL or platelet-transfusion-dependence (requiring at least 4 platelet transfusions in the 8 weeks prior to study entry, see definition of platelet transfusion units at 8.2.1); neutrophil count less than 1000/μL; hemoglobin less than 10 g/dL or red cell transfusion- dependence (requiring at least 4 transfusions of PRBCs (adult patient 4 units PRBC, pediatric patients at least 10ml/kg/transfusion) in the eight weeks prior to study entry.

4.1.3 Failed or declined treatment with androgens (danazol or oxymetholone).

4.1.4 Age ≥ 6 years old.

4.1.5 Weight ≥10kg.

4.2 Exclusion Criteria

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

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

4.2.3 Any cytogenetic abnormality associated with poor prognosis in FA, including gains of chromosome 3q, gains of chromosome 1q, deletions of chromosome 7, and complex cytogenetics (88-90) identified from bone marrow aspirate. Patients with known biallelic mutations in BRCA2 (FANCD1).

4.2.4 Active malignancy or likelihood of recurrence of malignancies within 12 months

4.2.5 Moribund status such that death within 7 to 10 days is likely. Comorbidities of such severity that in the view of the investigator it would likely preclude the patient's ability to tolerate eltrombopag.

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

4.2.7 Creatinine > 2.5 times ULN

4.2.8 Direct Bilirubin > 3.0mg/dL, indicating congenital abnormalities in the bilirubin level

4.2.9 SGOT (AST) or SGPT (ALT) > 5 times the ULN normal

4.2.10 Known liver cirrhosis in severity that would preclude tolerability of eltrombopag as evidenced by albumin < 35g/L

4.2.11 Known immediate or delayed hypersensitivity to EPAG or its components

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

4.2.13 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 EPAG. 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 is she 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.

4.2.14 History of thromboembolic events.

4.2.15 Unable to take oral medication

4.2.16 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 diseases (e.g. uncontrolled hypertension, history of labile hypertension), history of known structural abnormalities (e.g. cardiomyopathy).

4.2.17 History of HIV positivity.

4.2.18 History of alcohol/drug abuse.

4.2.19 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)

Based on the empirical data and clinical observations detailed in Section 2.4.4, the eltrombopag dosing regimen for patients with Fanconi anemia has been determined as follows and summarized in Table 1. For patients participating in the extension phase of the study, laboratory testing will be conducted upon initiation of dose escalation (see Section 6.5.1 and Table 11). The principal investigator and/or study team will review these results and confirm their acceptability before proceeding with the dose escalation.

- **Non-Asian Populations:**
  - Children aged 6-11:
    - EPAG will be administered starting at 75 mg daily for the first 6 months.
    - Subject to tolerance (refer to Sections 5.2 and 5.3 for details), the dosing protocol for patients enrolled in the extension phase of the study will include an optional dose escalation strategy. Specifically, an increase of 75 mg will be implemented after the initial 6-month period, reaching a cap of 150 mg daily. This escalation applies to patients entering the extension protocol and those already participating in the extension protocol, after re-consent/consent by the participant, LAR, or parent/guardian, as applicable.
  - Subjects aged 12 and older:
    - EPAG will be administered starting at 150 mg daily for the first 6 months.
- Subject to tolerance (refer to Sections 5.2 and 5.3 for details), the dosing protocol for patients enrolled in the extension phase of the study will include an optional dose escalation strategy. Specifically, an increase of 75 mg will be implemented after the initial 6-month period, followed by an increase of 75 mg four weeks after the first dose increase, reaching a cap of 300 mg daily. This escalation applies to patients entering the extension protocol and those already participating in the extension protocol after re-consent/consent by the participant, LAR, or parent/guardian, as applicable.
- **East Asian Populations:**

Recognizing the pharmacogenomic factors where Asian individuals may metabolize drugs distinctively compared to Caucasian cohorts due to genetic variances impacting drug-metabolizing enzymes, the dosing strategy is carefully tailored. Although this is a significant consideration, it is not universally applicable to all drugs or individuals within these populations. For example, the study by Platzbecker et al. (71) demonstrated that the mean plasma trough levels of EPAG at a 50 mg dose were comparable between Asian and non-Asian patients, with only a marginal increase at doses exceeding 50 mg daily. Thus, the dosing guidelines below are formulated to maximize therapeutic efficacy while minimizing the risk of adverse effects, ensuring patient safety and drug efficacy.

- Children aged 6-11:
  - EPAG will be administered starting at 37.5 mg daily for the first 6 months.
  - Subject to tolerance (refer to Sections 5.2 and 5.3 for details), the dosing protocol for patients enrolled in the extension phase of the study will include an optional dose escalation strategy. Specifically, an increase of 37.5 mg will be implemented after the initial 6-month period, followed by two increases of 37.5 mg at 4 and 8 weeks after the first dose increase, reaching a cap of 150 mg daily. This escalation applies to patients entering the extension protocol and those already participating in the extension protocol after re-consent/consent by the participant, LAR, or parent/guardian, as applicable .
- Subjects aged 12 and above:
  - EPAG will be administered starting at 75 mg daily for the first 6 months.
  - Subject to tolerance (refer to Sections 5.2 and 5.3 for details), the dosing protocol for patients enrolled in the extension phase of the study will include an optional dose escalation strategy. Specifically, an increase of 75 mg will be implemented after the initial 6-month period, followed by two increases of 75 mg at 4 and 8 weeks after the first dose increase, reaching a cap of 300 mg daily. This escalation applies to patients entering the extension protocol and those already participating in the extension protocol after re-consent/consent by the participant, LAR, or parent/guardian, as applicable.

Table 1. Daily Eltrombopag Dose

Age groups	Initial daily dose	Daily dose escalation (optional)	Maximum daily dose
Non-Asian Populations			
6-11	75 mg	Increase of 75 mg after 6 months	150 mg
≥12	150 mg	Increase of 75 mg after 6 months, followed by an increase of 75 mg at 4 weeks after the first dose increase	300 mg
East Asian Populations			
6-11	37.5 mg	Increase of 37.5 mg after 6 months, followed by two increases of 37.5 mg at 4 and 8 weeks after the first dose increase*	150 mg

≥12	75 mg	Increase of 75 mg after 6 months, followed by two increases of 75 mg at 4 and 8 weeks after the first dose increase*	300 mg

* Dose escalation is subject to tolerance (refer to Sections 5.2 and 5.3 for details)

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 6 months as originally planned. Interruptions will be recorded in the medical record. Whether interruptions are reported (e.g., as deviations or adverse events) will 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 reported as deviations, but will be recorded in the medical record.

5.2 Dose Delays, Adjustments or Discontinuation of eltrombopag for Hematologic Side Effects

5.2.1 Platelet counts

Table 2. Criteria for the adjustment of the daily dose of EPAG based on platelet counts

Platelet Count	Dose Adjustment or Response
>400,000/μL (untransfused)	• Stop the dose escalation if the maximum dose has not been reached. • Decrease dosage by 25mg (or 12.5mg in children under 12) every 2 weeks (± 3 days) to lowest dosage that maintains platelet count ≤ 400,000/μL.
>600,000/μL (untransfused)	• Stop the dose escalation if the maximum dose has not been reached. • Discontinue eltrombopag for one week (± 3 days); if platelets fall to ≤400,000/μL, restart at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

5.2.2 Hemoglobin levels

Table 3. Criteria for the adjustment of the daily dose of EPAG based on hemoglobin levels

Hemoglobin	Dose Adjustment or Response
>15 g/dL (untransfused)	• Stop the dose escalation if the maximum dose has not been reached. • Decrease dosage by 25mg (or 12.5mg in children under 12) every 2 weeks (± 3 days) to lowest dosage that maintains hemoglobin ≤ 15 g/dL.
>16 g/dL (untransfused)	• Stop the dose escalation if the maximum dose has not been reached. • Discontinue eltrombopag for one week (± 3 days); if hemoglobin falls to ≤15 g/dL, restart at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

5.2.3 Thrombosis and 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. Patients with platelet counts of >50,000/μL at the time of thrombosis will be treated with enoxaparin or another appropriate anticoagulant as clinically indicated until the platelet count drops below 20,000/μL with discontinuation of EPAG. They will be treated for the thrombotic event as otherwise clinically-indicated.

5.2.4 New morphological abnormalities on peripheral blood smear

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

5.3 Dose Delays, Adjustments or Discontinuation for Non-Hematologic Side Effects

5.3.1 Infection

Subjects who experience an infection requiring intravenous antibiotics will not have EPAG discontinued. They will continue the dose escalation if the maximum dose has not been reached, or maintain the dose level if the maximum dose has been reached. If the subject experiences infection severe enough to require vasopressors or intubation, the drug will be withheld until the patient is stable.

5.3.2 Liver function abnormalities

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

Elevation	Recommended dose modification
≤ 6 x ULN	• Continue the dose escalation if the maximum dose has not been reached. • Maintain the dose level if the maximum dose has been reached. • Optional Temporary Hold: Eltrombopag dosing may be temporarily held if clinically indicated, even if the criteria for mandatory dose modification have not been met.

>6 – 12 x ULN	• Treatment:
	  ○ Stop the dose escalation if the maximum dose has not been reached.
	  ○ Maintain the dose level if the maximum dose has been reached.
	  ○ Optional Temporary Hold: Eltrombopag dosing may be temporarily held if clinically indicated, even if the criteria for mandatory dose modification have not been met.
	• Monitoring and reinstitution of treatment:
	  ○ Repeat liver function tests as soon as possible, preferably within 48-72 hours, from awareness of the abnormal results.
	  ○ If abnormal laboratory values are confirmed upon the repeat test, monitor liver function tests weekly or more frequently if clinically indicated until

	resolved to ≤5.0 x ULN.
>12 – 20 x ULN	• Treatment: o Stop the dose escalation if the maximum dose has not been reached. o Interrupt the treatment. • Monitoring and reinstitution of treatment: o Repeat liver function tests as soon as possible; preferably within 48-72 hours from awareness of the abnormal results. o Monitor liver function tests weekly, or more frequently if clinically indicated until resolved to ≤5.0 x ULN then: ▪ If resolved in ≤14 days, maintain the dose level. ▪ If resolved in >14 days: □ Maintain the dose level if attribution to EPAG is unrelated or unlikely (i.e., similar elevation in liver function tests encountered before treatment with eltrombopag). □ Restart eltrombopag at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose if attribution to EPAG is possible, probable or definite (i.e., no similar elevation in liver function tests encountered before treatment with eltrombopag).
>20.0 x ULN	• Treatment: o Discontinue the subject from the study drug treatment. • Monitoring: o Repeat liver function tests as soon as possible, preferably within 48-72 hours from awareness of the abnormal results. o Monitor liver function tests weekly or more frequently if clinically indicated until they resolved to baseline or stabilized over 4 weeks.

5.3.3 Gastrointestinal symptoms

Table 5. Eltrombopag dose modifications for gastrointestinal symptoms

Symptoms	Recommended dose modification
Nausea	**Grade 1-2** • Continue the dose escalation if the maximum dose has not been reached. • Maintain EPAG dose level if the maximum dose has been reached. **Grade 3** • Treatment: o Stop the dose escalation if the maximum dose has not been reached. o Interrupt the treatment. • Monitoring and reinstitution of treatment: o Reassess symptoms daily, or more frequently if clinically indicated until resolution, then: ▪ If resolved in ≤14 days, maintain EPAG dose level. ▪ If resolved in >14 days, restart EPAG at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose.
Vomiting	**Grade 1-2** • Continue the dose escalation if the maximum dose has not been reached. • Maintain EPAG dose level if the maximum dose has been reached. **Grade 3** • Treatment: o Stop the dose escalation if the maximum dose has not been reached.

	○ Interrupt the treatment. • Monitoring and reinstitution of treatment: ○ Reassess symptoms daily, or more frequently if clinically indicated until resolution, then: ▪ If resolved in ≤14 days, maintain EPAG dose level. ▪ If resolved in >14 days, restart EPAG at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose. **Grade 4** • Discontinue the subject from the study drug treatment. • Reassess symptoms daily or more frequently if clinically indicated until they resolved to baseline.
Diarrhea	**Grade 1-2** • Continue the dose escalation if the maximum dose has not been reached. • Maintain EPAG dose level if the maximum dose has been reached. **Grade 3** • Treatment: ○ Stop the dose escalation if the maximum dose has not been reached. ○ Interrupt the treatment. • Monitoring and reinstitution of treatment: ○ Reassess symptoms daily, or more frequently if clinically indicated until resolution, then: ▪ If resolved in ≤14 days, maintain EPAG dose level. ▪ If resolved in >14 days, restart EPAG at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose. **Grade 4** • Discontinue the subject from the study drug treatment. • Reassess symptoms daily or more frequently if clinically indicated until they resolved to baseline.
Constipation	**Grade 1-2** • Continue the dose escalation if the maximum dose has not been reached. • Maintain EPAG dose level if the maximum dose has been reached. **Grade 3** • Treatment: ○ Stop the dose escalation if the maximum dose has not been reached. ○ Interrupt the treatment. • Monitoring and reinstitution of treatment: ○ Reassess symptoms daily, or more frequently if clinically indicated until resolution, then: ▪ If resolved in ≤14 days, maintain EPAG dose level. ▪ If resolved in >14 days, restart EPAG at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose. **Grade 4** • Discontinue the subject from the study drug treatment. • Reassess symptoms daily or more frequently if clinically indicated until they resolved to baseline.
Abdominal pain	**Grade 1-2** • Continue the dose escalation if the maximum dose has not been reached. • Maintain EPAG dose level if the maximum dose has been reached. **Grade 3**

	• Treatment:
	  ○ Stop the dose escalation if the maximum dose has not been reached.
	  ○ Interrupt the treatment.
	• Monitoring and reinstitution of treatment:
	  ○ Reassess symptoms daily, or more frequently if clinically indicated until resolution, then:
	  ▪ If resolved in ≤14 days, maintain EPAG dose level.
	  ▪ If resolved in >14 days, restart EPAG at a dose level 25mg/day (or 12.5mg/day for children under 12) lower than the prior dose.

5.4 Taper of eltrombopag in the Extension Protocol

Once a robust blood count recovery occurs in the extension part (i.e. platelets >50,000/μL, Hb>10 g/dL in the absence of transfusions, and neutrophils > 1,000 for more than 8 weeks), or once subjects have reached steady state response defined as stable counts for 6 months, EPAG dose may be tapered according to table 6, per PI's discretion. If platelets drop to <30,000/μL, Hb to <9g/dL, or ANC to <500/μL then EPAG will be either re-initiated or the dose will be increased to the last effective strength. Additionally, EPAG may be tapered if patients continue to meet criteria for response (as defined in section 8.2), but fail to show further clinically-significant improvement in their counts over a period of 6 months (stable disease). If during taper or after discontinuation of EPAG, any lineage falls back below original study inclusion criteria values or if the patient again requires transfusions, EPAG will be reinstituted at the previously-effective dose.

Table 6. Eltrombopag taper in the extension protocol

Age group	Maximum initial dose	Step 1	Step 2	Step 3	Step 4
Non-Asian Populations					
6-11	150 mg	75	37.5	25	stop
≥12	300 mg	75	75	75	stop
East Asian Populations					
6-11	150 mg	75	37.5	25	stop
≥12	300 mg	75	75	75	stop

Step = 8 weeks

5.5 Extended Access to Study Drug

Subjects who demonstrate response by protocol criteria at 6 months (± 14 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 section 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 increased, tapered, discontinued, and/or re-started as per sections 5.1, 5.2, 5.3, and 5.4.

5.6 Permitted Supportive Care

- Transfusion supportive care (e.g., blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) as clinically indicated.
- 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.

5.7 Non-Permitted Medications

- Romiplostim (N-Plate)
- IL-11 (Neumega)
- Androgens (e.g., danazol and oxymetholone) within 4 weeks prior to starting EPAG.
- 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.
- Growth stimulating agents (e.g., somatropin).

5.8 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 Cmax by 2-fold and AUCinf 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,

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 (irinotecan), 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.

5.9 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 meal containing no drugs or products with polyvalent cations such as antacids, dairy products, or 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 (i.e. aluminum, calcium, magnesium, iron, selenium and zinc) such as mineral supplements and dairy products.

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

5.10 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 MONITORING

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

6.1 Screening and Baseline Evaluation within 16 weeks prior to Starting EPAG (Table 10):

Screening and baseline evaluation will be done as part of this protocol. Minimal risk activities may be performed before the subject has signed a consent, including e-mail, written, in person or telephone communications with prospective subjects, and review of existing medical records, such as H&P, laboratory studies, etc. (Please see request for waiver of consent for pre-screening activities in Section 10.5).

Patients will sign the consent prior to any other screening and baseline tests/procedures done as part of this protocol. Should a test/procedure result be available in CRIS from another NHLBI protocol, or be part of the subject's medical records, results from this test/procedure may be used if within the appropriate timelines (i.e., 16 weeks or otherwise stated below) for eligibility and baseline evaluation.
The following tests/procedures will be done as part of the screening and baseline evaluation process within 16 weeks of initiating treatment with EPAG:

- Medical history and physical examination
- Ophthalmology consult for evaluation of cataract
- Pediatric consult
- Clinical photography for physical characteristics associated with Fanconi anemia
- Concurrent medication review
- Complete blood count with differential
- Peripheral blood smear
- Reticulocyte count
- DAT (direct antiglobulin test)
- Chromosome breakage analysis in lymphocytes and/or fibroblasts (e.g., DEB testing) to confirm diagnosis of Fanconi anemia (not required if already performed elsewhere at any time, and records available).
- Genetic testing for mutations associated with Fanconi anemia (not required if already performed elsewhere at any time, and records available).
- 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)
- Total Protein

- CK
- Uric Acid
- LDH
- Coagulation and thrombosis screens (PT, PTT)
- Type and screen
- Viral serologies for hepatitis A, B (including HBsAg, HBsAb and HB DNA PCR), C, HIV, HSV, EBV and CMV (positive serologies will have viral nucleic acid testing performed)
- Folate level
- B12 level
- Vitamin D, 25 Hydroxy, Total
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- 24-hour urine collection to determine total iron (at PI's discretion if indicated for iron metabolism and excretion studies)
- HLA typing (not required if already performed elsewhere at any time, and records available
- Pregnancy test (blood or urine HCG in women of childbearing potential)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Flow cytometry of peripheral blood lymphocytes for phenotyping (analysis of T, B and NK subsets)
- Immunoglobulins
- Thyroid function panel (T3, T4, and TSH)
- Bone marrow aspiration and core biopsy will be processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins; d) Aspirate will be used for chromosomal analysis via standard cytogenetic techniques, as required to evaluate for MDS/AML and cytogenetic abnormalities; e) Aspirate may be used for MDS FISH analysis (optional). In addition, bone marrow research sample (10 cc of bone marrow aspirate can be collected at the time of screening; sample will be destroyed if subject not eligible for treatment under this protocol)
- Skin biopsy
- Electrocardiogram (EKG)
- Hard copy or electronic HRQL survey administration

6.2 Baseline Evaluation within 7 days prior to Starting EPAG (Table 10):

Once a subject is considered eligible for this study based on evaluation above, evaluation listed below will be performed to determine the subject's baseline and confirm continued eligibility within 7 days prior to starting treatment. This is required given the high variability in hematopoietic and hepatic parameters in patients with Fanconi Anemia, and the unclear safety profile of eltrombopag in pregnancy. Research blood will also be obtained at this visit as outlined in section 7. If continued eligibility is confirmed, the patient will be provided with a supply (up to 6 months) of EPAG and the first dose will be administered.

If the patient is unable to return to the NIH within 16 weeks of the initial visit, evaluation listed below, except for research samples which will be omitted in this case, will be performed by their home physician's outside lab. If continued eligibility is confirmed, a supply (up to 6 months) of EPAG will be shipped to the patient to initiate treatment. If shipping is prohibited in the specified country, the subject will be taken off study and may be re-evaluated at a later time.

- Concurrent medication review
- Complete blood count with differential
- Type and screen (optional if baseline evaluation is not performed at the NIH)
- Peripheral blood smear (optional if baseline evaluation is not performed at the NIH)
- Reticulocyte count (optional if baseline evaluation is not performed at the NIH)
- Acute care panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen). Glucose is optional if baseline evaluation is not performed at the NIH
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin and Direct Bilirubin). Alkaline phosphatase is optional, and either total bilirubin or direct bilirubin may be obtained if baseline evaluation is not performed at the NIH.
- Pregnancy test (blood or urine HCG in women of childbearing potential)
- Nutrition consult (optional if baseline evaluation is not performed at the NIH)
- Research samples as detailed in section 7

6.3 On Study Monitoring (through 6 months)

On study monitoring intervals are counted from the day subjects start EPAG. Subjects may be followed by their home physician or at the Clinical Center. Clinical 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 filed in the research charts, and all lab data will be recorded. Medical history and physical assessments by a physician at the NIH Clinical Center or the referring local health care provider are required every month (± 14 days) for both USA and international subjects.

- Complete blood counts with differential (every month ± 14 days)
- ALT, AST, Total Bilirubin or Direct Bilirubin (every month ± 14 days)

- Iron panel, including transferrin, % saturation, total serum iron and ferritin (every month ± 14 days), optional

6.4 Landmark 3-Month and 6-Month Monitoring

Subjects must be evaluated at the NIH Clinical Center at the 3- and 6-month (± 14 days) time points (Table 10)

- History and physical examination
- Concurrent medication review
- Complete blood counts with differential
- Peripheral blood smear
- Reticulocyte count
- Type and screen
- Coagulation and thrombosis screens (PT, PTT).
- 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)
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- Vitamin D, 25 Hydroxy, Total
- Chromosome breakage analysis in lymphocytes (e.g., DEB testing) (at 6 months only)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies
- Blood or urine pregnancy test (woman of childbearing potential only)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Flow cytometry of peripheral blood lymphocytes for phenotyping (analysis of T, B and NK subsets)
- Bone marrow aspiration and core biopsy will be done for safety evaluation and processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins; d) Aspirate will be used for bone marrow chromosomal analysis via standard cytogenetic techniques; e) Aspirate may be used for MDS FISH analysis (optional)
- Hard copy or electronic HRQL survey administration
- Blood for pharmacokinetic evaluation of EPAG levels at PI's discretion. Samples drawn

pre-dose, and at 2, 4, 6, and 8 hours after the dose, with an optional final sample at 24 hours after dose (3-month time point, unless do not meet requirements per Appendix B then at 6 month visit)

- Document drug accountability
- Research samples as detailed in section 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. All subjects, regardless of their country of residence, will be evaluated at the Clinical Center every 6 months (± 30 days) while they remain on extended access and continue periodic clinical and laboratory monitoring in their home physician's office or the NIH (see Section 6.5.1). At each landmark NIH visit, subjects living in the USA will be given a 3-month supply of EPAG, the maximum quantity that can be ordered each time, and a second supply will be shipped at 3 months to ensure sufficient provisions until their 6-month evaluation at the NIH. Subjects living outside the USA will be provided with a 6-month supply of EPAG. This provision aligns with the current policies permitting the distribution of a six-month medication supply for international patients, thereby accommodating the restrictions on international shipping of EPAG. Progress notes and 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 filed in the research charts, and all lab data will be recorded.

6.5.1 Testing required for patients on EPAG while on extended access phase (up to 3 years after the 6-month visit) (Table 11):

- Complete blood counts with differential (monthly ± 14 days)
- ALT, AST, total, direct and indirect bilirubin (every 2 weeks ± 7 days during the dose escalation period)
- ALT, AST, total bilirubin or direct bilirubin (monthly ± 14 days after the dose escalation period)
- Ferritin (full iron panel, including ferritin, transferrin, % saturation and total serum iron, is preferable but not required) (monthly ± 14 days), optional
- History and physical examination (every 6 months ± 30 days)
- Ophthalmology consult (every 12 months ± 60 days) at PI's discretion
- Pediatric consult (every 6 months ± 30 days) at PI's discretion
- Concurrent medication review (every 6 months ± 30 days)
- Peripheral blood smear (every 6 months ± 30 days)
- Reticulocyte count (every 6 months ± 30 days)

- Type and screen (every 6 months ± 30 days)
- Coagulation and thrombosis screens (PT, PTT) (every 6 months ± 30 days)
- Vitamin D, 25 Hydroxy, Total" (every 6 months ± 30 days)
- "Chromosome breakage analysis in lymphocytes (e.g., DEB testing)" (every 6 months ± 30 days)

- Hepatic panel (Alkaline phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin) (every 6 months ± 30 days)
- Acute care panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen) (every 6 months ± 30 days)
- Iron panel (ferritin, transferrin, % saturation, total serum iron) (every 6 months ± 30 days)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 6 months ± 30 days)
- Pregnancy test (blood or urine HCG in women of childbearing potential) (every 6 months ± 30 days)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells (every 6 months ± 30 days)
- Flow cytometry of peripheral blood lymphocytes for phenotyping (analysis of T, B and NK subsets) (every 6 months ± 30 days)
- Bone marrow aspiration and core biopsy will be done for safety evaluation and processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins; d) Aspirate will be used for bone marrow chromosomal analysis via standard cytogenetic techniques (every 6 months ± 30 days); e) Aspirate may be used for MDS FISH analysis (optional)
- Hard copy or electronic HRQL survey administration (every 6 months ± 30 days)
- Document Drug Accountability (every 6 months ± 60 days)
- Research samples as detailed in section 7 (every 6 months ± 30 days)

6.5.2 Testing required for patients who have EPAG discontinued during the extended access phase (see Table 6) (up to 3 years after the 6-month visit) (Table 12):

- History and physical examination (every 12 months ± 60 days)
- Complete blood counts with differential (monthly ± 14 days for 6 months off eltrombopag, then every 3 months ± 14 days until they reach off study criteria as per section 8.6 or the study is closed)

- Type and screen (every 12 months ± 60 days)
- Coagulation and thrombosis screens (PT, PTT) (every 12 months ± 60 days)
- Vitamin D, 25 Hydroxy, Total" (every 12 months ± 60 days).
- "Chromosome breakage analysis in lymphocytes (e.g., DEB testing)" (every 12 months ± 60 days).
- Hepatic panel (Alkaline phosphatase, ALT, AST, total bilirubin, and direct bilirubin) (every 6 months ± 30 days)
- Iron panel (ferritin, transferrin, % saturation, total serum iron) (every 12 months ± 60 days)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies (every 12 months ± 60 days)
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells (every 12 months ± 60 days)
- Flow cytometry of peripheral blood lymphocytes for phenotyping (analysis of T, B, and NK subsets) (every 12 months ± 60 days)
- Bone marrow aspiration and core biopsy will be done for safety evaluation and processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins; d) Aspirate will be used for bone marrow chromosomal analysis via standard cytogenetic techniques (every 12 months ± 60 days); e) Aspirate may be used for MDS FISH analysis (optional)
- Hard copy or electronic HRQL survey administration (every 12 months ± 60 days)
- Research samples as detailed in section 7 (every 12 months ± 60 days)

Patients who discontinue EPAG according to the response criteria per section 5.3 who need to have EPAG reinitiated due to counts falling will go back onto the monitoring 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 section 8.6, but this visit will not be required. If they do not return to the NIH, we will contact their primary hematologist for information on their current hematologic status 6 months following their final dose of 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 (Table 12):

- History and physical examination
- Complete blood count with differential
- Peripheral blood smear
- Reticulocyte count
- Type and screen
- Coagulation and thrombosis screens (PT, PTT)
- 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)
- Iron panel (ferritin, transferrin, % saturation, total serum iron)
- 24-hour urine collection to determine the total iron content at PI's discretion if indicated for iron metabolism and excretion studies
- Flow cytometry of the peripheral blood to quantitate GPI-negative cells
- Bone marrow aspiration and core biopsy will be done for safety evaluation and processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins; d) Aspirate will be used for bone marrow chromosomal analysis via standard cytogenetic techniques (every 12 months ± 60 days); e) Aspirate may be used for MDS FISH analysis (optional)
- Lymphocyte peripheral blood phenotyping (analysis of T, B, and NK subsets via flow cytometry)
- Research samples as detailed in section 7

6.7 Genetic Analysis

Approach: 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). Germline somatic mutation analysis will be performed using a targeted CLIA certified "Inherited bone marrow failure panel". 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. Additionally, pharmacogenomics studies may be performed in selected patients. These analyses will be done using a CLIA certified 'Pharmacogenomics' panel.

Intended use: The purpose of the genetic analysis will be to identify, if possible, a disease-defining mutation and to rule-out alternative diagnoses; however, it is understood that some patients will not have an identifiable mutation upon screening. The presence of a disease-defining mutation is not required for study inclusion, nor is its absence considered exclusionary (see §4.1.1). The purpose of the pharmacogenomics studies will be to deepen our understanding of patients' responses to eltrombopag by investigating how genes implicated in drug metabolic pathways influence varied responses to eltrombopag among Fanconi anemia patients (see §7.2).

Privacy and Confidentiality: The results of all genetic testing will be kept confidential. Fanconi anemia-specific results (i.e. identification of a disease-causing mutation and pharmacogenomics data) will be linked to the anonymized, study-specific identifier assigned to patient at the time of enrollment to allow subgrouping of patient data during the data analysis phase and for subgrouping of any banked biospecimens. These data will be stored on secure, NIH-maintained servers, accessible only to Key Study Personnel, and only for purposes of research. After review, the results of genetic testing will be scanned into the medical record (CRIS).

7. EXPLORATORY RESEARCH STUDIES

7.1 Clinical Research Studies

Intended use: During the extended phase of this study, we may increase dosages of eltrombopag as outlined in Table 1. These studies are not used in assessing the primary endpoint but are undertaken as exploratory investigations.

Exploratory clinical endpoints

- Assess and compare the changes in hematological response (e.g., hemoglobin, platelet counts and absolute neutrophil counts), marrow cellularity and marrow CD34+ cell percentages pre- and post-dosage escalation of eltrombopag during the extended phase of the study.
- Assess and compare the changes in incidence and severity of hematologic (see section 5.2) and non-hematologic side effects (see section 5.3) pre- and post-dosage escalation of eltrombopag during the extended phase of the study.

7.2 Laboratory Research Studies

Intended use: During the initial phase of this study (baseline, 3 and 6 months), 60ml of blood (3 mL/kg not to exceed 60 mL of blood for pediatric subjects), and 10 mL of bone marrow aspirate will be obtained for the correlative laboratory research studies listed below. During the extended

phase of this study, 60mL of blood (3 mL/kg not to exceed 60 mL of blood for pediatric subjects), and 10 mL of bone marrow aspirate will be obtained every 6 months (for patients on eltrombopag) or 12 months (for patients who have their eltrombopag discontinued during the extended phase) for the correlative laboratory research studies listed below. 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 investigations. Except for the urine studies all tests will be performed on each subject's material.

Exploratory laboratory endpoints

- 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)
- Hematopoietic progenitor colony, long term-culture-initiating cell, and immunodeficient mouse engraftment assays for primitive cell content and function
- Evaluation of clonal dynamics (single/bulk cell whole exome/genome sequencing)
- mRNA expression studies (RNA seq) on blood or marrow cells
- 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)
- Pharmacogenomics studies (selected patients)
- Whole genome/exome/epigenome sequencing
- Evaluation of DNA repair activity in HSPCs (γ-H2AX Assay and comet assay at baseline, 3 and 6 months).

  Intended use of genome/exome/epigenome sequencing: The purpose of this analysis is for safety evaluation, such as the presence of MDS defining mutations at baseline or arising from prolonged use of EPAG, and for ancillary research, such as evaluation of DNA repair activity and epigenetic changes associated with the use of EPAG.

  Although whole exome/genomic/epigenomic sequencing may be performed in this study, we are primarily interested in mutations that may cause or contribute to the subject's rare disease. 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."

  Privacy and Confidentiality: The results of all genetic testing will be kept confidential and linked to the anonymized, study-specific identifier assigned to patient at the time of enrollment to allow subgrouping of patient data during the data analysis phase and for subgrouping of any banked biospecimens. These data will be stored on secure, NIH-maintained servers, accessible only to Key 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).

Storage: Research samples will be stored coded in the secure laboratory of Andre Larochelle, M.D., Ph.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***s are to assess the safety and efficacy of 6 months of EPAG administration in improving bone marrow function in patients with FA with persistent severe cytopenias.

Secondary objectives include assessment of relapse, clonal evolution, toxicities with extended duration of therapy, the impact of treatment and treatment response on quality of life, and the impact of EPAG on organ systems commonly involved in FA (e.g. skin lesions, endocrine dysfunction, and incidence of new head/neck, oropharyngeal, gastrointestinal, anogenital or skin cancers).

Exploratory laboratory objectives include HSPC immunophenotyping, progenitor and reconstitution assays, mutational/clonal dynamics, transcriptomic studies, cytokine/chemokine/soluble receptor profiling, pharmacokinetics and pharmacogenomics on selected patients, and evaluation of DNA repair activity in HSPCs.

8.2 Endpoints

8.2.1 Primary endpoints

Primary efficacy endpoint: will be the proportion of drug responders at 6 months.

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

- Peripheral blood platelet treatment response will be defined as platelet count increases to 20,000/μL above baseline at six months, or stable platelet counts with transfusion independence for those participants that were transfusion dependent prior to treatment for a minimum of 8 consecutive weeks prior to response assessment.
- Peripheral blood erythroid response will be defined as an increase in hemoglobin by > 1.5 g/dL or a reduction in the units of PRBC transfusions by at least 50% during the eight consecutive weeks prior to response assessment, compared with the pretreatment transfusion number in the previous 8 weeks.
- Peripheral blood neutrophil response will be defined in those with a pretreatment absolute neutrophil count (ANC) of $<0.5 \times 10^9$/L as at least a 100% increase in ANC, or an ANC increase $>0.5 \times 10^9$/L.
- Bone marrow hematopoietic stem and progenitor cell treatment response will be defined as ≥2-fold increase in normal marrow CD34+ cells by CD34 immunohistochemistry or flow cytometry, and/or ≥ 2-fold increase in normal marrow cellularity as measured by standard stains (H&E) of bone marrow biopsy/aspirate sections

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

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

8.2.2 Secondary endpoints

Secondary endpoints will include:

- Hematological responses at 3 months
- Relapse during the extension phase
- Clonal evolution.
- Toxicities with extended duration of therapy.
- The impact of treatment and treatment response on quality of life.

- The impact of EPAG on other organ systems commonly involved in FA (e.g. skin lesions, endocrine dysfunction, and incidence of new head/neck, oropharyngeal, gastrointestinal, anogenital or skin cancers by clinical assessment at baseline, 3 and 6 months).

8.3 Sample Size

Because the efficacy of EPAG 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 Simon's Two-Stage Minimax Design (91) with a response probability of 20% or less to terminate the treatment. The sample size is determined by testing the null hypothesis H0: $p \leq 20\%$ versus the alternative H1: $p > 20\%$ with type I error of 0.05. If the true response rate is 45%, the study using the following two-stage design has 85% power. In the first stage, 12 subjects will be accrued and we will stop the study if no more than 2 subject responds to the treatment within 6 months. If 3 or more subjects respond to the treatment within 6 months at the first stage, then an additional 13 subjects will be accrued, bringing the total number of subjects to n=25. Up to 10 additional subjects may be enrolled to account for early discontinuation prior to the 6-month endpoint due to non-treatment related reasons and to account for screen failures. Subjects with non-treatment related early discontinuation and those who fail initial screening will not be evaluable for the primary efficacy and safety endpoints. We will not reject the null hypothesis of $p \leq 20\%$ if the total number of responders within 6 months is 8 or less. We will reject the null hypothesis if there are 9 or more responders within 6 months.

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 complete risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their corresponding 95% confidence intervals. Exploratory subgroup analysis will be used to compare the possible different response rates among male, female and different age categories.

Longitudinal measurements of continuous hematologic response variables and related biomarker variables will be plotted over the duration of the study. We will estimate the mean trajectories over time for a given continuous variable and assess the effect of increased dosage of eltrombopag on the response variable using flexible regression methods and mixed models for repeated measures.

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. (92), 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 7 summarizes the threshold numbers for stopping the study.

Table 7. Threshold Numbers for Stopping the Study

Number of subjects	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
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. The following table summarizes the proportion of stopped studies under a number of scenarios for p:

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 6-month is below 20%, and the probability of stopping a study is high when the true proportion of TRSAE within the 6-month 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 section 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
- 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 Fanconi anemia
- Any new cytogenetic abnormality associated with poor prognosis in FA, including gains of chromosome 3q, translocation of chromosome 1 and deletions of chromosome 7(88, 89) identified from bone marrow aspirate
- Subject non-compliance
- Lost to follow-up
- 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. Once off study, subjects will be referred back to the referring physician or consented to the NHLBI 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, Andre Larochelle, M.D., Ph.D.

NIH Intramural IRB: Accrual and safety data will be monitored and reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient consent form 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, follow up of subjects, or data analysis.

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

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

1) To verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject;

2) To verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;

3) To compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and

4) To help ensure investigators are in compliance with the protocol.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

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

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

Food & Drug Administration
Document Control Center
Center for Drug Evaluation and Research
Division of Hematology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266
(301) 796-0683 (phone)

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

Jay Chonkar, Manager, Clinical Sciences
Novartis Pharmaceuticals Corporation
One Health Plaza
East Hanover, NJ 07936-1080, USA
Mobile: +19739013128
jay.chonkar@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 4.0 (Table 8) and attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease (Table 9). A copy of the criteria can be downloaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Table 8. 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
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
4	Life threatening	Life- threatening consequences; urgent intervention indicated
5	Death	Death related to AE

Table 9. 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).

Grade (1 to 4) AEs will be recorded and resolved when the corresponding laboratory value normalizes (grade 0), with the following two exceptions:

- Hypertension: Hypertension CTCAE v4 Grade 1 is not an applicable category for this study and therefore only hypertension grade 2 or greater, that require medical intervention, will be recorded. These AEs will be resolved when the corresponding blood pressure value normalizes (grade 0).
- Hematologic laboratory values: In view of the underlying disease (bone marrow

failure in Fanconi anemia), all patients will enter the study with abnormally low blood counts that would meet criteria for grade 2 or 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.

The criteria to discontinue eltrombopag are listed in Sections 5.2 and 5.3.
Unscheduled laboratory results or patient reports that have been sent in addition to the protocol required ones will be reviewed for AE's and will be recorded on secure network drives or in approved alternative sites that comply with NIH information security standards, and not in the study 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 collected as an SAE.

9.2.4 Reporting Events

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

Andre Larochelle, M.D., Ph.D.,
CMTB, NHLBI, NIH, Clinical Center
10 Center Dr. Building 10, Room CRC 5-5132
Bethesda, MD 20892-1452
Tel: 301-814-8289 Fax: 301-496-8396
E-mail: larochea@nhlbi.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 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 and timelines.

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 e-mail (clinicalsafetyop.phuseh@novartis.com) within 24 hours.

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 e-mail (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.

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.

Coded data may be sent to associate investigators and collaborators outside of the NIH with IRB approval; local institutional approval may be required. 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. Larochelle. 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.

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.

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. Biospecimens may be destroyed only when permitted by the clinical director and the IRB.

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.

10. Human Subject Protection

10.1 Rationale for Subject Selection

With an estimated carrier frequency of 1:181, about 4 persons/million are born each year with FA in the USA (93). The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on gender, race or ethnicity.

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 AUC0-∞ 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
- Fanconi Anemia Research Fund website
- NHLBI website
- Clinical Center patient recruitment website with study specific webpage (using approved flyer and language)
- ResearchMatch
- Flyers – used as needed hard copy and electronically
- Targeted letter to physician specialists – sent nationally and internationally
- Twitter messages and chats with study investigators
- Facebook Posts on Clinical Center and NHLBI accounts
- Fanconi Anemia SunShine Camp (for families)
- Google Ad Words
- 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 NHLBI Office of Patient Engagement and Recruitment (PRO) will work with study investigators on meeting accrual goals. Reviews of the accrual will be performed monthly. If needed, 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 and internationally if feasible; additional Twitter and 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: Please see IRB approved Public Service Announcement (PSA) and flyers for study 17-H-0121.

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

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

10.2 Participation of Pediatric Patients

As outlined in section 2.2. bone marrow failure is one of the hallmarks of Fanconi anemia and it usually manifests in the first decade of life. We are currently treating pediatric patients with EPAG on 3 different protocols: in the combination with immunosuppression (12-H-0150), headed by Dr. Neal Young, and protocols 11-H-0134 for patients with moderate aplastic anemia and 13-H-0133 for patients with refractory aplastic anemia. 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 FA treated with EPAG have not been performed. 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. Subjects must be able to swallow tablets. To ensure a safe drug administration we propose enrolling patients 6 years of age or older.

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 (UGT)1A1 and organic anion-transporting polypeptide (OATP)1B1, which may lead to indirect hyperbilirubinemia. If bilirubin is elevated, perform fractionation. Evaluate abnormal serum liver tests with repeat testing within 3 to 5 days. If the abnormalities are confirmed, monitor serum liver tests weekly until resolved or stabilized. Discontinue PROMACTA if ALT levels increase to greater than or equal to 3 x ULN in patients with normal liver function or greater than or equal to 3 x baseline (or greater than 5 x ULN, whichever is the lower) in patients with pre-treatment elevations in transaminases and are:

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

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

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

Thrombotic/Thromboembolic Complications

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

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

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

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

Cataracts

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

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

Clinical Experience:

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

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

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

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

Rash was reported in 7% of patients.

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

USE IN SPECIFIC POPULATIONS

Pregnancy

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.

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

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

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

Nursing Mothers

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

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

Pediatric Use

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

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

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 (94).

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

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

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

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

Adverse Events Considered to be Expected for Reporting Purposes in cITP Adults

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

Additional Adverse Events Considered to be Expected for Reporting Purposes in cITP Pediatric Patients (Aged 1 to 17 years) in addition to those seen in cITP in Adults

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

spontaneously

Adverse Events Considered to be Expected for Reporting Purposes in SAA

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

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

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

Common: Skin discoloration
Vascular disorders Very common: Hematoma

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

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

Additional Eltrombopag risks

Patients with Fanconi anemia are at risk of developing chromosomal abnormalities and progressing to another form of bone marrow failure syndrome called myelodysplasia. As described in the scientific justification section of this protocol we hypothesize that eltrombopag may also improves DNA repair mechanism in hematopoietic stem cell *in vivo*. Thus, counteracting or delaying the naturally occurring process of malignant transformation in FA patients. However, eltrombopag stimulates stem cells and potentially also pre-malignant stem cells. Therefore, it is hypothetical that eltrombopag accelerates malignant transformation.

Nonclinical Pharmacology

Studies conducted in vitro have shown that EPAG is an effective agonist binding to *mpl*, the thrombopoietin receptor (TPO-R), to stimulate thrombopoiesis. It binds *mpl* at a position distinct from the ligand binding site, and thus does not compete with TPO for binding to its receptor (38). In vivo, EPAG increases platelet number in the chimpanzee (the only nonclinical species which is pharmacologically responsive to eltrombopag). These findings, coupled with supporting clinical efficacy data, suggested that eltrombopag is an orally active TPO-R agonist that functions in a similar manner to endogenous thrombopoietin (TPO). Additionally, in vitro electrophysiology studies have been performed and in vivo safety pharmacology studies assessed the effects of EPAG 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)(95, 96)

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 HRQL

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

10.3.5 Related to 24-Hour Urine Collection

No major risks are involved with urine collection. There is minimal risk associated with collecting urine, including accidental spillage during collection. Participants will be instructed with safe collection techniques.

10.3.6 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 (including subjects unable to consent)

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

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

10.5 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 may 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 may 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.

Request for waiver of consent for screening activities

Prior to the subject signing the consent for this study pre-screening activities listed in section 6.1 may be performed. We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in this research protocol. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

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 7 years of age (inclusive) 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. Assent will be obtained as appropriate based on age:

1) 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.
2) Children who are 7 years of age (inclusive) but less than 12 years of age (exclusive): Verbal assent will be obtained as appropriate.
3) Children who are 12 years of age (inclusive) but less than 18 years of age (exclusive): Will be asked to sign an assent form.

The parent or guardian will sign on the designated line on the informed consent providing permission for their child to participate. 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.

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 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects
   a. Analysis of samples and data from this study involves no additional risks to subjects.

(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects
   a. Samples and data will be kept in secure locations in the laboratory of Dr. Andre Larochelle. 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.

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

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 Collaborator(s)

Dr. Winkler is currently working at Agios Pharmaceutical and will be analyzing coded/non-identifiable data as a collaborator in this protocol. Dr. Winkler's role in the research will be limited to data analysis.

11. PHARMACEUTICALS

11.1 Eltrombopag (Promacta®):

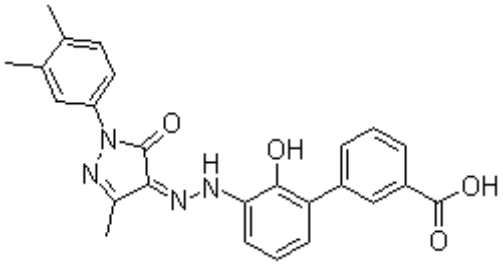
Supply: Novartis

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 manufactured 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**:

  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.

12. REFERENCES

1. Alter BP. Fanconi anemia and the development of leukemia. *Best Pract Res Clin Haematol.* 2014;27(3-4):214-21.
2. Kottemann MC, and Smogorzewska A. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature.* 2013;493(7432):356-63.
3. Ceccaldi R, Parmar K, Mouly E, Delord M, Kim JM, Regairaz M, Pla M, Vasquez N, Zhang QS, Pondarre C, et al. Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. *Cell Stem Cell.* 2012;11(1):36-49.
4. Garaycoechea JI, and Patel KJ. Why does the bone marrow fail in Fanconi anemia? *Blood.* 2014;123(1):26-34.
5. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, and Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. *Blood.* 1994;84(5):1650-5.
6. Rosselli F, Sanceau J, Gluckman E, Wietzerbin J, and Moustacchi E. Abnormal lymphokine production: a novel feature of the genetic disease Fanconi anemia. II. In vitro and in vivo spontaneous overproduction of tumor necrosis factor alpha. *Blood.* 1994;83(5):1216-25.
7. Rathbun RK, Christianson TA, Faulkner GR, Jones G, Keeble W, O'Dwyer M, and Bagby GC. Interferon-gamma-induced apoptotic responses of Fanconi anemia group C hematopoietic progenitor cells involve caspase 8-dependent activation of caspase 3 family members. *Blood.* 2000;96(13):4204-11.
8. Rathbun RK, Faulkner GR, Ostroski MH, Christianson TA, Hughes G, Jones G, Cahn R, Maziarz R, Royle G, Keeble W, et al. Inactivation of the Fanconi anemia group C gene augments interferon-gamma-induced apoptotic responses in hematopoietic cells. *Blood.* 1997;90(3):974-85.
9. Wang J, Otsuki T, Youssoufian H, Foe JL, Kim S, Devetten M, Yu J, Li Y, Dunn D, and Liu JM. Overexpression of the fanconi anemia group C gene (FAC) protects hematopoietic progenitors from death induced by Fas-mediated apoptosis. *Cancer Res.* 1998;58(16):3538-41.
10. Li X, Yang Y, Yuan J, Hong P, Freie B, Orazi A, Haneline LS, and Clapp DW. Continuous in vivo infusion of interferon-gamma (IFN-gamma) preferentially reduces myeloid progenitor numbers and enhances engraftment of syngeneic wild-type cells in Fancc-/- mice. *Blood.* 2004;104(4):1204-9.
11. Dufour C, Capasso M, Svahn J, Marrone A, Haupt R, Bacigalupo A, Giordani L, Longoni D, Pillon M, Pistorio A, et al. Homozygosis for (12) CA repeats in the first intron of the human IFN-gamma gene is significantly associated with the risk of aplastic anaemia in Caucasian population. *British journal of haematology.* 2004;126(5):682-5.
12. Lin FC, Karwan M, Saleh B, Hodge DL, Chan T, Boelte KC, Keller JR, and Young HA. IFN-gamma causes aplastic anemia by altering hematopoietic stem/progenitor cell composition and disrupting lineage differentiation. *Blood.* 2014;124(25):3699-708.
13. Selleri C, Maciejewski JP, Sato T, and Young NS. Interferon-gamma constitutively expressed in the stromal microenvironment of human marrow cultures mediates potent hematopoietic inhibition. *Blood.* 1996;87(10):4149-57.

14. Felli N, Pedini F, Zeuner A, Petrucci E, Testa U, Conticello C, Biffoni M, Di Cataldo A, Winkles JA, Peschle C, et al. Multiple members of the TNF superfamily contribute to IFN-gamma-mediated inhibition of erythropoiesis. *Journal of immunology.* 2005;175(3):1464-72.
15. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, and Auerbach AD. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood.* 2003;101(4):1249-56.
16. Alter BP, Giri N, Savage SA, Peters JA, Loud JT, Leathwood L, Carr AG, Greene MH, and Rosenberg PS. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. *British journal of haematology.* 2010;150(2):179-88.
17. Gregory JJ, Jr., Wagner JE, Verlander PC, Levran O, Batish SD, Eide CR, Steffenhagen A, Hirsch B, and Auerbach AD. Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. *Proc Natl Acad Sci U S A.* 2001;98(5):2532-7.
18. Waisfisz Q, Morgan NV, Savino M, de Winter JP, van Berkel CG, Hoatlin ME, Ianzano L, Gibson RA, Arwert F, Savoia A, et al. Spontaneous functional correction of homozygous fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. *Nat Genet.* 1999;22(4):379-83.
19. Smith AR, and Wagner JE. Current clinical management of Fanconi anemia. *Expert Rev Hematol.* 2012;5(5):513-22.
20. Dalle JH, and Peffault de Latour R. Allogeneic hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. *Int J Hematol.* 2016;103(4):373-9.
21. Scheckenbach K, Morgan M, Filger-Brillinger J, Sandmann M, Strimling B, Scheurlen W, Schindler D, Gobel U, and Hanenberg H. Treatment of the bone marrow failure in Fanconi anemia patients with danazol. *Blood Cells Mol Dis.* 2012;48(2):128-31.
22. Diamond LK, and Shahidi NT. Treatment of aplastic anemia in children. *Semin Hematol.* 1967;4(3):278-88.
23. Shahidi NT, and Diamond LK. Testosterone-induced remission in aplastic anemia of both acquired and congenital types. Further observations in 24 cases. *N Engl J Med.* 1961;264(953-67.
24. Dufour C, and Svahn J. Fanconi anaemia: new strategies. *Bone Marrow Transplant.* 2008;41 Suppl 2(S90-5.
25. Paustian L, Chao MM, Hanenberg H, Schindler D, Neitzel H, Kratz CP, and Ebell W. Androgen therapy in Fanconi anemia: A retrospective analysis of 30 years in Germany. *Pediatr Hematol Oncol.* 2016;33(1):5-12.
26. Zeigler FC, de Sauvage F, Widmer HR, Keller GA, Donahue C, Schreiber RD, Malloy B, Hass P, Eaton D, and Matthews W. In vitro megakaryocytopoietic and thrombopoietic activity of c-mpl ligand (TPO) on purified murine hematopoietic stem cells. *Blood.* 1994;84(12):4045-52.
27. Qian H, Buza-Vidas N, Hyland CD, Jensen CT, Antonchuk J, Mansson R, Thoren LA, Ekblom M, Alexander WS, and Jacobsen SE. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell.* 2007;1(6):671-84.
28. Yoshihara H, Arai F, Hosokawa K, Hagiwara T, Takubo K, Nakamura Y, Gomei Y, Iwasaki H, Matsuoka S, Miyamoto K, et al. Thrombopoietin/MPL signaling regulates hematopoietic

stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell.* 2007;1(6):685-97.
29. Murray LJ, Luens KM, Estrada MF, Bruno E, Hoffman R, Cohen RL, Ashby MA, and Vadhan-Raj S. Thrombopoietin mobilizes CD34+ cell subsets into peripheral blood and expands multilineage progenitors in bone marrow of cancer patients with normal hematopoiesis. *Exp Hematol.* 1998;26(3):207-16.
30. Alexander WS, Roberts AW, Nicola NA, Li R, and Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietic receptor c-Mpl. *Blood.* 1996;87(6):2162-70.
31. Grozovsky R, Begonja AJ, Liu K, Visner G, Hartwig JH, Falet H, and Hoffmeister KM. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. *Nat Med.* 2014.
32. Kuter DJ, Goodnough LT, Romo J, DiPersio J, Peterson R, Tomita D, Sheridan W, and McCullough J. Thrombopoietin therapy increases platelet yields in healthy platelet donors. *Blood.* 2001;98(5):1339-45.
33. Li J, Yang C, Xia Y, Bertino A, Glaspy J, Roberts M, and Kuter DJ. Thrombocytopenia caused by the development of antibodies to thrombopoietin. *Blood.* 2001;98(12):3241-8.
34. de Bruin AM, Demirel O, Hooibrink B, Brandts CH, and Nolte MA. Interferon-gamma impairs proliferation of hematopoietic stem cells in mice. *Blood.* 2013;121(18):3578-85.
35. de Laval B, Pawlikowska P, Petit-Cocault L, Bilhou-Nabera C, Aubin-Houzelstein G, Souyri M, Pouzoulet F, Gaudry M, and Porteu F. Thrombopoietin-increased DNA-PK-dependent DNA repair limits hematopoietic stem and progenitor cell mutagenesis in response to DNA damage. *Cell Stem Cell.* 2013;12(1):37-48.
36. de Laval B, Pawlikowska P, Barbieri D, Besnard-Guerin C, Cico A, Kumar R, Gaudry M, Baud V, and Porteu F. Thrombopoietin promotes NHEJ DNA repair in hematopoietic stem cells through specific activation of Erk and NF-kappaB pathways and their target, IEX-1. *Blood.* 2014;123(4):509-19.
37. Garnock-Jones KP. Eltrombopag: a review of its use in treatment-refractory chronic primary immune thrombocytopenia. *Drugs.* 2011;71(10):1333-53.
38. Imbach P, and Crowther M. Thrombopoietin-receptor agonists for primary immune thrombocytopenia. *The New England journal of medicine.* 2011;365(8):734-41.
39. Erickson-Miller CL, Delorme E, Tian SS, Hopson CB, Landis AJ, Valoret EI, Sellers TS, Rosen J, Miller SG, Luengo JI, et al. Preclinical activity of eltrombopag (SB-497115), an oral, nonpeptide thrombopoietin receptor agonist. *Stem Cells.* 2009;27(2):424-30.
40. Emmons RV, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, and Shulman NR. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. *Blood.* 1996;87(10):4068-71.
41. Bussel JB, Cheng G, Saleh MN, Psaila B, Kovaleva L, Meddeb B, Kloczko J, Hassani H, Mayer B, Stone NL, et al. Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. *N Engl J Med.* 2007;357(22):2237-47.
42. Cheng G, Saleh MN, Marcher C, Vasey S, Mayer B, Aivado M, Arning M, Stone NL, and Bussel JB. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. *Lancet.* 2011;377(9763):393-402.

43. Saleh MN, Bussel JB, Cheng G, Meyer O, Bailey CK, Arning M, Brainsky A, and Group ES. Safety and efficacy of eltrombopag for treatment of chronic immune thrombocytopenia: results of the long-term, open-label EXTEND study. *Blood.* 2013;121(3):537-45.
44. Desmond R, Townsley DM, Dumitriu B, Olnes MJ, Scheinberg P, Bevans M, Parikh AR, Broder K, Calvo KR, Wu CO, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood.* 2014;123(12):1818-25.
45. Olnes MJ, Scheinberg P, Calvo KR, Desmond R, Tang Y, Dumitriu B, Parikh AR, Soto S, Biancotto A, Feng X, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. *The New England journal of medicine.* 2012;367(1):11-9.
46. Erickson-Miller CL, DeLorme E, Tian SS, Hopson CB, Stark K, Giampa L, Valoret EI, Duffy KJ, Luengo JL, Rosen J, et al. Discovery and characterization of a selective, nonpeptidyl thrombopoietin receptor agonist. *Exp Hematol.* 2005;33(1):85-93.
47. Nakamura T, Miyakawa Y, Miyamura A, Yamane A, Suzuki H, Ito M, Ohnishi Y, Ishiwata N, Ikeda Y, and Tsuruzoe N. A novel nonpeptidyl human c-Mpl activator stimulates human megakaryopoiesis and thrombopoiesis. *Blood.* 2006;107(11):4300-7.
48. Kim MJ, Park SH, Opella SJ, Marsilje TH, Michellys PY, Seidel HM, and Tian SS. NMR structural studies of interactions of a small, nonpeptidyl Tpo mimic with the thrombopoietin receptor extracellular juxtamembrane and transmembrane domains. *J Biol Chem.* 2007;282(19):14253-61.
49. Cheruku PS, Cash A, Dunbar CE, Young NS, and Larochelle A. The Thrombopoietin Receptor Agonist Eltrombopag Has DNA Repair Activity in Human Hematopoietic Stem and Progenitor Cells. *Blood.* 2015;126(23):2407-.
50. Cheng H, Cheruku PS, Alvarado L, Cash A, Dunbar CE, Young NS, and Larochelle A. Interferon-γ Perturbs Key Signaling Pathways Induced By Thrombopoietin, but Not Eltrombopag, in Human Hematopoietic Stem/Progenitor Cells. *Blood.* 2016;128(22):3870-.
51. Bussel JB, Provan D, Shamsi T, Cheng G, Psaila B, Kovaleva L, Salama A, Jenkins JM, Roychowdhury D, Mayer B, et al. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2009;373(9664):641-8.
52. Gernsheimer TB, George JN, Aledort LM, Tarantino MD, Sunkara U, Matthew Guo D, and Nichol JL. Evaluation of bleeding and thrombotic events during long-term use of romiplostim in patients with chronic immune thrombocytopenia (ITP). *Journal of thrombosis and haemostasis : JTH.* 2010;8(6):1372-82.
53. Catala-Lopez F, Corrales I, Martin-Serrano G, Tobias A, and Calvo G. Risk of thromboembolism with thrombopoietin receptor agonists in adult patients with thrombocytopenia: systematic review and meta-analysis of randomized controlled trials. *Medicina clinica.* 2012;139(10):421-9.
54. AbbVie Inc. NC, IL 60064 USA Kaletra Prescribing Information. http://www.rxabbvie.com/pdf/kaletratabpi.pdf.
55. Company VH, and Research Triangle Park N. Viracept Package Insert. April 2012.
56. LP AP, and Wilmington D. Prilosec Package Insert. Spetember 2012.
57. Merck Sharp & Dohme Corp. aso, Merck & Co. I, and Whitehouse Station N, USA Grygiel Prescribing Information. https://www.merck.com/product/usa/pi_circulars/i/isentress/isentress_pi.pdf.

58. UCB I, and Smyrna G. Keppra Package Insert.
59. Bussel JB, de Miguel PG, Despotovic JM, Grainger JD, Sevilla J, Blanchette VS, Krishnamurti L, Connor P, David M, Boayue KB, et al. Eltrombopag for the treatment of children with persistent and chronic immune thrombocytopenia (PETIT): a randomised, multicentre, placebo-controlled study. *The Lancet Haematology.* 2015;2(8):e315-25.
60. Grainger JD, Locatelli F, Chotsampancharoen T, Donyush E, Pongtanakul B, Komvilaisak P, Sosothikul D, Drelichman G, Sirachainan N, Holzhauer S, et al. Eltrombopag for children with chronic immune thrombocytopenia (PETIT2): a randomised, multicentre, placebo-controlled trial. *Lancet.* 2015;386(10004):1649-58.
61. Wilkinson AC, Ishida R, Kikuchi M, Sudo K, Morita M, Crisostomo RV, Yamamoto R, Loh KM, Nakamura Y, and Watanabe M. Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature.* 2019;571(7763):117-21.
62. Cui L, Moraga I, Lerbs T, Van Neste C, Wilmes S, Tsutsumi N, Trotman-Grant AC, Gakovic M, Andrews S, Gotlib J, et al. Tuning MPL signaling to influence hematopoietic stem cell differentiation and inhibit essential thrombocythemia progenitors. *Proc Natl Acad Sci U S A.* 2021;118(2).
63. Tsutsumi N, Masoumi Z, James SC, Tucker JA, Winkelmann H, Grey W, Picton LK, Moss L, Wilson SC, Caveney NA, et al. Structure of the thrombopoietin-MPL receptor complex is a blueprint for biasing hematopoiesis. *Cell.* 2023;186(19):4189-203 e22.
64. Vlachodimitropoulou E, Chen Y-L, Garbowski M, Koonyosying P, Psaila B, Sola-Visner M, Cooper N, Hider R, and Porter J. Eltrombopag: a powerful chelator of cellular or extracellular iron(III) alone or combined with a second chelator. *Blood.* 2017;130(17):1923-33.
65. Kao YR, Chen J, Narayanagari SR, Todorova TI, Aivalioti MM, Ferreira M, Ramos PM, Pallaud C, Mantzaris I, Shastri A, et al. Thrombopoietin receptor-independent stimulation of hematopoietic stem cells by eltrombopag. *Sci Transl Med.* 2018;10(458).
66. Zhao Z, Sun Q, Sokoll LJ, Streiff M, Cheng Z, Grasmeder S, Townsley DM, Young NS, Dunbar CE, and Winkler T. Eltrombopag mobilizes iron in patients with aplastic anemia. *Blood.* 2018;131(21):2399-402.
67. Nowell P, Bergman G, Besa E, Wilmoth D, and Emanuel B. Progressive preleukemia with a chromosomally abnormal clone in a kindred with the Estren-Dameshek variant of Fanconi's anemia. *Blood.* 1984;64(5):1135-8.
68. Thurston VC, Ceperich TM, Vance GH, and Heerema NA. Detection of monosomy 7 in bone marrow by fluorescence in situ hybridization. A study of Fanconi anemia patients and review of the literature. *Cancer Genet Cytogenet.* 1999;109(2):154-60.
69. Alter BP, Scalise A, McCombs J, and Najfeld V. Clonal chromosomal abnormalities in Fanconi's anaemia: what do they really mean? *Br J Haematol.* 1993;85(3):627-30.
70. Fan X, Desmond R, Winkler T, Young DJ, Dumitriu B, Townsley DM, Gutierrez-Rodrigues F, Lotter J, Valdez J, Sellers SE, et al. Eltrombopag for patients with moderate aplastic anemia or uni-lineage cytopenias. *Blood Adv.* 2020;4(8):1700-10.
71. Platzbecker U, Wong RS, Verma A, Abboud C, Araujo S, Chiou TJ, Feigert J, Yeh SP, Gotze K, Gorin NC, et al. Safety and tolerability of eltrombopag versus placebo for treatment of thrombocytopenia in patients with advanced myelodysplastic syndromes or acute myeloid leukaemia: a multicentre, randomised, placebo-controlled, double-blind, phase 1/2 trial. *Lancet Haematol.* 2015;2(10):e417-26.

72. Will B, Kawahara M, Luciano JP, Bruns I, Parekh S, Erickson-Miller CL, Aivado MA, Verma A, and Steidl U. Effect of the nonpeptide thrombopoietin receptor agonist Eltrombopag on bone marrow cells from patients with acute myeloid leukemia and myelodysplastic syndrome. *Blood.* 2009;114(18):3899-908.
73. Erickson-Miller CL, Kirchner J, Aivado M, May R, Payne P, and Chadderton A. Reduced proliferation of non-megakaryocytic acute myelogenous leukemia and other leukemia and lymphoma cell lines in response to eltrombopag. *Leuk Res.* 2010;34(9):1224-31.
74. Kalota A, and Gewirtz AM. A prototype nonpeptidyl, hydrazone class, thrombopoietin receptor agonist, SB-559457, is toxic to primary human myeloid leukemia cells. *Blood.* 2010;115(1):89-93.
75. Mavroudi I, Pyrovolaki K, Pavlaki K, Kozana A, Psyllaki M, Kalpadakis C, Pontikoglou C, and Papadaki HA. Effect of the nonpeptide thrombopoietin receptor agonist eltrombopag on megakaryopoiesis of patients with lower risk myelodysplastic syndrome. *Leuk Res.* 2011;35(3):323-8.
76. Roth M, Will B, Simkin G, Narayanagari S, Barreyro L, Bartholdy B, Tamari R, Mitsiades CS, Verma A, and Steidl U. Eltrombopag inhibits the proliferation of leukemia cells via reduction of intracellular iron and induction of differentiation. *Blood.* 2012;120(2):386-94.
77. Sugita M, Kalota A, Gewirtz AM, and Carroll M. Eltrombopag inhibition of acute myeloid leukemia cell survival does not depend on c-Mpl expression. *Leukemia.* 2013;27(5):1207-10.
78. Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, Heller G, Wolden S, O'Reilly RJ, and Boulad F. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. *British journal of haematology.* 2008;140(6):644-55.
79. Masserot C, Peffault de Latour R, Rocha V, Leblanc T, Rigolet A, Pascal F, Janin A, Soulier J, Gluckman E, and Socie G. Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. *Cancer.* 2008;113(12):3315-22.
80. Myers KC, and Davies SM. Hematopoietic stem cell transplantation for bone marrow failure syndromes in children. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2009;15(3):279-92.
81. Rackoff WR, Orazi A, Robinson CA, Cooper RJ, Alter BP, Freedman MH, Harris RE, and Williams DA. Prolonged administration of granulocyte colony-stimulating factor (filgrastim) to patients with Fanconi anemia: a pilot study. *Blood.* 1996;88(5):1588-93.
82. Townsley D, Dumitriu B, Scheinberg P, Desmond R, Feng X, Rios O, Weinstein B, Valdez J, Winkler T, Desierto M, et al. Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia Accelerates Count Recovery and Increases Response Rates. *Blood.* 2015;126(23):LBA-2-LBA-.
83. Ohara A, Kojima S, Hamajima N, Tsuchida M, Imashuku S, Ohta S, Sasaki H, Okamura J, Sugita K, Kigasawa H, et al. Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood.* 1997;90(3):1009-13.
84. Socie G, Mary JY, Schrezenmeier H, Marsh J, Bacigalupo A, Locasciulli A, Fuehrer M, Bekassy A, Tichelli A, and Passweg J. Granulocyte-stimulating factor and severe aplastic

anemia: a survey by the European Group for Blood and Marrow Transplantation (EBMT). *Blood.* 2007;109(7):2794-6.
85. Locasciulli A, Arcese W, Locatelli F, Di Bona E, Bacigalupo A, and Italian Aplastic Anaemia Study G. Treatment of aplastic anaemia with granulocyte-colony stimulating factor and risk of malignancy. Italian Aplastic Anaemia Study Group. *Lancet.* 2001;357(9249):43-4.
86. Tichelli A, Schrezenmeier H, Socie G, Marsh J, Bacigalupo A, Duhrsen U, Franzke A, Hallek M, Thiel E, Wilhelm M, et al. A randomized controlled study in patients with newly diagnosed severe aplastic anemia receiving antithymocyte globulin (ATG), cyclosporine, with or without G-CSF: a study of the SAA Working Party of the European Group for Blood and Marrow Transplantation. *Blood.* 2011;117(17):4434-41.
87. Scagni P, Saracco P, Timeus F, Farinasso L, Dall'Aglio M, Bosa EM, Crescenzio N, Spinelli M, Basso G, and Ramenghi U. Use of recombinant granulocyte colony-stimulating factor in Fanconi's anemia. *Haematologica.* 1998;83(5):432-7.
88. Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, and Neitzel H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. *Blood.* 2003;101(10):3872-4.
89. Peffault de Latour R, and Soulier J. How I treat MDS and AML in Fanconi anemia. *Blood.* 2016;127(24):2971-9.
90. Sebert M, Gachet S, Leblanc T, Rousseau A, Bluteau O, Kim R, Ben Abdelali R, Sicre de Fontbrune F, Maillard L, Fedronie C, et al. Clonal hematopoiesis driven by chromosome 1q/MDM4 trisomy defines a canonical route toward leukemia in Fanconi anemia. *Cell Stem Cell.* 2023;30(2):153-70 e9.
91. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials.* 1989;10(1):1-10.
92. Geller N, Follmann D, Carter S, and Leifer E. *Advances in Clinical Trial Biostatistics*. CRC Press; 2003.
93. Rosenberg PS, Tamary H, and Alter BP. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi Anemia in the United States and Israel. *American journal of medical genetics Part A.* 2011;155A(8):1877-83.
94. Matthys G, Park JW, McGuire S, Wire MB, Bowen C, Williams D, Jenkins J, and Peng B. Clinical pharmacokinetics, platelet response, and safety of eltrombopag at supratherapeutic doses of up to 200 mg once daily in healthy volunteers. *Journal of clinical pharmacology.* 2011;51(3):301-8.
95. Matthys G, Park JW, McGuire S, Wire MB, Bowen C, Williams D, Jenkins J, and Peng B. Clinical pharmacokinetics, platelet response, and safety of eltrombopag at supratherapeutic doses of up to 200 mg once daily in healthy volunteers. *J Clin Pharmacol.* 2011;51(3):301-8.
96. Bauman JW, Vincent CT, Peng B, Wire MB, Williams DD, and Park JW. Effect of hepatic or renal impairment on eltrombopag pharmacokinetics. *J Clin Pharmacol.* 2011;51(5):739-50.

13. APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Table 10: Schedule of Events (Pre-enrollment through first 6 months of treatment)

Visit/Time Point	Screening[1]	Baseline[6]	Month (±14days)[2]					Month 6[3]	Month 12 Off-study[4]
			1	2	3[3]	4	5		
Clinical Assessments									
Consent	X								
Medical History[5]	X		X	X	X	X	X	X	X
Physical Examination[5]	X		X	X	X	X	X	X	X
Ophthalmology Consult	X								
Pediatric Consult	X								
Nutrition Consult	X[18]	X[18]							
Clinical Photography	X								
Concurrent Medication Review	X	X			X			X	
Laboratory Evaluations									
Complete Blood Count with Differential	X	X	X	X	X	X	X	X	X
Type and Screen	X	X			X			X	X
Peripheral Blood Smear	X	X[7]			X			X	X
Reticulocyte Count	X	X[7]			X			X	X
DAT (Direct Antiglobulin Test)	X								
Chromosomal Breakage Analysis (Lymphocytes and/or Fibroblasts)	X[8]							X	
Genetic Testing	X[8]								
Acute Care Panel (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen)	X	X[9]			X			X	X
Mineral (Phosphorus, Magnesium, Albumin, and Calcium)	X				X			X	X
Hepatic Panel (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)	X	X[10]			X			X	X

Visit/Time Point	Screening[1]	Baseline[6]	Month (±14days)[2]					Month 6[3]	Month 12 Off-study[4]
			1	2	3[3]	4	5		
Hepatic (ALT, AST, Total Bilirubin, OR Direct Bilirubin)			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				X			X	X
Viral Serologies (HepA, HepB [HBsAg, HBsAb, DNA PCR], HCV, HIV, HSV, EBV and CMV)	X								
Folate Level	X								
B12 Level	X								
Vitamin D, 25 Hydroxy, Total	X				X			X	
Ferritin[11]	X		X	X		X	X		
Iron Panel (Ferritin, Transferrin, % Saturation, Total Serum Iron)	X				X			X	X
24-hour Urine Collection to Determine the Total Iron[12]	X				X			X	X
HLA Typing	X[8]								
Pregnancy Test (Blood or Urine HCG in Women of Child Bearing Potential)	X	X			X			X	
Flow Cytometry of the Peripheral Blood to Quantitate GPI-Negative Cells	X				X			X	X
Flow Cytometry of Peripheral Blood Lymphocytes for Phenotyping (Analysis of T, B, and NK Subsets)	X				X			X	X
Immunoglobulins	X								
Thyroid Function Panel (T3, T4, and TSH)	X								

Visit/Time Point	Screening[1]	Baseline[6]	Month (±14days)[2]					Month 6[3]	Month 12 Off-study[4]
			1	2	3[3]	4	5		
Procedures									
Bone Marrow Aspiration and Core Biopsy[13]	X				X			X	X
Bone Marrow Chromosomal Analysis via Standard Cytogenetic Techniques	X				X			X	X
MDS FISH Panel[14]	X				X			X	X
Skin Biopsy	X								
Electrocardiogram (EKG)	X								
Research Testing and Assessments									
HRQL Survey Administration[15]	X				X			X	
Pharmacokinetics (PK)					X[16]			X	
First dose of Medication	X[17]								
Document Drug Accountability					X			X	
Research Samples		X[18]			X			X	X

[1] within 16 weeks prior to starting EPAG
[2] all time points are counted from the day EPAG is initiated and must be completed within a range of ± 14 days
[3] 12-week and 6-month evaluations must be completed within a range of ± 14 days
[4] optional 12-month off-study evaluation may be completed within a range of ± 30 days
[5] medical history and physical assessments by a physician at the NIH Clinical Center or the referring local health care provider are required every two weeks (± 7 days) for the initial 12 patients, including both USA and international subjects.
[6] within 7 days prior to starting EPAG
[7] optional for the baseline evaluation (i.e., within 7 days prior to starting EPAG) if not performed at the NIH
[8] not required if already performed elsewhere and records are available
[9] glucose is optional for the baseline evaluation (i.e., within 7 days prior to starting EPAG) if not performed at the NIH
[10] alkaline phosphatase is optional and either total bilirubin or direct bilirubin may be obtained for the baseline evaluation (i.e., within 7 days prior to starting EPAG) if not performed at the NIH
[11] full iron panel (including transferrin, % saturation and total serum iron) is preferable but not required
[12] at PI's discretion if indicated for iron metabolism and excretion studies
[13] bone marrow aspirate and core biopsy will be processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins
[14] an MDS FISH panel (MDS, Diagnostic FISH and/or MDS, Specified FISH) at the times indicated is optional and will be done at PI's discretion
[15] subjects or parent proxies who can read English or Spanish will complete the hard copy or electronic survey, either: 1) Parent Proxy HRQL Survey for subjects who are under the age of 7 (exclusive); 2) Pediatric HRQL Survey for subjects who are 7 years of age (inclusive) but less than 18 years of age (exclusive); 3) Adult HRQL Survey for subjects who are 18 years of age (inclusive) or more.
[16] per Appendix B, if subjects do not meet requirements for PK at month 3, then PK can be performed at month 6, at PI's discretion

[17] after confirmation that all inclusion criteria are met
[18] include blood research samples outlined in section 7 only; marrow research samples are obtained at screening with the diagnostic marrow to avoid a repeat procedure

Table 11: Schedule of Events for patients on EPAG while on the extended phase (up to 3 years after the 6 month visit)

Visit/ Time Point	Every 2 weeks (±7 days)	Monthly (±14 days)	Every 6 Months (±30 days)	Every 12 months (±60 days)
Clinical Assessments				
Medical History		X	X	
Physical Examination		X	X	
Ophthalmology Consult				X[1]
Pediatric Consult			X[1]	
Concurrent Medication Review			X	
Laboratory Evaluations				
Complete Blood Count with Differential		X		
Peripheral Blood Smear			X	
Reticulocyte Count			X	
ALT, AST, Total Bilirubin, Direct Bilirubin and Indirect Bilirubin	X[2]			
ALT, AST, Total Bilirubin, or Direct Bilirubin		X		
Hepatic Panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)			X	
Acute Care Panel: Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen			X	
Ferritin		X[1]		
Iron Panel (Ferritin, Transferrin, % Saturation, Total Serum Iron)			X	
Type and Screen			X	
Coagulation and Thrombosis Screens (PT, PTT)			X	
Vitamin D, 25 Hydroxy, Total			X	
Chromosome Breakage Analysis in Lymphocytes (e.g., DEB testing)			X	
24-hour Urine Collection to Determine the Total Iron			X[1]	
Pregnancy Test (Blood or Urine HCG in Women of Child Bearing Potential)			X	
Flow Cytometry of the Peripheral Blood to Quantitate GPI-Negative Cells			X	
Flow Cytometry of Peripheral Blood Lymphocytes for Phenotyping (Analysis of T, B and NK Subsets)			X	
Procedures				
Bone Marrow Aspiration and Core Biopsy[3]			X	

Bone Marrow Chromosomal Analysis via Standard Cytogenetic Techniques			X	
MDS FISH Panel			X[1]	
Research Testing and Assessments				
HRQL Survey Administration[4]			X	
Document Drug Accountability			X	
Research Samples			X	

[1] optional/at PI's discretion

[2] during the dose escalation period

[3] bone marrow aspirate and core biopsy will be processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins (every 6 months ± 30 days)

[4] Subjects or parent proxies who can read English or Spanish will complete the hard copy or electronic survey, either: 1) Parent Proxy HRQL Survey for subjects who are under the age of 7 (exclusive); 2) Pediatric HRQL Survey for subjects who are 7 years of age (inclusive) but less than 18 years of age (exclusive); 3) Adult HRQL Survey for subjects who are 18 years of age (inclusive) or more.

Table 12: Schedule of Events for patients who have EPAG discontinued while on extended phase (up to 3 years after the 6-month visit**)**

Visit/ Time Point	Monthly (±14 days)	Every 6 Months (±30 days)	Every 12 months (±60 days)
Clinical Assessments			
Medical History			X
Physical Examination			X
Laboratory Evaluations			
Complete Blood Count with Differential	X[1]		
Hepatic Panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin		X	
Iron Panel (Ferritin, Transferrin, % Saturation, Total Serum Iron)			X
Type and Screen			X
Coagulation and Thrombosis screens (PT, PTT)			X
Vitamin D, 25 Hydroxy, Total			X
Chromosome breakage analysis in Lymphocytes (e.g., DEB testing)			X
24-hour Urine Collection to Determine the Total Iron			X[2]
Flow Cytometry of the Peripheral Blood to Quantitate GPI-Negative Cells			X
Flow Cytometry of Peripheral Blood Lymphocytes for Phenotyping (Analysis of T, B and NK Subsets)			X
Procedures			
Bone Marrow Aspiration and Core Biopsy[3]			X
Bone Marrow Chromosomal Analysis via Standard Cytogenetic Techniques			X
MDS FISH Panel[4]	X		
Research Testing and Assessments			
HRQL Survey Administration[5]			X
Research Samples			X

[1] per section 6.5.2, monthly (± 14 days) for 6 months off EPAG, then every 3 months (± 14 days) for robust responders who discontinue EPAG until they reach off study criteria as per section 8.6 or the study is closed

[2] at PI's discretion

[3] Bone marrow aspirate and core biopsy will be processed as follows: a) Aspirate and biopsy will be used in standard stains for morphologic analysis and quantitation of cellularity; b) Aspirate and biopsy will be used in special stains to assess reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other special stains as indicated to classify any abnormalities; c) Aspirate will be used in flow cytometric phenotyping for cell surface or intracellular proteins (every 6 months ± 30 days)

[4] an MDS FISH panel (MDS, Diagnostic FISH and/or MDS, Specified FISH) at the times indicated is optional and will be done at PI's discretion

[5] Subjects or parent proxies who can read English or Spanish will complete the hard copy or electronic survey, either: 1) Parent Proxy HRQL Survey for subjects who are under the age of 7 (exclusive); 2) Pediatric HRQL Survey for subjects who are 7 years of age (inclusive) but less than 18 years of age (exclusive); 3) Adult HRQL Survey for subjects who are 18 years of age (inclusive) or more.

APPENDIX B: PHARMACOKINETIC STUDIES

Collection of samples for PK Assessments

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

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.

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.