Protocol with Statistical Analysis Plan Cover Page:

Official Title:	A Phase I/II, Intra-Patient Dose-Escalation Study of the Selective GlyT1 Inhibitor, Bitopertin for Steroid-Refractory Diamond-Blackfan Anemia.
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Title: A Phase I/II, Intra-Patient Dose-Escalation Study of the Selective GlyT1 Inhibitor, Bitopertin for Steroid-Refractory Diamond-Blackfan Anemia.

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Investigational Agent:

Drug Name:	Bitopertin (DISC-1459; RG1678; RO4917838)
IND Number:	165778
Sponsor:	NHLBI Office of Clinical Director (OCD)
Manufacturer:	ThermoFisher (formerly known as Patheon)

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STATEMENT OF COMPLIANCE

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

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

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

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

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	A phase I/II, intra-patient dose-escalation study of the selective GlyT1 inhibitor, bitopertin for steroid-refractory Diamond-Blackfan anemia.
Study Description:	Diamond-Blackfan anemia (DBA) is an inherited bone marrow failure syndrome characterized by selective erythroid defects. In DBA, a defect in erythroid ribosome biosynthesis creates an asynchrony between protein synthesis of globin chains and heme, wherein the continued production of free heme without sufficient globin is toxic to cells. Bitopertin prevents the uptake of glycine through the GlyT1 transporter reducing the synthesis of 5-aminolevulinic acid, the rate-limiting step in heme synthesis, which in turn leads to a significant reduction in intracellular heme. We hypothesize that bitopertin will rescue DBA by rebalancing heme and globin chain production and reducing the toxicity to the hematopoietic cells that the excess heme causes. Thus, we propose a phase I/II (pilot), single-arm, intra-patient dose-escalation trial of bitopertin for the treatment of steroid-refractory DBA.
Objectives:	Primary Objective: Efficacy of bitopertin in treating Diamond-Blackfan anemia Secondary Objectives: • Examine the safety and tolerability of bitopertin in treating Diamond-Blackfan anemia. • Examine the maintenance of response and relapse rates of Diamond-Blackfan anemia during long-term bitopertin use • Examine the effects of long-term treatment of Diamond-Blackfan anemia with bitopertin on clonal evolution, survival, and health-related quality of life metrics Tertiary/Exploratory Objectives: • Evaluate the impact of bitopertin on stem cell and erythroid cell dynamics in the Diamond-Blackfan anemia through correlative and translational studies • Examine the pharmacology of bitopertin in Diamond-Blackfan anemia

Endpoints:	Primary endpoint: • response rate from drug initiation until 8 months (32 weeks) as measured by an increase in pre-transfusion hemoglobin and/or either decrease in transfusion rate or transfusion-independence Secondary endpoints: • toxicity profile at 8 months (CTCAE criteria) • intra-patient maximum tolerated dose at 8 months (32 weeks) from drug initiation • response rate after 3 months (12 weeks) at the maximum tolerated dose • response rate 3 years post-primary endpoint (extension) • rate of relapse ongoing for up to 3 years, beginning 8 months (32 weeks) from drug initiation as demonstrated by new or increasing transfusion requirements and/or according to clinical outcome • rate of clonal evolution on bitopertin annually for up to 3 years, beginning 8 months (32 weeks) from drug initiation as measured by karyotypic, histologic, and flow cytometric changes • rate of overall survival ongoing for up to 3 years, beginning 8 months (32 weeks) from drug initiation according to clinical outcomes • Health-related quality of life (HRQL) annually for up to 3 years, beginning 8 months (32 weeks) from drug initiation based on questionnaire-based inventory of HRQL
Study Population:	Enroll up to 25 eligible patients (up to 30 recruited) regardless of gender or ethnicity, 18 years of age or older at the time of enrollment, with Diamond-Blackfan anemia, relapsed from, refractory to, or intolerant of steroid therapy
Phase:	Phase I/II
Sites Enrolling Participants:	Single-site study to be conducted at the NIH Clinical Center, Bethesda, MD
Study Intervention:	Oral bitopertin, taken once daily escalating from 5 mg to the lesser of 60 mg or the maximally tolerated dose
Study Duration:	7 years
Participant Duration:	Up to 4.2 years (8 months to primary endpoint with an optional, additional 3-year extension phase for responding participants and an optional 6-month follow-up post drug discontinuation)

1.2 Schema

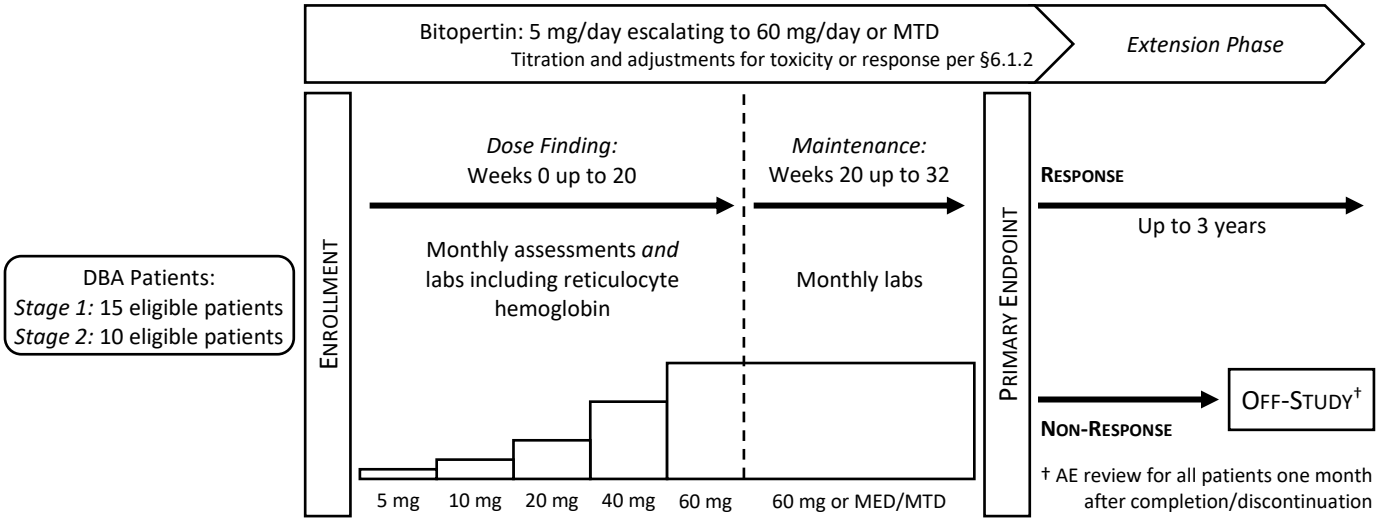


Figure 1: Study enrollment and treatment schema

1.3 Schedule of Activities (SOA)

1.3.1 Schedule of Activities (Pre-enrollment through first 8 months of treatment)

Visit/Time Point	Screening[1]	Baseline[2] (Day 0)	Week (± 10 days[4])							Month 8 [5]	Month 14 Off-study[6]
			4	8	12	16	20	24	28		
Clinical Assessments											
Consent	X										
Medical history / Interval history (diary review at month 8)	X	X								X	X
Physical examination	X									X	X
Telehealth visit (including drug diary review)			X	X	X	X	X	X	X		
PHQ-8 and C-SSRS inventory	X									X	
Concurrent medication review	X	X	X	X	X	X	X	X	X	X	X
Transfusion records review	X	X	X	X	X	X	X	X	X	X	X
Procedures											
Bone marrow aspiration and core biopsy[7]	X[8]									X	X
Bone marrow chromosomal analysis	X[8]									X	X
Electrocardiogram (ECG)	X	X									
Laboratory Evaluations											
Complete blood count with differential	X	X[3]	X	X	X	X	X	X	X	X	X
Reticulocyte count and hemoglobin	X	X[3]	X	X	X	X	X	X	X	X	X
Peripheral blood smear	X	X								X	X
Hemoglobin F quantification	X	X								X	X
DAT (direct antiglobulin test)	X	X								X	X
Chromosomal breakage analysis	X	X[9]									
Genetic testing	X	X[9]									
Acute care (Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen)	X	X								X	X
Mineral (phosphorus, magnesium, albumin, calcium)	X	X								X	X
Hepatic (alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein)	X	X[3]								X	X
CK	X	X								X	X
Uric Acid	X	X								X	X

Visit/Time Point	Screening[1]	Baseline[2] (Day 0)	Week (± 10 days[4])							Month 8 [5]	Month 14 Off-study[6]
			4	8	12	16	20	24	28		
Laboratory Evaluations (continued)											
LDH	X	X								X	X
Coagulation and thrombosis screens (PT, aPTT, INR)	X	X									
Viral serologies: HepA, HepB (HBsAg, HBsAb), HCV, HIV, HSV, EBV and CMV	X	X									
Folate level	X	X									
B_{12} level	X	X									
Iron panel: ferritin, transferrin, % saturation, total serum iron	X	X								X	X
HLA typing (if not performed & available)		X									
Pregnancy test (blood or urine HCG in women of childbearing potential)	X	X								X	
Immunoglobulins		X									
Thyroid function panel (T3, T4, and TSH)		X									
Research Testing and Assessments											
Pharmacokinetic/Pharmacodynamic studies (select patients)			X	X	X	X	X	X	X	X	
Research blood		X								X	X
HRQL survey administration		X[10]								X[10]	X
Document drug accountability										X	

[1] may use existing results of tests performed within 3 months (12 weeks)
[2] may use screening results as baseline if obtained within 3 months (12 weeks) of starting drug, unless indicated otherwise
[3] may use results if obtained within 7 days of screening
[4] all timepoints must be completed within a range of ± 10 days *except* for the 8-month and optional 14-month timepoints and are measured from the date of drug initiation
[5] 32-week (8-month) evaluations must be completed within a range of ± 14 days
[6] optional 14-month off-study evaluation may be completed within a range of ± 30 days. All noted evaluations are recommended, but not required per subject preference. All patients will be contacted 30 days (± 5 days) after completion/discontinuation of bitopertin therapy to review any interval adverse events since discontinuing study drug regardless of response or participation in the 14-month evaluation.
[7] stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess iron, reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities
[8] not required if already performed previously at the NIH within two years prior to signing consent as per section **8.1.2**
[9] not required if already performed elsewhere and records available
[10] only subjects who read English or Spanish will complete the survey

1.3.2 Schedule of Activities (Extended access phase, beginning after 8 months of treatment)

Visit/ Time Point	Monthly[1] (±10 days)	Every 3 months (±30 days)	Every 12 months (±30 days)	Off study[2] (optional)
Clinical Assessments				
Interval medical history (Telehealth for 3-months visits, In-person for 12-month visits)		X	X	X
Physical examination			X	X
PHQ-8 and C-SSRS inventory			X	
Concurrent medication review		X	X	
Transfusion records review		X	X	
Laboratory Evaluations				
Complete blood count with differential	X		X	X
Peripheral blood smear			X	X
Reticulocyte count	X		X	X
Hemoglobin F quantification			X	X
Hepatic panel: alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein			X	X
Mineral panel: phosphorus, magnesium, albumin, calcium			X	X
Acute care panel: Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen			X	X
Pregnancy test (blood or urine HCG in women of childbearing potential)			X	
Iron panel: ferritin, transferrin, % saturation, total serum iron			X	X
Procedures				
Bone marrow aspiration and core biopsy[3]			X	X
Bone marrow chromosomal analysis via standard cytogenetic techniques			X	X
Research Testing and Assessments				
Research Blood			X	X
HRQL survey administration			X	X
Document Drug Accountability			X	

[1] for ***robust*** responders, monthly testing may be spaced to every 3 months (±30 days) as long as a robust response is maintained without dose adjustments
[2] optional evaluation may be conducted 6 months (± 30 days) after subject is off treatment. All noted evaluations are recommended, but not required per subject preference. All subjects will be contacted 30 days (± 5 days) after completion of drug treatment to review any interval adverse events since discontinuing study drug regardless of response or participation in the optional off-study evaluation.
[4] stained for standard morphologic analysis and quantitation of cellularity with hematoxylin and eosin, and special stains to assess iron, reticulin and collagen, primitive stem and progenitor cells via CD34 immunohistochemistry, and other lineage-specific or special stains as indicated to classify any abnormalities

2 INTRODUCTION

2.1 Study Rationale

Diamond-Blackfan anemia is an inherited marrow failure syndrome leading to chronic and potentially severe anemia. Although steroids are effective for treating many patients, over half will be resistant to therapy, and of the responders, many will experience relapse or steroid intolerance. Currently, the only universal therapy is chronic red blood cell transfusions, and the only curative treatment is hematopoietic stem cell transplantation, both of which carry significant risks of morbidity and mortality. Consequently, a new, non-transplant therapy amenable to long-term safety and tolerability is needed for patients with this disorder. Bitopertin is a novel agent that changes the levels of an essential amino acid, glycine, in developing red blood cells, and in doing so can restore the biochemical balances disrupted by DBA-associated genetic changes. Oral bitopertin may be able to stimulate sufficient erythropoiesis in DBA patients to avoid transfusions and thus fulfill a long-standing need in DBA therapy. This study will attempt to determine whether bitopertin is effective and safe in the treatment of DBA.

2.2 Background

2.2.1 Pathophysiology of Diamond-Blackfan Anemia

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

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

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

normal steady-state conditions, the synthesis of the protein components of hemoglobin are exquisitely matched to that of the iron-carrying heme moiety (5). However, the ribosomal defect in the primitive erythroid cells of DBA patients leads to an asynchrony between globin chain translation and heme biosynthesis (6). An excess of free heme thus develops in these primitive erythroid cells, which in turn leads to an accumulation of protein-free iron. The consequence of these imbalances is the production of reactive oxygen species within the sensitive developing erythroid precursors, leading to a block in differentiation and the clinical presentation of pure red cell aplasia. In addition, a major component of DBA therapy is chronic transfusions (see section **2.2.2**), which in time cause whole-body iron loading, and likely exacerbate the iron-mediated oxidative stress in developing erythroid cells, in addition to the other clinical consequences of iron overload, as discussed below.

Although it is only within the erythrocytes that the subtle imbalance in ribosomal biology leads directly to such a profoundly toxic metabolic disruption, it is unlikely that the ribosomal effects of DBA-associated mutations are restricted to the developing erythroid lineages in the bone marrow. Likely similar but more subtle disruptions in protein synthesis in other lineages, especially at critical points in embryogenesis and early development, lead to some of the phenotypic changes and dysmorphisms seen in some patients with DBA, particularly with certain mutations.

Although the primary presentation is isolated anemia, improved supportive care has increased the life expectancy of DBA patients and uncovered progressive defects in other consistent with a long-term hematopoietic stem cell defect (7). This may be a consequence of the cumulative effects of disordered protein synthesis slowly disrupting other pathways, but with less toxic impact than excess erythroid heme. Additionally, there is growing evidence that the effects of heme accumulation and iron toxicity are not confined intracellularly. Erythrocytes cells develop in erythroblastic islands (EI), aggregates of developing erythroid progenitors surrounding a central macrophage. Within the context of close cellular contact, the oxidative stresses can spread from the affected progenitor, first poisoning adjacent progenitors, the associated macrophage, and eventually other primitive cells within the greater hematopoietic niche (8).

The role of heme biosynthesis and oxidative stress has been further supported by work on non-ribosomal DBA. *Feline leukemia virus subgroup C receptor-related protein 1* (FLVCR1), is a transport protein that facilitates the export of free cytoplasmic heme. Its mutation has been linked to a non-canonical form of DBA (9). In *Flvcr1* mutated mice, free heme cannot be efficiently cleared from the cells during hemoglobin synthesis, leading to heme accumulation reminiscent of the RP-mutated forms of DBA, with similar downstream consequences (10, 11). Furthermore, investigations of both FLVCR1 and GATA1 have demonstrated a feedback loop between the two proteins, with the assistance of various chaperones that further regulate heme synthesis, heme efflux, and globin translation, further underscoring the importance of balancing the components of hemoglobin and providing a model by which ribosome biology, cell signaling, and metabolism intersect to produce disease (12, 13).

2.2.2 Clinical Features and Current Treatment Options

Typically, DBA presents as an isolated, macrocytic anemia in early childhood. As it is a bone marrow failure syndrome (BMFS), this anemia is characterized by a lack of marrow precursors and reticulocytes (circulating immature erythrocytes). For the overwhelming majority of patients

(>90%), the hematopoietic defects are noted within the first year of life. The following features make up the diagnostic tetrad that defines DBA:

1. presentation within the first year of life;
2. macrocytic anemia without other significant cytopenias;
3. reticulocytopenia; *and*
4. a paucity of bone marrow erythroid precursors, but with otherwise preserved marrow architecture.

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

In addition to the hematologic changes, DBA is often associated with congenital abnormalities. Whereas *RPS19* patients do not demonstrate consistent patterns of developmental defects (14), considerably higher rates of physical malformations are identified in patients with *RPL5* (83%) and *RPL11* (73%) mutations, with *RPL5* tending towards more severe phenotypes, including cardiac malformations (15). *RPL11* and *RPL5* mutations are strongly associated with hand defects (often thumb) and craniofacial abnormalities (especially cleft lift and palate), respectively (15, 16). Although approximately one-half of *RPS19* patients will have an identifiable congenital abnormality, there is no specific genotype-phenotype correlation, and these abnormalities usually occur in isolation, whereas *RPL5* and *RPL11* patients frequently present with multiple abnormalities.

DBA presents early in life; however, it is a lifetime condition. As supportive care and treatments have improved life expectancy, it has become apparent that DBA is associated with other complications that tend to present later in life. With time, additional, progressive cytopenias may develop (7). The most frequent of these is thrombocytopenia; however, neutropenia has been reported, with some patients progressing to aplastic anemia. Additionally, DBA patients are at increased risk for developing myelodysplastic syndrome (MDS) at a rate over 300-fold higher than expected and acute myeloid leukemia (AML) at an almost 30-fold higher rate than expected, with diagnoses occurring as early as the first or second decades of life for what is typically considered an adult cancer (17, 18). Indeed, when considering hematopoietic stem cell transplant (HSCT) as definitive therapy (see below), all related donors need to be tested for DBA, even if clinical signs and symptoms are absent, as donation from an affected patient carries the risk of engraftment failure, primary graft failure even beyond the peri-transplant period, or subsequent evolution to MDS and/or AML.

DBA is a cancer-predisposition syndrome beyond hematologic malignancies. Patients are at risk for carcinomas and sarcomas, including, but not limited to, gastric and colon cancer, lymphoma, osteogenic sarcoma, and breast cancer (17-19). Yet, like other BMFS, oncologic treatments are often complicated by prolonged cytopenias and other chemotherapeutic toxicities. Cancer surveillance and optimal treatment modifications for these patients remains uncertain.

Effective treatment of DBA is currently limited to three options:

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

The current standard of care for DBA is systemic corticosteroids. Ideally steroid initiation is delayed until after the first year of life to avoid the most serious developmental effects, especially in a population already at risk for altered growth and development. Initial response to steroid therapy is often within weeks of starting therapy, and is observed in 75-80% of patients (19, 20); however, primary refractory disease is seen in approximately one-fifth of patients. Even in responders, relapse or discontinuation of therapy due to side effects is common, and durable, steroid-independent remission is rare. In long-term longitudinal studies of the Diamond-Blackfan Anemia Registry (DBAR), 40% of patients remain steroid-dependent, and 40% are transitioned to chronic transfusion therapy for either steroid intolerance or relapsed/refractory disease. Only one-fifth of patients maintain remission without steroids or HSCT (19). Long-term steroid therapy carries significant side-effects and risks, including growth retardation, metabolic syndrome with hyperglycemia and diabetes, osteopenia complicated by pathologic fractures and avascular necrosis, hypertension, and cataracts.

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

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

transfusions. In addition, endocrine dysfunction can be significant and irreversible, especially in the thyroid, pancreas, and pituitary.

Liver biopsy has historically been used to measure liver iron concentration (LIC), and is generally considered the "gold standard" for assessing total body iron load and is more accurate and reliable than serum ferritin (21-23). However, this is an invasive procedure which carries significant risks of complications and is subject to considerable sampling error (24, 25). Consequently, it has fallen out of favor, especially for routine monitoring of iron loading in asymptomatic patients. With improvements in magnetic resonance imaging (MRI) and analysis, annual MRI surveillance has become the standard of care for any patients on or transitioning to chronic transfusion therapy. The presence of iron alters the signal characteristics of effective T2 (T2*) sequences, with an inversely proportional shortening of relaxation in relation to iron load. This shortening can be compared to established standards obtained via correlation with biopsy measurements to infer LIC. Liver MRI for LIC has proven to be reliable, reproducible, and well correlated with liver biopsy results (26, 27). With increasing access to MRI, even in relatively resource-poor settings, it has become the standard for measuring iron loading instead of liver biopsy (28-30). It also has proven to be a useful tool in the research setting, monitoring the impacts of various treatments upon iron loading (31, 32).

Chelation therapy with deferoxamine was revolutionary in the management of chronic transfusion-dependent anemias, including DBA. It has greatly reduced morbidity and mortality from the underlying disease and complications of transfusions. Newer oral chelation agents (deferiprone and deferasirox) have also proven efficacious, while improving quality of life through ease of administration compared to prolonged intravenous or subcutaneous infusions of deferoxamine. These agents also carry risks, including potentially fatal agranulocytosis associated with deferiprone reported in several DBA cases and leading to an FDA black-box warning label (33, 34). In addition, the chelation agents carry additional risks including such as sensorineural hearing loss and retinopathy, requiring clinical vigilance.

Alternatives to chronic steroids or chronic red cell transfusion therapy have proven elusive. Treatment with various hematopoietic cytokines or non-steroid forms of immunomodulation have not proven effective and have significant side effects. Ultimately, the only curative therapy is allogeneic hematopoietic stem cell transplantation. Although ongoing research into alternative donor transplants, graft-versus-host prophylaxis, and reduced-intensity conditioning have increased availability and reduced the morbidity and mortality of HSCT in DBA, it remains a high-risk, time intensive and expensive procedure, especially in the setting of iron overload seen in DBA. Alternative therapeutic modalities for half of the patients without a durable remission are greatly needed.

2.2.3 Bitopertin

Bitopertin (DISC-1459; RG1678; RO4917838) is an orally bioavailable, selective, small molecule inhibitor of the glycine transporter 1 (GlyT1). Originally developed for its ability to reduce glycine, a key component of neurotransmitter biosynthesis and a neurotransmitter itself, bitopertin has been investigated by R. Hoffman La-Roche in phase II and III clinical trials of obsessive-compulsive disorder (OCD) and schizophrenia (35-40), albeit without efficacy, however with excellent safety and tolerability (see §**2.3.1.1** for summary of trial safety data). Bitopertin is a thousand-fold more selective for GlyT1 over GlyT2 (41, 42).

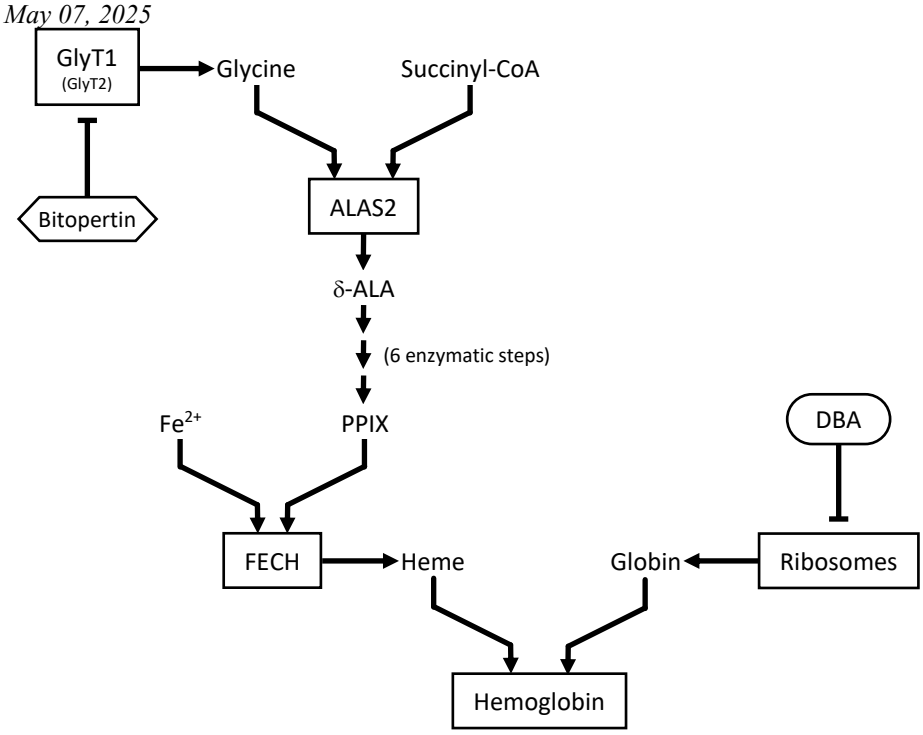


Figure 2: Hemoglobin synthesis is a balance of globin and heme synthesis.
δ-ALA, δ-aminolevulinic acid; ALAS2, aminolevulinic acid synthase 2; FECH, ferrochelatase; PPIX, protoporphyrin IX

Outside of the central nervous system, the expression of GlyT1 is tissue-restricted to erythroid cells, including erythroid precursors and reticulocytes (43-46). Expression is upregulated during erythropoiesis to accommodate the greatly increased glycine requirements imposed upon the developing erythroid cells during hemoglobinization (44, 45). Hemoglobinization, as noted above, consists of two parallel processes: the translation of the globin protein by the ribosome and the synthesis of the non-protein heme moiety (**Figure 2**). Heme synthesis begins with the combination of glycine and succinyl-CoA into δ-aminolevulinic acid (δ-ALA) by aminolevulinic acid synthase 2 (ALAS2). From there, δ-ALA undergoes a series of six enzymatic reactions to ultimately produce protoporphyrin IX (PPIX). PPIX is then complexed with ferrous iron to produce heme (47). Importantly, the initial synthesis of δ-ALA by ALAS2 is the primary rate-limiting step of this entire pathway. Heme synthesis is thus highly dependent upon maintenance of an adequate intracellular glycine pool, which itself is dependent upon timely upregulation of GlyT1. Although GlyT2 is also expressed on developing erythroid cells and throughout the body, its expression is not dynamically regulated. Thus, downstream heme synthesis is highly sensitive to GlyT1 activity specifically, and dependent on GlyT1 upregulation beginning approximately 12-24 hours prior to the start of globin synthesis.

Bitopertin inhibition of GlyT1 leads to a reduction in intracellular red blood cell glycine uptake in a dose-dependent manner in human subjects (BP20078, BP19292, **Figure 3**). By inhibiting GlyT1, bitopertin causes a marked reduction in the rate and overall production of heme within the developing erythroid cell progenitors. Mice lacking GlyT1 develop a microcytic, hypochromic anemia (44). In human trials with bitopertin, inhibition of GlyT1 consistently led to a mild decrease in hemoglobin, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

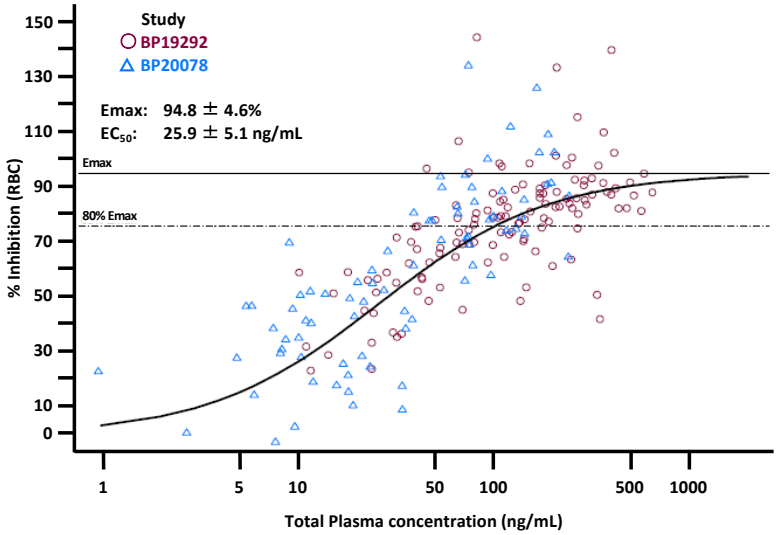


Figure 3: Inhibition of glycine uptake in red blood cells by bitopertin.

Of note, in a 4-month study of healthy volunteers (BP21441, **Figure 4**) there was a dose-dependent decrease in both reticulocyte MCH and MCV over time, plateauing at day 15. This was accompanied by a subsequent, lagging decrease in red blood cell MCH and MCV becoming apparent at day 15 and continuing to decrease through the treatment period. At the end of the treatment period, hemoglobin had changed relative to placebo by –1.34 g/dL (90%-CI: –1.97 to –0.72) and –1.85 g/dL (90%-CI: -2.49 to –1.22) for 30 and 60 mg dose levels respectively. This corresponds to a 9.3% (90%-CI: 5.0% to 13.5%) and 13.0% (90%-CI: 8.7% to 17.0%) relative reduction in hemoglobin for the 30 mg and 60 mg dose levels. Subjects at 10 mg dose level saw only a small (<0.5 g/dL) non-significant reduction in hemoglobin at the primary endpoint.

These results provide insight into the kinetics of the hematologic effects of bitopertin. Although the MCH had not reached a clear plateau at 120 days for each of the dose levels, especially at the highest (60 mg daily) dose, at all three levels, by day 120, the change in MCH from baseline had reached the same change from baseline in the reticulocyte hemoglobin. This delay reflects the 3- to 4-month lifespan of red blood cells, during which the newly formed, bitopertin-inhibited red blood cells replace the old cells. On the other hand, the near-immediate decline in reticulocyte hemoglobin that reaches maximal effect by 15 days (reflecting the approximate 2-week length of erythropoiesis), indicates that at all dose levels, bitopertin has rapid impact on the developing erythrocytes, limited only by the time it takes the previously developing cells to complete their maturation.

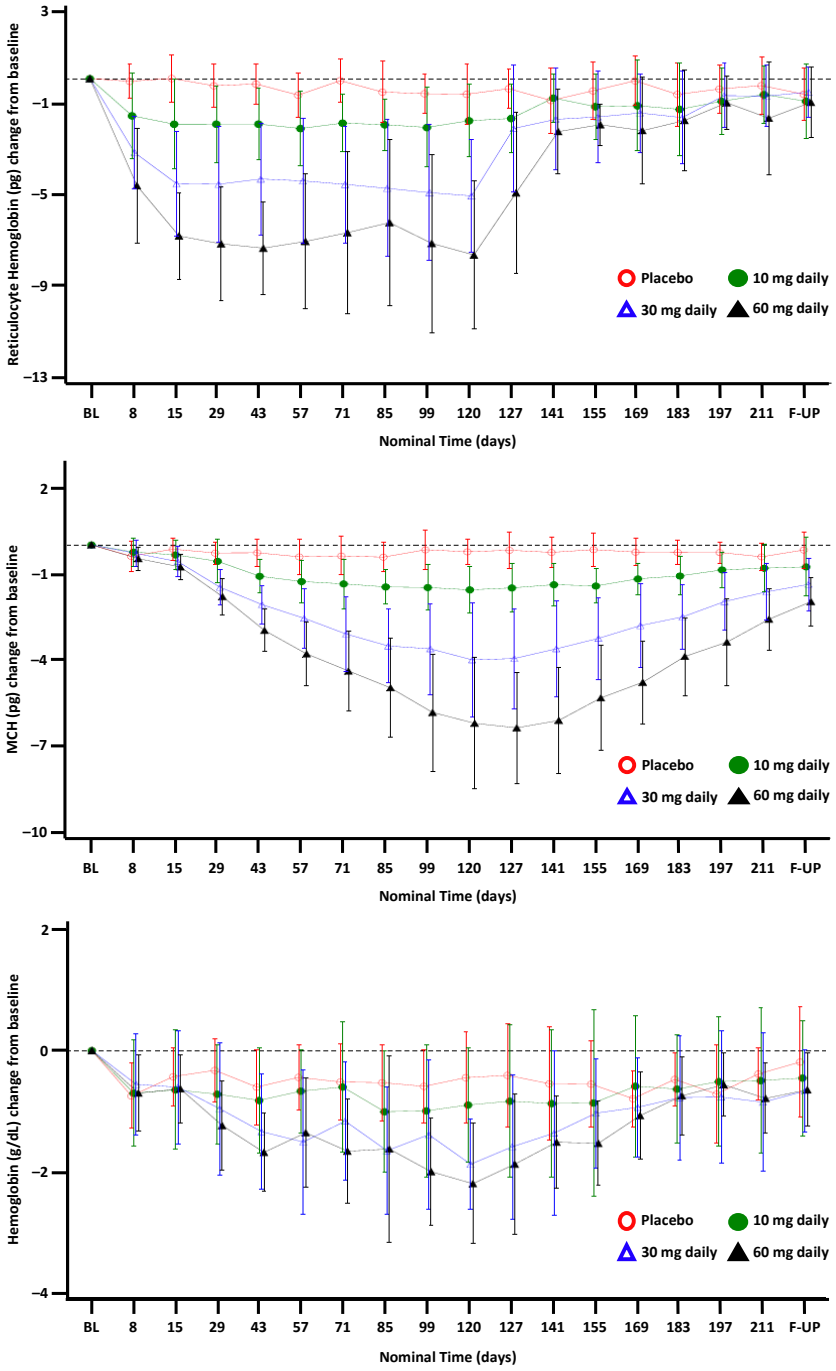


Figure 4: Dose-effect of bitopertin on hematologic parameters in healthy volunteers.
The change from baseline of reticulocyte hemoglobin (top), mean corpuscular hemoglobin (MCH, middle), and total hemoglobin (bottom) over time for patients treated with 120 days of placebo or indicated daily dose of bitopertin.

2.2.4 Rationale for bitopertin in DBA

Based upon the above findings, bitopertin represents an agent with potential clinical benefit for altering the biochemistry of the red blood cell. By targeting the rate limiting step of heme biosynthesis, it can reduce intracellular heme, whose excess relative to globin is hypothesized to be central to DBA pathogenesis. Furthermore, the observations that GlyT1 is predominantly expressed on developing erythroid progenitors and that bitopertin is specific to GlyT1 over GlyT2 suggest that it is possible to specifically target heme biogenesis in the erythroid compartment without significantly affecting glycine-dependent processes throughout the rest of the body. In addition, preclinical data in thalassemia confirm the mechanistic and cellular activity of bitopertin in organismal models, while unpublished data have led to a separate trial for cutaneous erythropoietic porphyria (48, 49). Furthermore, the addition of bitopertin to patient-derived DBA marrow cell cultures improved erythroid cultures and reduced cellular heme content (unpublished data, Janis Abkowitz). The use of bitopertin in a clinical trial for DBA is further supported by the minimal side effect profile of bitopertin in large clinical trials carried out to date. For these reasons, we have chosen bitopertin for further development as a potential therapy for DBA.

2.2.5 Scientific and clinical justification of the protocol

Without treatment, DBA progresses to critical anemia in over four-fifths of all cases. Even with treatment, approximately half of patients will either fail to respond, will not have a durable response, or will be unable to continue therapy due to steroid-associated toxicities. For these patients, chronic transfusions with the risk of cardiovascular, hepatic, endocrinologic, and other systemic organ dysfunction and a major impact on quality of life is the only management option. Chelation therapy is often insufficient to prevent iron overload organ toxicity and is poorly tolerated or associated with poor compliance in many patients, resulting in morbidity and mortality due to iron overload complications. HSCT remains the only definitive treatment for the hematologic complications of DBA. Yet, despite significant advances in preparative regimens, supportive care, and management of complications, HSCT continues to carry significant morbidity and mortality, especially in the heavily pre-transfused population. In addition, many patients do not have a sufficiently-matched related or unrelated donor available. As a result of complications of the disease and a paucity of effective alternative treatments, novel treatments for patients with DBA are acutely needed.

As discussed above (§2.2.1), DBA represents a spectrum of diseases caused by a variety of mutations in at least 21 genes affecting multiple different cellular pathways, but all converging on an excess of iron-containing heme pigment within the erythroid progenitors leading to iron accumulation and subsequently toxic levels of reactive oxygen species. With time, the accumulation of toxic intracellular and extracellular species may cause more general bone marrow failure with progressive cytopenias. Additionally, the chronic transfusions used to treat these patients further increases marrow iron loading, exacerbating the cellular damage in addition to the non-hematologic complications of therapy.

DBA, as also noted above, is a cancer-predisposition syndrome (§2.2.2) including both solid tumors and hematologic malignancies. Although the biology of this predisposition is poorly understood, it likely represents genotoxic stress from the same toxic processes that lead to erythroid aplasia. In the marrow the combined hematopoietic stresses of ineffective hematopoiesis, ongoing cellular damage leading to cytopenias likely driving clonal progression,

oxidative stress of free heme, and increased total body iron burden, especially within the marrow compartment, predisposes to myelodysplasia and full malignant transformation. Although beyond the immediate scope of this trial, we hypothesize that by reducing these combined stresses, bitopertin treatment might reduce the risk of hematopoietic malignancies in DBA. Furthermore, by reducing the need for HSCT, bitopertin may have the secondary clinical benefit of reducing the risk of treatment-related morbidity, malignancies, and mortality.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

2.3.1.1 Risks related to bitopertin

Over 4,000 subjects have received bitopertin in clinical trials including patients with schizophrenia, obsessive compulsive disorder (OCD), and cohorts of healthy volunteers. The safety data presented herein are primarily from healthy volunteers and patients with schizophrenia and OCD (35-40, 49, 50). A 16-week trial of twelve patients with beta thalassemia has also been conducted with a similar safety profile. Data regarding safety of bitopertin in DBA and other hematologic diseases does not exist, but "*major safety concerns are not expected given the safety profile in other populations*" per the manufacturer's experience (51).

In a first-in-human trial of healthy subjects (**NCT01636492**) at single doses up to four times those for this study (240 mg), no major safety concerns were identified.

In trials for patients with schizophrenia and OCD at doses up to 60 mg daily, both in combination (**NCT00616798**, **NCT01192906**, **NCT01192867**, **NCT01235559**, **NCT01235520**) and as a monotherapy (**NCT01234779**, **NCT01674361**), no major safety concerns were identified.

Trials encompassing both phase II (**NCT00616798**, **NCT01674361**), and phase III (**NCT01192867**, **NCT01192880**, **NCT01192906**, **NCT01234779**, **NCT01235520**, **NCT01235559**, **NCT01235585**), including full safety analyses of the phase III studies, no major safety concerns were identified. These trials (at the cut-off dates) included exposure of 2,587 patients with schizophrenia and 100 patients with OCD to bitopertin. The AEs noted during these trials are presented below in tabular form, separated according to phase II (**Table 1**) and phase III (**Table 2**).

2.3.1.1.1 Phase II Findings

In phase II trials, AEs in the nervous system disorders System Organ Class (SOC) were reported in a higher proportion of patients in the bitopertin 60 mg treatment group (31%) than in the placebo and bitopertin 10 mg and 30 mg groups (~12%). This difference was mainly attributable to the AEs of somnolence (placebo 3%, bitopertin 10 mg 7%, 30 mg 5%, 60 mg 10%), dizziness (placebo 3%, bitopertin 10 mg 1%, 30 mg 4%, 60 mg 12%) and headache (placebo 1%, bitopertin 10 mg 2%, 30 mg 2%, 60 mg 9%). Other AEs that occurred with increased frequency with bitopertin dose included nausea, vertigo, and blurred vision.

There were six SAEs during the phase II trials: suicidal ideation (2 patients), anxiety, panic attack, psychotic disorder, and suicide attempt. All six SAEs were in the bitopertin treatment groups and three were considered related to treatment (one in each bitopertin treatment group). No patients died during the study.

During the study, 9% of the patients in the bitopertin 30 mg and 60 mg groups withdrew due to AEs as compared with 1% in the placebo and bitopertin 10 mg groups with psychiatric disorders as the leading cause of discontinuation in the bitopertin 30 mg (1%) and 60 mg (4%) groups.

Table 1: Overview of AEs observed during phase II (NN20372 / NCT00616798)

Total no of patients (%)	**Placebo** ***N* = 80**	**Bitopertin 10 mg** ***N* = 82**	**Bitopertin 30 mg** ***N* = 81**	**Bitopertin 60 mg** ***N* = 78**
AEs [a]	32 (40)	34 (41)	33 (41)	36 (46)
severe [b]	0	2 (2)	2 (2)	7 (9)
related [c]	16 (20)	15 (18)	19 (23)	24 (31)
SAEs [a]	0	1 (1)	2 (2)	3 (4)
related [c]	0	1 (1)	1 (1)	1 (1)
AEs leading to treatment discontinuation	1 (1)	1 (1)	7 (9)	7 (9)
Deaths	0	0	0	0
AEs of special interest				
Suicide related AEs	0	0	1 (1)	2(3)
Dysphoria / depressed mood	0	0	0	1 (1)
Skin disorders of the hands and feet [d]	0	0	0	0
Eye disorders / blurred vision [d]	0	0	2(2)	1 (1)
Hemoglobin decrease	0	0	0	1 (1)

[a] Multiple occurrences of the same AE in an individual are counted once
[b] Multiple occurrences of the same AE in an individual are counted once under the highest reported intensity
[c] Sum of AEs considered remotely, possible, and probably related to treatment. Only the closest relationship to treatment is counted for multiple occurrences of the same AE in one individual.
[d] Identified as an AE of interest based upon AE reported in a 4-month safety study in healthy volunteers

2.3.1.1.1.1 Laboratory Parameters in Phase II

No clinically significant bitopertin-dependent abnormalities in laboratory parameters other than the hematologic effects discussed above (**Figure 4**) were observed. There was an increase in the proportion of patients with hematuria with bitopertin treatment, especially in the 30 mg and 60 mg groups. The maximum increase in proportion was approximately 3-fold (proportion of patients with any assessment in the marked abnormality range: placebo 3%, bitopertin 10 mg 5%, bitopertin 30 mg 10%, bitopertin 60 mg 10%). Hematuria was observed in both genders but with a more than three-fold higher incidence in females than males, suggesting a possible confounding effect of menstruation.

2.3.1.1.1.2 Blood Pressure, Heart Rate, and ECG in Phase II

There was no signal on blood pressure, except for a lower standing systolic BP in the 60 mg group. There were no drug-placebo differences in orthostatic hypotension.

Table 2: Overview of AEs observed during phase III

Study (dose, patients)	Proportion (number) of patients with at least one AE		Proportion (number) of patient withdrawals due to AEs		Proportion (number) of patients with serious AEs		Deaths	
	Bitopertin	Placebo	Bitopertin	Placebo	Bitopertin	Placebo	Bitopertin	Placebo
WN25309 / NCT01192906 5 mg; *n* = 211 10 mg; *n* = 201 placebo; *n* = 209	66.8% (141) 52.7% (126)	75.1% (157)	8.1% (17) 6.0% (12)	11.5% (24)	5.2% (11) 8.0% (16)	7.2% (15)	5 mg - 1 death (cardiorespiratory arrest)	2 deaths (sudden death, homicide)
NN25310 / NCT01192867 10 mg; *n* = 208 20 mg; *n* = 208 placebo; *n* = 210	59.6% (124) 51.9% (108)	58.6% (123)	4.8% (10) 3.8% (8)	12.4% (26)	6.3% (13) 3.4% (7)	8.1% (17)	10 mg - 2 deaths (traffic accident, cardiorespiratory arrest) 20 mg - 2 deaths (myocardial infarction, completed suicide)	1 death (acute poisoning of neuroleptic)
WN25305 / NCT01235559 10 mg; *n* = 198 10 mg; *n* = 199 placebo; *n* = 199	56.1% (111) 56.8% (113)	56.3% (112)	5.6% (11) 7.0% (14)	8.5% (17)	4.0% (8) 3.5% (7)	3.0% (6)	10 mg - 4 deaths (alcohol poisoning, metastatic rectal cancer, duodenal ulcer hemorrhage, suicide)	1 death (bronchopneumonia)
WN25306 / NCT01235585 5 mg; *n* = 195 10 mg; *n* = 200 placebo; *n* = 193	63.1% (123) 63.5% (127)	68.9% (133)	8.7% (17) 9.0% (18)	10.4% (20)	3.6% (7) 3.5% (7)	7.8% (15)	none	2 deaths (myocardial infarction, cardiorespiratory arrest)
WN25308 / NCT01192880 10 mg; *n* = 206 20 mg; *n* = 208 placebo; *n* = 206	51.0% (105) 54.8% (114)	54.9% (113)	4.9% (10) 5.8% (12)	8.3% (17)	1.9% (4) 2.9% (6)	2.9% (6)	20 mg - 2 deaths (2 completed suicides)	none
WN25333 / NCT01234779 10 mg; *n* = 80 30 mg; *n* = 77 placebo; *n* = 80	53.8% (43) 67.5% (52)	58.8% (47)	10.0% (8) 13.0% (10)	5.0% (4)	none 3.9% (3)	none	none	none
NN25307 / NCT01235520 10 mg; *n* = 198 20 mg; *n* = 194 placebo; *n* = 196	52.5% (104) 57.7% (112)	48.0% (94)	8.6% (17) 9.3% (18)	8.7% (17)	6.6% (13) 4.6% (9)	5.1% (10)	10 mg - 1 death (acute renal failure 23 days after study drug discontinuation)	none

2.3.1.1.2 Phase III Findings

No major safety concerns were identified for bitopertin in patients with schizophrenia at doses of 10 to 60 mg/day in combination with antipsychotics in studies **NCT00616798 (NN20372)**, **NCT01192906 (WN25309)**, **NCT01192867 (NN25310)**, **NCT01235559 (WN25305)**, and **NCT01235520 (NN25307)**; and at doses of 10 mg and 30 mg as monotherapy in study **NCT01234779 (WN25333)**. Similarly, no major safety concerns were identified for bitopertin in patients with OCD in combination with SSRIs at doses of 10 to 30 mg in study **NCT01674361 (WN28137)** and profile is consistent with the safety results of the phase III studies in schizophrenia.

Overall, in the phase III studies conducted thus far, bitopertin treatment has not been associated with any significant safety or tolerability issues and did not alter the safety profile of the concomitant antipsychotics tested. Also, the number of patients with at least one AE up to end of treatment was comparable between placebo group and the bitopertin treatment groups and sometimes higher in the placebo group, mostly because of a higher incidence of nervous system disorders and psychiatric disorders. Most of the AEs were considered mild or moderate in severity in all studies. The most frequently affected SOCs were psychiatric disorders, nervous system disorders, infections and infestations, and gastrointestinal disorders. The most frequent AEs reported were headache, nasopharyngitis, somnolence, insomnia, anxiety, and dizziness. Taken together, there were no meaningful differences observed in the incidence of AEs of special interest (suicidality, abuse and withdrawal symptoms, mood changes, visual findings, and skin findings) across treatment groups in the phase III studies.

The incidence of SAEs was similar across the treatment groups, with the most frequent being in the "psychiatric disorders" and the "infections and infestations" System Organ Classes. **Table 2** summarizes the safety of bitopertin versus placebo in the phase III studies up to week 52 including SAE's, number of withdrawals due to AE's and number of deaths. Taken together, the safety profile of bitopertin in the extension and follow-up periods of the phase III trials appears similar across treatment groups as well.

2.3.1.1.2.1 Laboratory Parameters in Phase III

No clinically significant bitopertin-dependent abnormalities in laboratory parameters were observed apart from mild hemoglobin decrease and other hematologic changes noted previously.

2.3.1.1.2.2 Blood Pressure, Heart Rate, and ECG in Phase II

The results of vital signs and ECG assessments did not raise any safety concerns.

2.3.1.1.3 Non-serious adverse reactions

In studies involving healthy volunteers and patients with schizophrenia, the following events were identified as at least provisionally related to bitopertin administration.

Table 3: Summary of identified risks (non-serious) from bitopertin administration

Reaction	Description
Headache	Headache was usually mild in severity and reported by 9.8 % of the patients treated with bitopertin versus 6.7 % in the placebo group
Somnolence	Somnolence was reported more frequently in patients treated with bitopertin (5.2%) than placebo (3.7%)

Dizziness	Dizziness was reported more frequently in patients treated with bitopertin (4.2%) than placebo (3.6%)

As somnolence and dizziness have been reported with the use of bitopertin, such effects may affect tasks requiring alertness including the patient's ability to drive and operate machinery, and caution should be used when starting bitopertin or changing doses.

Other concerns common to experimental treatments are noted below, none of which have been identified in specific association with bitopertin:

- **Liver function tests:** A small number of modest and reversible liver function test elevations were observed in human volunteer studies. In 4 subjects, reversible AST/ALT elevations ≥ 3 ULN occurred: 3 of them started between 10 and 50 days after last treatment intake while one of them resolved during continued treatment. In the studies in patients with schizophrenia, there was no signal of abnormal liver function.
- **Allergic Reactions:** No allergic reactions have been reported in any of the human studies completed so far.
- **Dermatological AEs:** AEs were reported in healthy volunteers during the conduct of BP21441. They affected mostly the hands and feet: dry skin and skin peeling accompanied by somatosensory symptoms. In the Phase III studies in schizophrenia, there were no differences observed in the incidence of such events across treatments groups.
- **Immune system:** In the toxicology studies with bitopertin, there was no evidence of primary systemic effects on the immune system (no changes in WBC or lymphoid tissues). No immunological AEs were observed in Phase I or Phase II studies.
- **Fertility:** There is currently no data suggesting that bitopertin may affect fertility (no effect on reproductive performance and fertility or sperm in the rat, and no effects on organ weight or histopathological findings in sex organs in rat and monkey).
- **Embryo-fetal toxicity:** Bitopertin is not teratogenic; there was no evidence of a dysmorphogenic effect in embryo-fetal toxicity studies in the rat and the rabbit and no effects on reproductive performance and fertility were seen in the rat. During clinical trials adequate contraception must be ensured for all female subjects/patients of child-bearing potential.
- **Pre- and post-natal development:** In dose-range-finding studies on pre- and post-natal development in rats, pups were born alive but a dose-related effect on post-natal survival was observed. The effect is likely related to high exposure of pups at the time of birth and subsequent CNS side effects, preventing the neonate from suckling milk normally. In support of this hypothesis, a wash-out period of 2 days prior to birth is associated with 100% survival in rats.
- **Ophthalmology:** Detailed examinations in the toxicological studies (macroscopic examinations, indirect ophthalmoscopy, slit lamp, and histopathology) did not indicate any morphological effect of bitopertin on the eye. Several healthy volunteers complained of transient blurred vision or other visual AEs of mild intensity, however, no objective changes were found during the different ophthalmological assessments. During the **NCT00616798** study in schizophrenia, a few cases (n = 3) of blurred vision were the only ophthalmologic AEs reported. No visual findings were reported in the bitopertin arms during treatment or follow-up in study **NCT01234779**. There were no differences observed in the incidence of such events across treatment groups.

Although preclinical models do not suggest a risk, there is no clinical data on bitopertin use in pregnant subjects or developing fetuses, an effective contraceptive method is required for all subjects of childbearing potential.

2.3.1.2 Risks 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. For some patients, these procedures may involve the added risk of procedural sedation, if clinically indicated. These risks are viewed as acceptable in order to monitor for adverse effects upon the bone marrow, the primary target of this therapy, and to assess the effects of the therapeutic intervention.

2.3.1.3 Risks Related to ECG

Other than possibly experiencing some minor skin irritation from the electrodes, there are no anticipated risks related to complete the electrocardiogram and/or the echocardiogram.

2.3.1.4 Risks 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.

2.3.1.5 Risks Related to HRQL

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

2.3.1.6 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. The targeted genetic panel we are using for this study includes several genes on the ACMG list of actionable genes as well as genes not on the ACMG gene list that would change the subject's diagnosis. Identification of these genes that are incidental to our research aims, commonly referred to as "incidental findings", may cause emotional stress to the participant. 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 (see **Section 8.2.7, Samples for Genetic/Genomic Testing**).

Risks Related to Insurance or Employment Discrimination: The Genetic Information Nondiscrimination Act of 2008, also referred to as GINA, is a Federal law that prohibits discrimination in health coverage and employment based on genetic information. GINA generally will prohibit discrimination in health coverage and employment on the basis of genetic information. GINA, together with already existing nondiscrimination provisions of the Health Insurance Portability and Accountability Act, generally prohibits health insurers or health plan administrators from requesting or requiring genetic information of an individual or the individual's family members, or using it for decisions regarding coverage, rates, or preexisting conditions. The law also prohibits most employers from using genetic information for hiring, firing, or promotion decisions, and for any decisions regarding terms of employment.

(http://www.genome.gov/Pages/PolicyEthics/GeneticDiscrimination/GINAInfoDoc.pdf).

2.3.2 Known Potential Benefits

Although participating in this trial could lead to a response to bitopertin and an improvement in blood cell numbers with reduction in transfusions, the efficacy of bitopertin in DBA is unknown. As such, there is no known immediate benefit from participation in the trial.

Long-term, participation in the trial may result in an increased understanding of DBA, both from the patient's perspective and as community as whole. Whether bitopertin proves efficacious in the treatment of DBA, it is the hope that knowledge gained from the study will increase our understanding of DBA pathogenesis and if not lead to an immediate treatment, will provide novel pathways to explore development of future treatments.

2.3.3 Assessment of Potential Risks and Benefits

Currently, for DBA patients, steroid treatment with a high rate of relapse and intolerance, chronic transfusions with risks of cardiovascular, hepatic, endocrinologic, and other organ dysfunction, and HSCT are the only management options. If bitopertin is effective, the potential benefits to patients could be improvement of anemia (increased blood cell numbers) and/or reduction or abolition of transfusion requirements, resulting in improved quality of life and decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or susceptibility to infections. Potentially, treatment with other more toxic therapies such as HSCT may also be avoided or at least postponed. In addition, the knowledge and understanding of DBA pathogenesis expected to be gained from the study will allow us and others to explore development of future novel treatments.

In comparison, the clinical data collected from several thousand subjects, including those similar to the DBA subjects of this study, indicate that bitopertin is generally well-tolerated with an acceptable side effect profile. The risks from procedures and testing are comparable to those deemed acceptable in other such studies, and are generally not considered greater than those expected as part of the standard medical care for DBA. As such, the possibility of benefit from bitopertin outweighs the potential risks associated with the study drug and the procedures, even for subjects who ultimately do not directly benefit from the study treatment.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Determine the efficacy of bitopertin in steroid relapsed/refractory Diamond-Blackfan anemia	Efficacy as measured by the response rate and as demonstrated by either: • an increase in pre-transfusion hemoglobin or decrease in transfusion rate (*response*) • transfusion independence (*robust response*) within 8 months of initiating therapy, including a minimum of 3 months at the maximum or maximally tolerated dose (§**9.4.2.1**)	Clinically, the reduction in rate or volume of transfusions can have significant health benefits in chronically transfused patients, as well as a reduction in resource utilization and quality of life. Characteristics of red blood cell production and life span suggests that up to three months at an effective dose will be needed to identify responding patients.
Secondary		
Assess the safety and tolerability of bitopertin in relapse/refractory Diamond-Blackfan anemia	Safety as demonstrated by toxicity profile assessed at 8 months using CTCAE criteria. Tolerability as indicated by the maximally tolerated dose identified during intra-patient dose escalation prior to the primary endpoint.	Despite extensive prior clinical trial data, there is no prior experience regarding bitopertin in the DBA patient population. The possibility of disease-specific side effects needs to be investigated.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Assess the impact of long-term treatment of Diamond-Blackfan anemia with bitopertin as demonstrated through an extended-access phase (up to three years) in responding subjects	On a rolling window basis for up to three years from the primary (8-month) endpoint assess: • new and/or worsening treatment-related toxicities • maintenance of response / relapse rate • clonal evolution • overall survival • health-related quality of life metrics	The effects of long-term bitopertin, especially in Diamond-Blackfan anemia, are unknown. Relapse and/or evolution with subsequent effects on survival and quality of life are known late-term complications identified in treatment studies of other marrow failure diseases.
Tertiary/Exploratory		
Evaluate the impact of bitopertin on stem cell and erythroid cell dynamics in Diamond-Blackfan anemia	Translational and correlative studies of patient samples using specimens collected and banked throughout the duration of subject participation.	To generate new understanding of the pathogenesis of Diamond-Blackfan anemia, the role of heme in pathophysiology and mechanisms of potential activity of bitopertin in improving red blood cell production in DBA, with identification of potential novel pathways for future therapeutic development.
Examine the pharmacology of bitopertin in Diamond-Blackfan anemia	Pharmacokinetic and pharmacodynamic studies during dose escalation phase and at major landmark timepoints.	The pharmacology of bitopertin has not been specifically studied in DBA. This will also allow for correlation of *in vivo* drug activity with response.

4 STUDY DESIGN

4.1 Overall Design

The study is designed as a single-site, non-randomized, single-arm, dose-escalating pilot study of the oral selective GlyT1 inhibitor bitopertin in Diamond-Blackfan anemia (**Figure 1**) to test the hypothesis: ***bitopertin improves the production of red blood cells in Diamond-Blackfan anemia patients.*** Subjects will undergo a 5-month (20-week) dose finding phase with monthly assessments and planned intra-patient dose escalation to identify either a patient-specific maximum tolerated dose (MTD) or minimum effective dose (MED). This will be followed by a 3-month (12-week) maintenance phase. The primary endpoint (see §**9.4.2**) is measured at 8 months (32 weeks ± 14 days). Subjects who cannot tolerate the medication or fail to respond by the primary endpoint will be taken off study drug. Subjects who ***respond*** at or before the primary endpoint (pre-transfusion ("nadir") hemoglobin increase of 1.5 g/dL or more and/or transfusion independence for the 8 weeks preceding response assessment, see §**9.4.2.1**) will be able to continue bitopertin on the extension part of this protocol for up to an additional 3 years. During the 3-year extension phase, subjects will remain on the same dose of bitopertin. The drug may be titrated for toxicity or for waning efficacy up to the subject-specific MTD or the 60 mg daily study maximum, whichever is less (see §**6.1.2.3**).

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

4.2 Scientific Rationale for Study Design

The primary objective of this trial will be to evaluate the efficacy of bitopertin as a single agent in subjects with Diamond-Blackfan anemia, specifically patients with relapsed or steroid-refractory disease, or who cannot tolerate standard therapy with corticosteroids. The secondary objectives of this trial will include assessments of the safety and tolerability of bitopertin, the impact of bitopertin upon rates of relapse and clonal evolution, as well as toxicities of extended therapy and the impact of treatment upon health-related quality of life. Tertiary/exploratory objectives include analysis of the impact of bitopertin upon stem cell and erythroid cell dynamics and iron homeostasis. To achieve these objectives, we have designed an intra-patient dose-escalation phase I/II study.

Given the kinetics of bitopertin in altering hemoglobin concentration in developing red blood cells (see §**2.2.3**), we expect it will take approximately two to four weeks for clinically significant changes in measurable hematologic parameters (reticulocyte hemoglobin, absolute reticulocyte count, MCH) at any given dose level. As our goal is to identify the minimal effective dose (MED), we incorporate monthly dose escalations (see §**6.1.2**), with parameters for holding dose escalation in the event that there is evidence of early efficacy (i.e., increased reticulocyte counts, see §**6.1.2.1** and **Figure 5**) and for loss of efficacy (see §**6.1.2.3.2**).

We have incorporated a three-month "maintenance phase" (weeks 20-32, **Figure 1**) prior to primary endpoint assessments. This time period is to ensure adequate observation once the subjects have reached either the 60 mg daily study maximum dose, the maximum tolerated dose (MTD), or the minimum effective dose (MED). With both the intra-patient dose escalation scheme and during maintenance phase, each subject will be assured of at least four months (16 weeks) at their individualized final target dose. Prior trials of eltrombopag in aplastic anemia, unilineage cytopenias, MDS, Fanconi anemia, Dyskeratosis Congenita, and DBA have indicated

that this is sufficient time to identify responding patients, even if they have not yet reached criteria for a robust response (i.e., remain transfusion-dependent, see below §**9.4.2.1**).

4.3 Justification for Dose

4.3.1 Rationale for starting dose

As noted above (**Figure 4**, see §**2.2.3**), a reduction in reticulocyte hemoglobin, MCH and total hemoglobin was noted at doses as low as 10 mg daily; although this did not become clinically or statistically significant until 30 mg daily. However, reduction of these parameters by bitopertin is only a biomarker for the primary goal of bitopertin therapy in DBA: the reduction in heme synthesis to match the reduced globin synthesis observed in DBA, permitting completion of erythroid cell development. Therefore, it is possible that bitopertin may have effects upon erythroid development even in the absence of significant reduction in hemoglobin content. Indeed, the goal of reducing heme synthesis must be balanced with excess inhibition of erythropoiesis in general, although bitopertin has not been shown to completely block erythroid development due to the co-expression of the GlyT2 transporter and cell-autonomous sources of glycine. For this reason, bitopertin dose escalation will begin at 5 mg daily, lower than the lowest dose shown to cause detectable changes in hemoglobin content in previous trials.

4.3.2 Rationale for dose escalation and range

As the effective doses are not established for hematologic uses of bitopertin, we will perform a dose escalation for each patient (intra-patient dose escalation) to establish the minimal effective dose (MED) of bitopertin for treating DBA. Prior safety data have not identified any significant toxicity of bitopertin, and the 60 mg daily study maximum dose is well below maximum doses used in prior human studies. There are no data or inherent reasons to suggest the DBA patients would have a different side effect profile; however, the safety of bitopertin has not been previously established in DBA. Therefore, the dose escalation will also examine whether there is a maximum tolerated dose (MTD) less than the planned 60 mg daily study maximum.

The planned 60 mg daily study maximum has previously shown to lead to an approximate 25% reduction in reticulocyte hemoglobin. Ascending dose studies (BP20281, **NCT01613040**) in healthy volunteers demonstrated tolerance of doses approximately 3 times this dose (180 mg and 175 mg, respectively). However, despite these significantly higher doses, hematologic effects were not markedly greater than those seen in studies with 60 mg daily doses, suggesting a saturating dose effect. Indeed, in pharmacologic studies (BP21441, **NCT01613040**), both 60 mg and 175 mg daily dosing produced steady-state plasma drug levels no less than four-fold higher (≥442 ng/mL) than the maximum inhibitory dose (95 ng/mL) for GlyT1 inhibition identified in pharmacodynamic studies (**NCT01636492** and BP20078, **Figure 3**). Based upon this, and to minimize the risk of side effects, we have selected a maximum study dose of 60 mg daily.

4.4 Rationale for treatment duration (8-month primary endpoint), extended access to bitopertin for responding patients

The average lifespan of red blood cells is approximately 3 months, and the time between initiation of erythroid commitment from hematopoietic stem cells until final release of anucleate red blood cells into the circulation is several weeks. Therefore, to ensure adequate time for response, including complete turnover of the red blood cell compartment, we have selected an 8-month (32-week) primary endpoint. As all subjects will have reached the 60 mg dose level (or MED/MTD) no later than 16 weeks after starting bitopertin, this will ensure that all subjects will

receive a minimum of 4 months (16 weeks) at the target dose. Although some subjects may reach an MED or MTD before the 4-month (16-week) point, for the purpose of standardized analysis, the primary endpoint will be fixed for all subjects at 32 weeks from the start of bitopertin. However, to account for subjects reaching MED/MTD before 4 months, and therefore receiving a maximum for different lengths of time, we will also perform a secondary analyses examining response after 4 months (16-weeks) from reaching maximum dose and time-to-first-response.

Assuming no increased rates of spontaneous somatic reversion of inherited mutations, an extremely rare but reported event in DBA (52-55), hematopoiesis in patients with DBA will continue to rely on stem cells with the underlying genetic defect. Therefore, it is likely that patients with DBA will require continuing bitopertin treatments to improve their anemia. To examine this, and to establish the safety of extended use of bitopertin, will we provide all patients with a response (robust or not) at the primary endpoint the option of enrolling on extended access for up to an additional three years of bitopertin treatment. As the intent is to develop an alternative to chronic transfusion therapy, patients who do not achieve at least transfusion independence by the end of the first year of extension will be taken off study. 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.

5 STUDY POPULATION

5.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. ***Diamond-Blackfan anemia*** defined as chronic anemia presenting on or before the third year of life, associated with reticulocytopenia and greatly reduced or absent bone marrow erythroid precursors, supported by, but *not* requiring either:
    a. familial history
    b. gene mutation testing demonstrating a known disease-causing mutation or a mutation of disease-associated gene in combination with clinical characteristics of DBA

    Patients with late-onset DBA (diagnosed after the third year of life) may also be included if (but only if) gene mutation testing confirms a disease-causing mutation, as above (see section **8.1.2** for acceptable testing).
2. ***Clinically-significant anemia*** as defined as either:
    a. hemoglobin less than 9.0 g/dL
    b. red cell transfusion of at least 2 units PRBC for adults in the eight weeks prior to study enrollment
3. Relapsed and/or steroid-refractory disease or patient intolerance of systemic corticosteroids
4. Age ≥ 18 years at the time of consent

Although all patients without a molecular diagnosis (i.e., genetic testing positive for a disease-causing lesion) will undergo targeted gene panel testing for mutations in all known genes associated with DBA , the diagnosis of DBA is a clinical one, and as such, consent and enrollment does not require results from these genetic tests in the absence of any features that would suggest an alternative diagnosis (e.g., Fanconi Anemia, Dyskeratosis Congenita, etc.).

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Treatment with androgens (danazol or oxymetholone) or corticosteroids less than 4 weeks prior to initiating bitopertin.
    - Stable physiologic dose steroid replacement for adrenal insufficiency or other similar conditions is not an exclusion criterium.
2. Hypersensitivity to bitopertin or its components
3. Patient Health Questionnaire-8 (PHQ-8) score greater than or equal to 10 or imminent suicidal risk identified by the Columbia-Suicide Severity Rating Scale (C-SSRS) as defined as suicidal ideation with intent (grade 4 or 5) within the last year or any suicidal behavior within the last five years
4. Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy
5. Life expectancy of less than 3 months from any cause
6. 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 ECG, other clinically significant cardio-vascular disease (e.g., uncontrolled hypertension, history of labile hypertension), history of known structural abnormalities (e.g. cardiomyopathy).
7. Known active or uncontrolled infections not adequately responding to appropriate therapy.
    - Note, HIV infection is not exclusive of trial participation if the infection is effectively controlled with medications not known to interfere with bitopertin metabolism or be metabolized by pathways known to be altered by bitopertin. HIV RNA viral load must be undetectable at the time of enrollment, and CD4 cell count must be ≥ 200/μL. Patients must remain on antiretroviral therapy throughout study participation and must be periodically monitored for suppression of viral load and CD4 cell count.
    - If drug-drug interactions between antiretroviral (e.g. HIV), antiviral (e.g., hepatitis), or antifungal medications and bitopertin are suspected (such as lopinavir, ritonavir or other strong CYP3A4 inhibitors, see section **6.5.3**), these must be addressed by a qualified clinical pharmacist or pharmacologist, and any

changes to antiretroviral therapy need to be approved in consultation with an Infectious Disease and/or HIV specialist prior to enrollment.

8. Evidence for MDS or AML as defined by WHO criteria, or any active malignancy or likelihood of recurrence of malignancies within 12 months
9. Patients who have received chemotherapeutic treatment or other specific antineoplastic drugs or radiation therapy within 6 months of study entry
10. Female subjects who are nursing or pregnant (positive serum or urine β-human chorionic gonadotrophin (β-hCG) pregnancy test) at screening/baseline visit
11. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, not using highly effective methods of contraception during dosing and for 30 days after the last dose of bitopertin. 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 childbearing potential if they have had over 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile age appropriate (e.g., generally 40-59 years), history of vasomotor symptoms (e.g., hot flushes) in the absence of other medical justification or have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment should she be considered not of childbearing potential.
        - Sexually active males unless they use a condom during intercourse while taking the study treatment and for 30 days after stopping study treatment and should not father a child in this period. A condom is required to be used also by vasectomized men as well as during intercourse with a male partner in order to prevent delivery of the drug via seminal fluid.
12. Active alcohol/drug abuse
13. Unable to understand the investigational nature of the study or give informed consent and does not have a legally authorized representative or surrogate that can provide informed consent

14. Unable to take the oral study drug
15. Concurrent participation in an investigational study within 30 days prior to enrollment or within 5-half-lives of the study drug, whichever is longer. Note: parallel enrollment in a disease registry is permitted.

5.3 Inclusion of Vulnerable Participants

This research provides the prospect of direct benefit; therefore, inclusion of vulnerable patients may be justified as per below. The benefits to the participants could be improvement of anemia resulting in improved quality of life and 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.

5.3.1 Adult subjects who lack capacity to consent to research participation

Given the rareness of this disease, the possibility of benefit, and the desire to consider any eligible subjects, adults with permanent decisional impairments may be enrolled. There are no known additional risks specific to this group that would not apply to other groups of participants, and not allowing participants who cannot provide consent would deny them these potential benefits this protocol offers. A Legally Authorized Representative (LAR) shall provide consent. All requirements of NIH HRPP Policy 403 shall be complied with. Adults with decisional impairments that are not deemed permanent shall not be enrolled until those impairments have resolved and the subject can again provide their own consent, or such impairment is now considered permanent. Subjects with capacity having been consented to the research who have a loss of capacity may continue to participate in the trial if it is deemed safe to do so by the PI and Medically Accountable Investigator. If such loss is considered temporary (i.e., they are expected to regain capacity), reconsent of the subject by the LAR is not required; however, the LAR should be engaged to advocate on behalf of the subject during the period of incapacity. If the loss is considered permanent, then the subject shall be reconsented through the LAR.

5.3.2 Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study if they meet the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* will be made available. Please see section **10.1.3** for consent of NIH Staff.

5.4 Inclusion of Pregnant Women, fetuses or neonates

Women who are pregnant, are attempting to become pregnant, plan to become pregnant within the treatment period (3 years, 8 months from drug initiation), or are of childbearing potential but cannot use effective contraceptive methods will not be included in this trial (see sections **5.2**, **7.2.1**, and **8.4.10** for discussion of subjects who become pregnant).

Fetuses and neonates will not be included in this trial.

5.5 Lifestyle Considerations

Specific lifestyle and/or diet changes are not required for participation in this study. Alcohol consumption is allowed on this study except in the case of alcohol abuse as noted above (see **Section 5.2, Exclusion Criteria**).

5.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but do not subsequently begin study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a disqualifying laboratory result may be rescreened. Rescreened participants will be assigned a new unique participant number.

5.7 Strategies for Recruitment and Retention

5.7.1 Recruitment goals

As discussed in Section 9.2, Sample Size Determination, we plan to enroll 25 eligible patients in a two-stage process: first, 15 patients in stage 1 and then 10 patients in stage 2 if response rates meet the predetermined continuation thresholds. Based upon experience with this patient population, we expect to recruit 30 patients (18 in stage 1, 12 in stage 2) to accommodate for screening fails. No gender, race, ethnicity, or age (apart from those defined by inclusion criteria, section 5.1) will be specifically targeted, and all recruitment efforts will be designed to target as broad and balanced audience as reasonably possible.

With an incidence of approximately 1 in 100,000-200,000 live births (19), and an expectation of approximately 25-35 new diagnoses a year in the USA, of which 15-20 will likely prove refractory to treatment, and in conjunction with recruitment through patient networks and ongoing natural history studies at the NIH, accrual is projected to take 2.5-3 years. We recently successfully completed enrollment of 15 subjects in a trial of an oral drug for DBA over a 15-month time frame.

5.7.2 Recruitment efforts

The study may use the following strategies of recruitment including:

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

as the NIH recruitment listserv.

- Recruitment post cards may be developed and printed for use in NIH and partnering specialists as well as community use.

The DIR Patient Recruitment Office (PRO) will work with study investigators to ensure accrual goals are being met. Biannual reviews of accrual, at a minimum will be performed by the PI and the PRO. If recruitment goals are lagging, an enhanced plan of recruitment will be triggered, which may include a communication blitz to known and newly contacted hematologists and oncologists throughout the country via email correspondence additional Twitter feeds and chats, Facebook posts and recirculation of updated and/or new patient recruitment flyers/cards. All recruitment materials and tools that will be used to recruit potential subjects will be IRB-approved.

5.7.3 Costs

Study drug will be provided to patients for no cost. All procedures (study-mandated and/or standard-of-care clinical) performed at the National Institutes of Health Clinical Center will be at no cost to participants. If testing beyond what is routinely required for clinical monitoring needs to be done outside of the NIH (at home) for the protocol, then NIH may arrange to have this done free of charge.

5.7.4 Compensation

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 and NIH HRPP Policy 302, and (for NIH staff participants) NIH HRPP Policy 404. Broadly, the NIH will cover the cost of long-distance domestic (within the US) travel with some restrictions and certain expenses for local travel. International sections of travel are generally not covered. The NIH will cover lodging.

Subjects will not be otherwise compensated for their participation in this study.

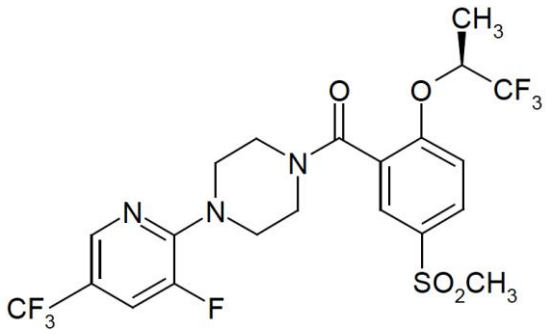
6 STUDY INTERVENTION

6.1 Study Interventions(s) Administration

6.1.1 Physical and Chemical Properties

Generic Name	Bitopertin
Code Number	RO4917838
Chemical Name	(S)-[4-(3-Fluoro-5-trifluoromethyl-pyridin-2-yl)-piperazin-1-yl]-[5-methanesulfonyl-2-(2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-methanone Also known as: [4-(3-Fluoro-5-trifluoromethyl-pyridin-2-yl)-piperazin-1-yl]-[5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-methanone

Chemical Structure



Molecular Formula	$C_{21}H_{20}F_7N_3O_4S$
Molecular Weight	543.46 g. mol^{-1}
Description	Bitopertin is a white, fine powder or fine powder with lumps.
	Three crystalline forms of bitopertin have been identified to date. The form, which is thermodynamically the most stable at room temperature, Form A, was chosen for further development.
Physicochemical properties	Mp: approx. 143°C
	Not hygroscopic
	Partition coefficient: Log D (at pH 7.4) = 3.03
	pKa (of the corresponding protonated species) < 2
Solubility	Bitopertin exhibits a low solubility in water at 25°C: < 0.001 mg/mL (pH 1.0 – pH 9.0) or non-polar organic solvents, e.g., n-heptane (0.1 mg/mL).
	Higher solubility can be reached in polar organic solvents: e.g., ethanol (35.2 mg/mL), ethylacetate (328.6 mg/mL), and acetonitrile (495.6 mg/mL).
Stability	Stability studies showed that bitopertin is very stable in solid state against heat and light. In solution bitopertin is stable in a wide pH range (3-11) and under oxidative conditions but slightly sensitive against light in solution.
	The drug substance is stable when stored under the recommended storage conditions “Do not store above 25°C”.

6.1.2 Dosing and Administration

All patients will begin at a fixed dose of 5 mg bitopertin orally once daily and escalate on a fixed schedule, with adjustments to escalation as outlined below. Once a predefined or a maximally tolerated dose (MTD) is reached, the patient shall continue this dose through maintenance (weeks 20-32) and extended access (if response is observed), unless a dose adjustment is indicated as below.

6.1.2.1 Dose Escalation

Escalation shall be monthly (every 4 weeks, ± 10 days) up to a maximum dose of 60 mg bitopertin orally once daily. With the following dose intervals (see **Figure 1**):

Table 4: Dose escalation

Level	Dose (once daily)	Timing
1	5 mg	0 – 3.9 weeks (month 1)
2	10 mg	4 – 7.9 weeks (month 2)
3	20 mg	8 – 11.9 weeks (month 3)
4	40 mg	12 – 15.9 weeks (month 4)
5	60 mg	16 – 19.9 weeks (month 5)

At each monthly (4 week) interval, a complete blood count (CBC) with differential and a reticulocyte analysis (including reticulocyte hemoglobin) will be collected prior to taking the higher dose. Ideally, this evaluation will be performed sufficiently in advance such that the results will be available prior to escalation; however, in the absence of clinically significant toxicities, the subject will escalate the dose as planned.

If the absolute reticulocyte count (ARC) is 60,000 / µL or higher, the subject will hold at the current dose level (or return to that level if escalation has already occurred) for an additional 4 weeks. If, after 8 weeks at that dose, response criteria are met (see section **9.4.2.1, Response Definitions**) this will be considered the minimum effective dose (MED) and shall be the treatment dose throughout the remainder of the study unless modifications are indicated (as per section **6.1.2.3**). If response criteria are not met, the subject will then escalate to the next dose level as previously planned (**Figure 5**). De-escalation/reduction for toxicity, loss of efficacy, etc. are discussed below (section **6.1.2.3**).

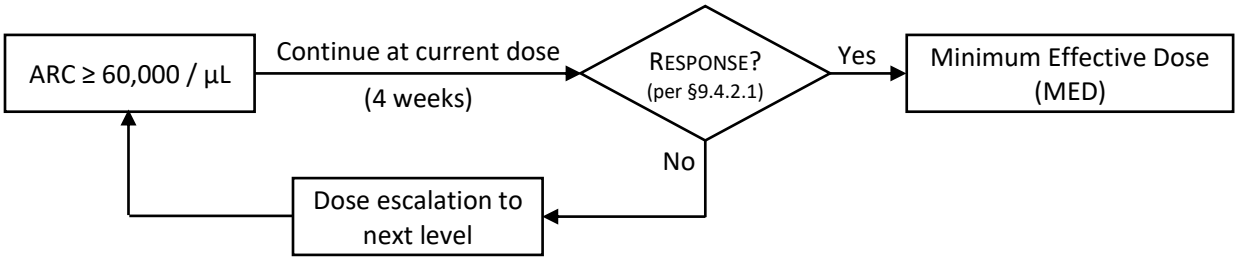


Figure 5: Dose escalation schema for reticulocytosis

6.1.2.2 Dose Limiting Toxicity (Study-wide Maximum Tolerated Dose)

No specific dose limiting toxicities have been identified for bitopertin (see **Section 2.3.1.1, Risks Related to Bitopertin** for reported side effects and risks). For this trial, any one occurrence of the stopping rule criteria listed in section **9.4.4.1** will be considered a dose limiting toxicity (DLT), and the dose level lower will be considered the MTD and the new study maximum (in place of 60 mg daily). In the event that a DLT is identified, any subjects at a higher dose will be immediately reduced to the new study maximum. Titration for the remaining subjects to this new maximum will continue as above, including the identification of subject-specific MTDs (see below). The primary endpoint will remain 8 months (see section **9.4** for statistical considerations, including section **9.4.3** for patients reaching maximum dose prior to the pre-planned 20 weeks).

6.1.2.3 Dose Modifications

Table 5: Dose levels

Level	Dose
–1	5 mg every other day
1	5 mg daily
2	10 mg daily
3	20 mg daily
4	40 mg daily
5	60 mg daily

Dose changes should not be more than one dose level up (see **Table 5: Dose levels**) unless otherwise specified. Reductions will generally be by one dose level, but may be more if it is the judgement of the PI that such a reduction is in the best clinical interests of the subject (i.e., resuming treatment after holding for severe toxicities).

For subjects at dose level 1 (5 mg daily) meeting criteria for dose reduction, a non-standard dose level ("–1") consisting of 5 mg every other day may be attempted before removing the subject for intolerance.

For non-hematologic toxicities, the toxicity will be classified (see section 8.4.4 for grading and attribution), and the following actions will be taken accordingly:

- **Grade 2** toxicities will be monitored. If ***probably*** or ***definitely*** related to bitopertin, do not escalate dose until resolved. If the toxicity is a laboratory abnormality, recheck in 7-10 days until resolved. If not resolved within 1 month, the dose will be considered the subject's maximum tolerated dose (MTD) unless the event can clearly be determined to be unrelated to the study drug.
- **Grade 3** toxicities will result in immediate cessation of drug until resolved unless the event can clearly be determined to be unrelated to the study drug. Once resolved, restart bitopertin at the previously tolerated drug level, and attempt reescalation if tolerated. If not resolved within one month, or reescalation is unsuccessful, the prior tolerated dose will be the subject's MTD.
- **Grade 4** laboratory abnormalities that are expected to resolve will result in immediate cessation of drug until resolved unless the event can clearly be determined to be unrelated to the study drug. Monitor every 7-10 days until resolved. Once resolved, restart at the previously tolerated dose level. Do not attempt reescalation. The prior tolerated dose will be the MTD.
- Any other **Grade 4** toxicities including laboratory abnormalities ***not*** expected to resolve, will result in immediate cessation of drug unless the event can clearly be determined to be unrelated to the study drug. The subject will be monitored until resolved (or deemed stable, if not expected to resolve), at which time the subject will be removed from the study.

See **Section 8.4, Adverse Events and Serious Adverse Events** for the recording, management, and reporting of adverse events.

See below (section 6.1.2.3) for management of hematologic toxicities.

6.1.2.3.1 Loss of Efficacy

If response is lost (see **Section 9.4.2.1, Response Definitions**) within a month of a dose reduction, but in the absence of toxicity, the dose shall be increased back to the prior effective dose. This dose will be continued for at least 4 weeks.

If response is lost on a previously stable dose, the dose shall be increased to the next dose level, provided that the subject is not already at the maximum dose level (60 mg daily) or at the previously identified MTD for that subject.

6.1.2.3.2 Hematologic Toxicity (Loss of Efficacy) After a Dose Increase

For subjects who have previously responded (see **Section 9.4.2.1, Response Definitions)** or those with an ARC greater than 60,000 / μL, hematologic toxicity is defined by the following criteria:

- a decline of ARC by 50%or more, *and*
- an ARC less than 60,000 / μL.

For subjects who have not responded, hematologic toxicity may be considered if there is either:

- a decline of ARC by 50% or more, *or*
- a return of ARC to baseline levels after experiencing an increase of at least 50% from baseline.

In non-responding patient, hematologic toxicity may be subtle, especially in cases where ARC declines after only a 50% increase. In such situations, dose de-escalation will be at the discretion of the principal investigator. However, it should be considered if there is also a concomitant rise in the immature reticulocyte count by 50% from baseline, and it should be ***strongly*** considered if the immature reticulocyte count has increased by 100% (doubled) or more from baseline.

In the event of hematologic toxicity (or if response is lost) within a month of a dose escalation, the dose shall be decreased back to the prior effective dose. This dose will be continued for at least 4 weeks.

6.1.2.4 Drug Administration

Bitopertin shall be taken once daily. The time shall be chosen by subject so as to be convenient but should be maintained throughout study participation within reasonable limits.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Drug will be shipped from the supplier (Disc Medicine) to the NIH Investigational Pharmacy. A complete supply for the initial dose escalation (weeks 0-20) will be dispensed by the pharmacy either directly to the patient or via a postal service (e.g., USPS, FedEx) via overnight delivery. Once subject has reached the maintenance dose, the drug will be dispensed in amounts sufficient for up to a 3-month supply (unless prior authorization has been received for larger dispenses on a per-subject basis). At interval in-person evaluations, subjects will return unopened containers and unused tablets for drug accounting and, if needed (end-of-study), disposal. Patients will be provided drug diaries to record dose and administration during the first 8 months (up to primary endpoint) of study participation. This diary will be reviewed at telehealth and in-person NIH visits (see sections 1.3.1 and 8.2). Virtual accounting will also be performed at refills (at the start

of maintenance and every three months during extension when not concurrent with an in-person clinical visit).

6.2.2 Formulation, Appearance, Packaging, and Labeling

The clinical formulation used in the initial Phase I studies and the Phase II study in schizophrenia (NN20372) was a hard gelatin capsule for oral administration. The capsule fill consists of bitopertin and the inactive ingredients lactose monohydrate, maize starch, sodium starch glycolate, povidone K 30, talc, and magnesium stearate.

In a relative bioavailability study, single oral doses of a 30 mg tablet (F10) vs. a 25 mg capsule (F06) and of a 10 mg tablet (F09) vs. a 10 mg capsule (F03) of bitopertin, exhibited similar dose-normalized exposures (90% confidence interval [CI] of $AUC_{0-\infty}$ and C_{max} within standard bioequivalence range of 80% − 125%) allowing a switch from the capsule to the film-coated tablet formulation [1031729].

For the clinical Phase III studies in schizophrenia and all other clinical studies performed in parallel to Phase III including the OCD study, film-coated tablets (with imprint [engraving] “Roche”) for oral administration were used.

The tablets are manufactured using wet granulation, blending, and tablet compression. They consist of bitopertin and the inactive ingredients lactose monohydrate, maize starch, croscarmellose sodium, microcrystalline cellulose, povidone K 30, talc and magnesium stearate.

The film coated tablets are available at the dosage strengths of 5 mg, 10 mg, and 30 mg of bitopertin. The formulations contain the same excipients with a common total tablet weight.

The tablet film-coat for the investigational drug is composed of a mixture of polyvinyl alcohol (partially hydrolyzed), titanium dioxide, macrogol 3350, talc, and iron oxide yellow. All excipients used in the formulations are compendial (USP/NF/Ph. Eur. and/or JP/JPE) grade. Film-coated tablets of bitopertin should be stored under the recommended storage conditions “Do not store above 30 °C”. For batch specific instructions and information on the shelf-life, see the packaging.

6.2.3 Product Storage and Stability

The drug should be stored under the recommended storage conditions on the packaging.

6.2.4 Preparation

Special preparation is not required. The drug is supplied ready-to-use.

6.3 Measures to Minimize Bias: Randomization and Blinding

Not applicable to this study

6.4 Study Intervention Compliance

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 Disc Medicine. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the Sponsor and/or Disc Medicine at the end of the study after drug accountability is performed. Patient inventories will

be obtained at each NIH CC visit after drug initiation and recorded on the appropriate Case Report Form (CRF). As noted above (section **6.2.1**), patients will be provided a diary to review at intervals during the primary endpoint to ensure correct dose and compliance with administration.

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and supplements.

All medications (prescription, over-the-counter, and supplemental) will be recorded at the initial visit and reviewed at the 8-month primary endpoint visit and any subsequent visits during extension. Any medication changes will also be noted at all telehealth visits during dose escalation and as needed, recorded on the appropriate CRF.

6.5.1 Permitted supportive care

- Transfusion supportive care (e.g., blood and platelets) as clinically indicated.
- Iron chelation therapy (e.g., deferasirox, deferoxamine, deferiprone) ongoing at the time of enrollment will be continued, but must be at a stable dose prior to study drug initiation. Weight-based adjustments, decreases for response, and/or discontinuing according to established standards of care will be allowed.
- 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 bitopertin.

6.5.2 Non-permitted medications

- Romiplostim (N-Plate), eltrombopag (Promacta), hetrombopag (Hengqu), avatrombopag (Doptelet), or any hematopoietic or erythropoietic stimulating agents including, but not limited to, epoetin alfa (Epogen, Procrit), epoetin beta, or darbepoetin (Aranesp).
- Disease (DBA)-targeted medications within 4 weeks prior to starting bitopertin
- IL-11 (Neumega)
- Oral or intravenous iron or iron-containing supplements
- Androgens (danazol and oxymetholone) within 4 weeks prior to starting bitopertin.
- Corticosteroids within 4 weeks prior to starting bitopertin, except at physiologic replacement dosing for adrenal insufficiency
- New iron chelation therapy should not be initiated during trial participation.
- 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 bitopertin 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 bitopertin is discontinued, unless in opinion of the Investigator and Sponsor, the medication will not interfere with the study treatment.

6.5.3 CYP3A4 Inhibitors and Inducers

Strong CYP3A4 inhibitors and inducers are not to be administered concomitantly with bitopertin. This includes, but is not limited to, ketoconazole, carbamazepine, clarithromycin, itraconazole, and lopinavir/ritonavir. Moderate inhibitors such as erythromycin are not contraindicated but should be avoided, if possible.

Note, several HIV and viral hepatitis medications are strong CYP3A4 inhibitors, and as such, any potential subjects on them will require consultation with a clinical pharmacologist. Any changes in treatment will only be done in consultation with an Infectious Disease specialist.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation from bitopertin ("off-drug") does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. The exception is for patients who are "off-drug" with the intention to come "off study" (see below) after a monitoring period, in which case only those study-indicated procedures and evaluations necessary to monitor ongoing AEs or to ensure patient safety need to be conducted. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE). However, if discontinuation of bitopertin reaches off-study criteria (see 7.2.1), the patient will be removed from the study.

Participants are free to withdraw from participation in the study at any time upon request.

7.2 Participant Discontinuation/Withdrawal from the Study

For patients discontinuing study participation ("off study"), data will be collected appropriate for relevant timepoint. Patients will be offered the option for the 6-month (± 30 day) follow-up evaluation. See sections **1.3** (SOA) and **8.2.5** for the appropriate off-study evaluations. Of note, these evaluations are per subject preference, and refusal of one or more evaluations and/or procedures shall be noted, but not constitute a protocol deviation.

7.2.1 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 bitopertin 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, bitopertin 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 bitopertin. We will offer patients a follow-up evaluation at the NIH 6 months following bitopertin discontinuation either due to lack of response, toxicity, or any other criteria for coming off study including patient choice, 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 bitopertin, 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 bitopertin not resolved by dose reduction
- Life threatening acute hypersensitivity reaction
- New or worsening bone marrow morphological abnormalities or cytopenia(s) indicative of transformation to MDS or AML
- Any Grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities or hematologic toxicities (see sections **2.3.1.1** and **8.4.1**) unless the event can clearly be determined to be unrelated to the study drug
- Significant progression of disease such as development of a solid tumor while on bitopertin, or a concomitant condition that would make the subject ineligible for further protocol participation
- Pregnancy or unwillingness to use acceptable forms of contraception
    - Pregnancy shall be reported as below (see section **8.4.10**), and the subject will remain on study solely for the purpose of monitoring the course of the pregnancy until either the conclusion of the pregnancy or the subject is enrolled in a separate monitoring protocol. No interventions, protocol procedures, or further research testing shall be performed except as deemed necessary for good clinical care.
- Initiation of non-protocol therapy for Diamond-Blackfan anemia
- Subject non-compliance
- Study completion

Failure to achieve and maintain transfusion-independence during extension: Subjects who experience a response at the primary 8-month endpoint but remain transfusion-dependent will be offered the opportunity to continue onto the extension phase (section **8.2.4**). If the subject has not achieved transfusion independence by the first annual evaluation during extension, they will be taken off drug, and undergo off-study evaluations as described below (section **8.2.5**). Subjects who become transfusion dependent during extension will undergo dose adjustments as above (section **6.1.2.3**), and will be similarly removed from drug if transfusion independence is not recovered within 12 months of the loss of efficacy. Subjects receive transfusions for surgical or emergent (i.e., trauma) indications will not be considered transfusion dependent if the episode is self-limited.

Loss of response during extension: Subjects who lose response during extension will undergo dose adjustments as above (section **6.1.2.3**). If the dose is titrated to the subject-specific MTD or 60 mg daily study maximum (whichever is less) and a response (robust or non) is not observed after four weeks at that dose, the subject will be taken off drug, and undergo off-study evaluations as described below (section **8.2.5**). As above, transfusion independence must also be recovered within 12 months of the initial loss of efficacy.

We will offer any subject going off-study during extension an optional follow-up evaluation at the NIH 6 months following bitopertin discontinuation, as described below (section **8.2.5**). Regardless of whether they return, we will contact all subjects or their primary hematologist 30 days (± 5 days) following their final dose of bitopertin for information on their current status and to review any intervening adverse events.

Screen failure: An unidentified, pre-existing, disqualifying condition is recognized after enrollment, or genetic testing ultimately identifies an alternative diagnosis from DBA.

Death: Death of subject

The reason for participant discontinuation or withdrawal from the study will be recorded on the appropriate CRF. Subjects who signed consent and began treatment with bitopertin but withdrew will not be replaced, except for patients who withdrew due to a screening failure or due to death before the primary endpoint not related to the study drug. In the case of early withdrawal (prior to the primary endpoint) who will not be replaced, a response assessment will be attempted (with a default of "non-response" if unable to assess) at the time of withdrawal and will be included in the study analyses as appropriate. In the case of patients who withdrew due to a screening failure or due to death before the primary endpoint not related to the study drug, their records and biospecimens will be retained per policy (per procedures laid out in section **10**), but they will not be included in analyses of the study outcome. Patients who withdraw due to death in extended access phase will be included in the primary outcome analysis and will not be replaced.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return within 6 months of any scheduled visits, and/or does not supply monitoring laboratory results for 8 months, and is unable to be contacted by the study site staff. Patients who cannot return for scheduled visits due to health restrictions or travel restrictions (such as in the case of states of emergency) but remains in contact with the study site staff and continues to supply the required evaluations will not be deemed lost to follow-up, although this will be reported to the IRB as a deviation as appropriate.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within three months and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.
- If a patient can be contacted but cannot travel due to health restrictions or a state of emergency, all efforts will be made to have the required evaluations performed at the local institute and biospecimens shipped to the NIH as needed.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

A complete list of all required tests, timing, and details can be found in the **Schedule of Activities (SOA)**. For a description of procedural details (e.g., bone marrow testing, etc.) see section **8.3**.

8.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic or disease assessment purposes.

8.1.2 Screening activities performed after a study consent has been signed

Screening may be performed under this study. Results of screening assessments (see section **1.3.1**) performed at outside facilities or on another NIH protocol within 3 months (12 weeks), unless otherwise specified below, may also be used to determine eligibility once a participant has signed the consent.

The screening to determine eligibility and exclusion criteria shall consist of:

- Genetic testing for DBA (if prior results not available or incomplete) consisting of one or more of the following, as appropriate (see section **8.2.7** for a description of genetic testing)
- Bone marrow aspiration and core biopsy (may use prior results if performed at the NIH within 2 years prior to signing consent and blood counts have remained stable since the prior marrow examination, see section **8.3**)
- ECG
- PHQ-8 and C-SSRS
- Physical exam including a neurologic exam. The neurologic exam shall include evaluation of mental status, optical and eye movement cranial nerves (CNII, CNIII, CNIV, CNVI), motor function, gait, stance, and coordination as well as any other clinically indicated assessments.
- Review of red blood cell transfusion records for 8 weeks (or more) prior to study entry

8.2 Study Evaluations & Procedures

8.2.1 Baseline labs

Screening results (see section **8.1.2**) may be used as baseline studies if performed within 12 weeks of starting drug, unless indicated otherwise. These labs will assess blood counts, metabolic function, organ function, research blood (as detailed in section **8.2.6**), and immunologic function (see section **1.3.1** for complete listing of tests)

8.2.2 On-Study evaluations (Dose escalation and maintenance phases)

Subjects may be followed by their home physician or at the Clinical Center. Lab tests not done at the NIH will be faxed or securely e-mailed to the Research Nurse. The PI will review outside test results, and these will be uploaded into CRIS. All lab data will be recorded with the appropriate CRF. Any progress notes from local assessments will be faxed or securely e-mailed to the research nurse and reviewed by the principal investigator. These evaluations will be conducted monthly (4 weeks ± 10 days), beginning at week 4 through week 28. They shall consist of a telehealth visit with PI, AI, or other qualified clinician designated by the PI including interval

history, depression and suicide screening, medication review, transfusion history, and review of any AEs.

The subject will also have blood work (see section **1.3,** Schedule of Activities (SOA)) consisting of complete blood counts with differential, a reticulocyte count with reticulocyte hemoglobin, and pharmacokinetic-pharmacodynamic (PK/PD) testing (selected patients). For the purposes of tracking, PK/PD samples will be registered in BSI, but will not be stored on site as with other research samples. These samples will instead be immediately processed and shipped for testing as outlined below.

For reticulocyte and PK/PD testing, the samples will be shipped to the NIH. This sample shall consist of two tubes with a minimum 3 mL of blood each in EDTA ("purple top" or "lavender top"), and shipped refrigerated but not frozen to arrive within 24-48 hours of collection at:

Attention: David Young or Research Nurse
10 Center Drive, Bld 10-CRC, Room 7C-306 (OP7 Clinic)
Bethesda, MD 20892

Email notification of the shipment should also be sent to: **david.young2@nih.gov**, **tania.machado@nih.gov**, **CC-NURSOP7@mail.nih.gov**, and **yusj@nhlbi.nih.gov**.

One tube will be relabeled for reticulocyte testing in the Department of Laboratory Medicine (DLM), and the other will be labeled and processed as per **APPENDIX A: Handling, Processing, and Shipment of Pharmacokinetic Samples**. Reticulocyte testing performed at the Mayo Clinic Laboratories or comparable CLIA-certified laboratory is also acceptable provided it includes reticulocyte hemoglobin quantification.

8.2.3 Primary endpoint (8-month) evaluation

Subjects will be evaluated at the 8-month (32 weeks ± 14 days) endpoint at the NIH Clinical Center. The evaluation shall include history, exam including a focused neurologic exam, review of records, bloodwork to assess counts and metabolic function, reticulocyte testing, pregnancy testing for continued eligibility, bone marrow studies (see section **8.3**), research blood (as detailed in **8.2.6**), and administer HRQL and PHQ-8 surveys and C-SSRS screening tool (see section **1.3.1**). This evaluation will assess response (according to a rolling basis up to and including the endpoint, see section **9.4.2**), review any adverse events since the last monthly evaluation, sign consent for enrollment in extended access if eligible and willing, and determine if any dose adjustments are necessary. The neurologic exam shall include evaluation of mental status, optical and eye movement cranial nerves (CNII, CNIII, CNIV, CNVI), motor function, gait, stance, and coordination as well as any other clinically indicated assessments.

8.2.4 Long-term follow-up (responding patients enrolled in extended access phase)

Patients fulfilling response criteria (per section **9.4.2**) by or at the 8-month visit will be offered participation in the extended access phase, and after signing consent, they will be permitted to continue bitopertin for an additional 3 years. Subjects taking bitopertin must be evaluated at the Clinical Center annually with monthly labs and quarterly telehealth visits (see below) while they remain on extended access.

8.2.4.1 Monthly extended access laboratory evaluations

Patients on extended access, shall continue monthly laboratory monitoring in their home physician's office or the NIH. Progress notes and lab tests not done in NIH will be faxed or

securely e-mailed to the Research Nurse. The PI will review outside test results, and these will be uploaded into CRIS. All lab data will be recorded with the appropriate CRF. This monthly monitoring shall occur every 4 weeks (± 10 days) and consist of blood counts and reticulocyte counts (see section **1.3.2**). Central reticulocyte hemoglobin testing is not required monthly during extended access unless clinically indicated. If a patient achieves a ***robust*** response, these labs may be spaced to every 3 months (±30 days) in alignment with the quarterly evaluations below as long as a robust response is maintained and the bitopertin dose remains unchanged.

8.2.4.2 Quarterly extended access remote evaluations

In additional to monthly laboratory assessment, patients on extended access shall have a quarterly remote (telehealth) visit to assess clinical status. This monitoring shall occur every 3 months (± 30 days) and will include: a review of interval history and events, depression and suicide screening, review of transfusion records, and concomitant medication review.

8.2.4.3 Annual extended access evaluations

Patients on extended access shall have annual (every 12 months ± 30 days) evaluations to be performed at the NIH Clinical Center. Similar to the 8-month primary endpoint evaluation, this evaluation shall include history, exam including a focused neurologic exam, review of records, bloodwork to assess counts and metabolic function, reticulocyte testing, pregnancy testing for continued eligibility, bone marrow studies (see section **8.3**), research blood (as detailed in **8.2.6**), and administer HRQL and PHQ-8 surveys and C-SSRS screening tool (see section **1.3.2**). This evaluation will assess ongoing response (according to a rolling basis up to and including the endpoint, see section **9.4.2**), review any adverse events since the last quarterly evaluation, and determine if any dose adjustments are necessary. The neurologic exam shall include evaluation of mental status, optical and eye movement cranial nerves (CNII, CNIII, CNIV, CNVI), motor function, gait, stance, and coordination as well as any other clinically indicated assessments.

8.2.5 Off study assessment

We will contact all patients or their primary hematologist 30 days (± 5 days) following their final dose of bitopertin for information on their current status and to review any intervening adverse events.

We will offer patients a follow-up evaluation at the NIH Clinical Center 6 months (± 30 days) following being taken off bitopertin treatment either due to lack of response or for the other reasons listed in section **7.1**, but this visit will not be required. If patients do return to the NIH, the assessment should include history, exam, review of records, bloodwork to assess counts and metabolic function, reticulocyte testing, bone marrow studies (see section **8.3**), research blood (as detailed in **8.2.6**), and administer HRQL survey (see section **1.3.2**). All off-study assessments detailed in the Extended Access Phase Schedule of Events (**1.3.2**) are preferred, but not required per patient preference. Omission of one or more of the evaluations shall be noted in the record, but not constitute a protocol deviation.

8.2.6 Correlative Studies for Research/Pharmacokinetic Studies

At the timepoints indicated above (baseline, 8-month primary endpoint, and annually during extended access), research blood including bone marrow specimens (when a bone marrow aspiration is performed) shall also be collected for translational and correlative research studies. Unless clinically indicated, the volume to be collected will be 60 mL of blood (anticoagulated

with either heparin or EDTA) and 10 mL of aspirated blood marrow in heparinized syringes, such that the total volume of blood (including bone marrow aspiration) collected for clinical and research purposes does not exceed 10.5 mL/kg or 500 mL, whichever is smaller, over any eight-week period, as per NIH Clinical Center policies (see **Table 6**).

Samples thus collected will be not used in assessing the primary endpoint but are undertaken as descriptive or exploratory ancillary studies (see "Tertiary / Exploratory Objectives", section **3**).

These studies will include:

- Extended peripheral blood and bone marrow flow cytometric phenotyping for cell surface or intracellular proteins (e.g., multiparametric flow cytometry to measure primitive hematopoietic stem cells) and assessment of reactive oxygen species
- Hematopoietic progenitor colony, long term-culture-initiating cell, and *in vitro* erythrocyte differentiation assays for primitive cell content and function
- Studies to investigate pathways that can improve erythroid differentiation in DBA
- Studies to examine ribosome function and biology
- Evaluation of clonal dynamics (single/bulk cell sequencing)
- mRNA expression studies (RNA seq) on blood or bone marrow cells
- Targeted ("myeloid panel") sequencing may be performed in this study to assess the presence of somatic mutations in genes known to be recurrently mutated in MDS/AML or novel recurrently mutated genes. See below (section **8.2.7**) for handling of genetic results and communication of such with subjects.
- Serum cytokine, chemokines and soluble receptor levels including but not limited to IFN-γ, TNF-α and TPO levels.
- Pharmacokinetic studies of bitopertin kinetics (selected patients, see **APPENDIX A**: Handling, Processing, and Shipment of Pharmacokinetic Samples)
- Should there be any remaining sample, it will be stored, as outlined in the consent and discussed below, for other exploratory laboratory research studies reviewed and approved by the IRB

Table 6: Biospecimens to be collected for research purposes

Test	Blood Volume (approximate)	Type of Tube	Collection Point
PK/PD (drug level) testing	3-4 mL	EDTA (lavender)	Weeks 4, 8, 12, 16, 20, 24, 28 (+/-10 days)
Bone marrow aspirate (research)	10 mL	Heparin-treated syringe	Baseline Month 8 (week 32) Annually (extended phase)
Research blood samples	60 mL	Heparin (green) or EDTA (lavender)	Baseline Month 8 (week 32) Annually (extended phase)

PK/PD samples will be logged in BSI for tracking but will not be stored, long-term. They will instead be processed and immediately shipped for testing.

8.2.7 Samples for Genetic/Genomic Analysis

8.2.7.1 Description of the scope of genetic/genomic analysis

Patients will undergo genetic testing as part of their initial enrollment assessments, if results are not available from a previous, outside assessment (see §**5.1** and §**8.1**). Functional testing (e.g., ribosomal assembly, adenosine deaminase, etc.) will be considered for purposes of supporting a diagnosis of DBA but are not sufficient to substitute for genetic testing.

Intended use: The purpose of this analysis will be to identify, if possible, a DBA disease-defining mutation and to rule-out alternative bone marrow failure diagnoses; however, it is understood that approximately half of all patients so screened will not have an identifiable mutation (see §**2.2**). The presence of a disease-defining mutation is not required for study inclusion, nor is its absence considered exclusionary (see §**5.1**). This testing will also be important to rule-out the presence of MDS-defining mutations that would be considered exclusionary (§**5.2**). This testing will take one of two forms:

> ***Patients with a familial history of a known mutation:*** For patients where a specific mutation known to cause DBA has been identified in a first-degree, biological relative (i.e., parent, sibling, or child), the appropriate gene-specific testing (e.g., direct sequencing, fluorescence-in-situ hybridization, *etc.*) will be performed by a CLIA-certified clinical laboratory.
>
> ***Patients without a familial history:*** For patients without a familial history of DBA, or for which a specific, disease-causing mutation has not previously been identified, germline somatic mutation analysis will be performed using the NeoGenomics "Inherited bone marrow failure panel" or a comparable CLIA-certified test encompassing known DBA genes (e.g., University of Chicago "Hereditary Myeloid Malignancy and Inherited Bone Marrow Failure Panel", Cincinnati Children's Hospital "Bone Marrow Failure Syndromes Panel by next-generation sequencing", *etc.*). Unbiased whole-genome or whole-exome sequencing from outside, CLIA-certified sources provided by the patient or referring institution may be accepted at the PI's discretion. Typically, these tests will be performed on peripheral blood mononuclear cell or whole blood samples, but bone marrow samples or skin biopsy fibroblasts are also acceptable.

In addition, testing for somatic mutations in genes known to be recurrently mutated in AML/MDS or clonal hematopoiesis may be performed on a research basis on either bone marrow or peripheral blood samples collected at varying timepoints. These results will be for research purposes only, and they will not be communicated to subjects except for instances described in section **8.2.7.3** below.

8.2.7.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

The results of all genetic testing will be kept confidential. DBA-specific results (*i.e.,* identification of a disease-causing mutation) will be linked to the coded, study-specific identifier assigned to patient at the time of enrollment to allow subgrouping of patient data in the data analysis phase and for subgrouping of any banked biospecimens. This data will be stored on secure, NIH-maintained servers, accessible only to study personnel, and only for purposes of research.

8.2.7.3 Management of Results

In this study, only targeted ("gene panel") sequencing will be performed on identifiable samples for the purpose of identifying eligible DBA mutations or disqualifying alternative mutations. Given the limited genes to be tested, incidental, secondary findings are not expected.

Patients will be notified if a DBA-defining (confirmatory) mutation that was previously unknown is identified, as this may have implications for reproductive genetic counseling.

Subjects will also be contacted if a clinically actionable gene variant is discovered. There are two classes of actionable secondary findings for the purpose of this study (see **Table 7**). First are findings for disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. The other are findings of disorders that suggest an alternative diagnosis from DBA, as these would be exclusionary for this protocol (screening failure) and may have significant impacts upon the management of their disease. Subjects will be contacted at this time and will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

Table 7: Actionable Genetic (Secondary) Findings

Syndrome, Condition, or Disease	Genes
Confirmatory of Diamond-Blackfan Anemia	
Diamond-Blackfan Anemia	*GATA1, RPL11, RPL15, RPL18, RPL23, RPL26, RPL27, RPL31, RPL35, RPL35A, RPL36, RPL5, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS28, RPS29, RPS7*
ACMG Reportable[1]	
Familial breast-ovarian cancer	*BRCA1, BRCA2, PALB2*
Li-Fraumeni syndrome	*TP53*
Lynch syndrome (hereditary nonpolyposis colorectal cancer)	*MLH1, MSH2, MSH6, PMS2*
PTEN hamartoma tumor syndrome	*PTEN*
Genes Indicating an Alternative Diagnosis (non-ACMG-reportable)[2]	
Congenital amegakaryocytic thrombocytopenia (CAMT)	*MPL*
Deficiency of ADA2 (DADA2)	*ADA2*
DOCK8 deficiency	*DOCK8*
Dyskeratosis Congenita	*TERC, TERT*
Fanconi Anemia	*FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM*
Familial Platelet Disorder (FPD)	*RUNX1*
GATA2 deficiency	*GATA2*
Neurofibromatosis	*NF1*

[1] This list is not intended to be exhaustive. If additional genes or syndromes are added to the ACMG list after this trial is initiated or if a gene panel is used that contains additional ACMG-reportable genes, they will also be reported accordingly.

Syndrome, Condition, or Disease	Genes

[2] Patients with syndromes or mutations covered by an NIH-sponsored trial (NHGRI: DADA2, FPD; NHLBI: Dyskeratosis Congenita, Fanconi Anemia; NCI: DOCK8, Neurofibromatosis) will also be offered referrals to the appropriate institute.

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

8.2.7.4 Genetic counseling

Genetic counseling is not a planned component of this study as all testing will be focusing on the pre-existing diagnosis. However, genetic counseling will be available should we identify a reportable gene per section **8.2.7.3** above.

8.3 Safety and Other Assessments

Safety of the study intervention will be monitored through a combination of laboratory assessments, physical examinations, telemedicine evaluations, and bone marrow studies (see **Section 1.3 Schedule of Activities** for timing of individual tests).

Procedures:

- Physical exam and review of records:
- Blood draws
- Bone marrow biopsy and aspirate
- ECG
- Questionnaires

Physical examinations: including vital signs and medication reviews (especially for interactions) with interval histories will be performed at baseline, and at landmark evaluations to assess the global health of each patient while on study. In addition, during dose escalation and at any timepoint when drug adjustments are needed, a telemedicine evaluation will be conducted to assess for any health changes or toxicities.

Blood draws/laboratory tests: Throughout the study, basic monthly laboratory evaluations will also be conducted to monitor for metabolic evidence of toxicities as well as disease response. For purposes of this protocol, the blood draws at monthly local visits are expected to consist of approximately 30-40 mL of blood (3-4 collection tubes). Blood collections at NIH Clinical Center Visits will include approximately 80 mL of peripheral blood collected at each visit. Effort shall be made to combine ("batch") samples as much as possible, to minimize the total blood drawn. The total blood collected, including bone marrow aspirates, shall not exceed 5 mL/kg or 275 mL, whichever is lesser, at any single visit, although it is not expected that the totals will approach this limit. The total blood and marrow collected over an eight-week period (including home samples) shall not exceed the lesser of 10.5 mL/kg or 550 mL. Exceptions to these limits shall only occur in the event of clinical need, in which case clinical testing should be prioritized over research testing, especially testing not directly related to the primary endpoint (i.e., response assessment) of this study.

The monthly studies are standard clinical studies and can be performed at any CLIA certified laboratory. In the case of reticulocyte hemoglobin analysis, samples may also be sent to the NIH DLM, as this is not a commonly performed test (see section **8.2.2** for shipping instructions).

Bone marrow studies: At baseline and landmark timepoints, bone marrow studies consisting of bone marrow biopsy and aspirate shall be performed through the NIH DLM to monitor for any

evidence of hematologic toxicities not detectable through peripheral blood testing. These shall include but not be limited to:

- stained with hematoxylin and eosin for standard morphologic analysis by histology and quantitation of cellularity
- special stains to assess reticulin and collagen, iron, and frequency of primitive stem and progenitor cells via immunohistochemistry
- other lineage-specific or special stains as indicated to classify any abnormalities
- karyotypic analysis
- flow cytometry
- research bone marrow samples as detailed in section **8.2.6**

Aspirates are obtained from the posterior iliac crest. After preparation of the skin with chlorhexidine and betadine, the epidermis, and periosteal layers are infiltrated with 1 % xylocaine. Individual aspirates are obtained through an 8-gauge needle. Approximately 30 mL, and not more than 60 mL, of bone marrow aspirate will be obtained at any single visit. As noted above, bone marrow aspirates will be included when calculating the total blood collected which shall not exceed 5 mL/kg or 275 mL, whichever is lesser, at any single visit.

In the case of a subject's inability to travel to the NIH for such an evaluation, these bone marrow evaluations may be performed at the local institution at the PI's discretion, but central review of the materials will be performed at the NIH DLM.

Electrocardiogram (ECG): An electrocardiogram (ECG) consisting of a full 12-lead (electrode) recording requiring approximately 5 minutes of relative immobility will be performed at screening to rule out cardiac abnormalities that would be exclusive of participation and at baseline to allow for comparison in the event of any cardiac-related AEs. The primary risk is discomfort during the recording or with the removal of the adhesive leads. All tracings shall be reviewed by a qualified practitioner and recorded in the medical record (CRIS). A single ECG can serve for both baseline and screening purposes as noted in sections **1.3.1** and **8.2.1**.

Questionnaires: Patients will undergo quality of life (HRQL) inventories which may be administered either by paper or electronic format at landmark evaluations as noted above. At enrollment, and if clinically indicated during the study participation, patients will also undertake a psychiatric inventory (PHQ-8 and C-SSRS) which will be administered by paper. These previously validated questionnaires will be administered electronically, via a secure portal, and responses stored in a secure electronic database (see sections **10.3** and **10.8**). Their collection will be noted in the CTDB, and the results reviewed by the PI for purposes of eligibility, safety, and monitoring, but they will not be included in the medical record (CRIS). Patients will have the option of completing the questionnaires on paper, after which the results will be scanned and stored as above, but the electronic format will be the preferred format. Due to the nature of the questionnaire and the need to properly analyze answers, the questionnaires will only be offered to those subjects who can speak and read English or Spanish.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (**21 CFR 312.32 (a)**).

8.4.2 Definition of Laboratory Adverse Event

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will include any change from laboratory assessments performed at baseline (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.

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 considered as AEs. However, as they are response criteria, transfusion frequency and volumes will be recorded in the medical record and on the appropriate CRF.

8.4.3 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.4.4 Classification of an Adverse Event

8.4.4.1 Severity of Event

AEs will be graded by severity utilizing the Common Terminology Criteria for Adverse Events (CTCAE). For internal consistency, the most current version at the writing of this protocol (version 5.0, published November 27, 2017) shall be used for the duration of this study and its analysis. The CTCAE can be downloaded at the above link or through the CTEP homepage at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm.

For AEs not specified by the CTCAE, the following guidelines will be used to describe severity:

Table 8: General classification of AEs

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 Activities of Daily Living (ADL)[1]

Grade	Category	Description
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2]
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

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

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

8.4.4.2 Relationship to Study Intervention

AEs must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related" or downgraded to "unlikely to be related" or "not related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

For the purpose of regulatory reporting requirements, causal relationships of definite, probable, and possible will be considered treatment-related, while unlikely and unrelated will be considered not treatment-related.

8.4.4.3 Expectedness

The principal investigator or a designated associate investigator with appropriate expertise will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention on the investigator's brochure.

In view of the underlying disease (Diamond-Blackfan anemia), all patients will enter the study with abnormally low blood counts that would meet criteria for grade 2 – and more commonly higher – toxicity. Also, platelet and red blood cell counts may be impacted by frequent transfusions. Therefore, 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 considered as AEs. However, as they are response criteria, transfusion frequency and volumes will be recorded in the medical record and on the appropriate CRF.

8.4.5 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Adverse events will start being recorded after informed consent has been obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after administration of the last dose of study drug or until study termination. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until adequate resolution or stabilization.

Adverse Events that meet the criteria of CTCAE v 5.0 grade 2 and above plus laboratory abnormalities that meet the definition of an AE will be captured on the appropriate case report form (CRF) regardless of relationship. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study drug and study procedure (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. These AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline (prior to first dose) and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent may require documentation of onset and duration of each episode per PI discretion.

When possible, signs and symptoms indicating a common underlying pathology or disease, should be recorded as one comprehensive event.

8.4.6 Adverse Event Reporting

8.4.6.1 Expedited Reporting

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

8.4.6.2 Reports to the NHLBI Clinical Director

The PI or designee will refer to NHLBI DIR guidelines to determine Office of Clinical Director (OCD) reporting requirements and timelines.

8.4.6.3 Interim Reports to the FDA

In addition to Federal, NIH, and NHLBI DIR reporting guidelines, the PI or designee with provide an interim safety update after month 8. This report shall include a summary of all AEs, AEs leading to drug discontinuation, AEs leading to drug interruption, and SAEs and AEs of special interest such as CNS toxicity.

8.4.7 Serious Adverse Event Reporting

The study investigator will immediately report to the study sponsor (NHLBI) any serious adverse event, whether considered study intervention related, including those listed in the protocol or investigator brochure, and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor. The study investigator will also notify the manufacturer of all SAE immediately upon learning of the event, and will provide a full report within a reasonable period of time needed for information collection (either through the online portal, or by emailing the appropriate form to **NorthAmerica_Medical@parexel.com**).

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

The study sponsor will be responsible for notifying the Food and Drug Administration (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. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (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 (**21 CFR 312.32**).

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of all adverse events and shall report the results of such evaluation to the NIH Institutional Review Board (IRB) as per Policy 801.

8.4.7.1 NHLBI DSMB Reporting

Reports of serious adverse events that are unexpected and thought to be related (possibly, probably or definitely) to the study 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.

8.4.8 NIH Intramural IRB Reporting of IND Safety Reports

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

Only IND Safety Reports that meet the definition of an unanticipated problem or is new information that might affect the willingness of subjects on the NIH study to enroll or remain in the study will need to be reported to the NIH Intramural IRB.

8.4.9 Events of Special Interest

AE of special interest (AESI) include the development while on study drug of suicidal ideation, suicide attempt, or any AE for a psychiatric condition leading to a new, unplanned prescription of a concomitant medication regardless of AE grade.

8.4.10 Reporting of Pregnancy

Pregnancy in a participant or the female partner of a male participant occurring up to 30 days after the participant's last dose of study drug must be reported to the contract research organization pharmacovigilance group (Parexel International) within 24 hours of the Investigator's knowledge of the pregnancy using a Pregnancy Report Form (email to **NorthAmerica_Medical@parexel.com)**.

Pregnancy *per se* is not considered an AE unless there is a cause to believe that the study interventions may have interfered with the effectiveness of a contraceptive medication or if the outcome of the pregnancy meets SAE criteria (miscarriage/fetal death or congenital anomaly/birth defect), in which case it should be reported in the same manner and timelines as an SAE. Hospitalization for normal delivery is not an SAE.

If a partner of a participant becomes pregnant, the Investigator should request consent from the partner to collect relevant safety information under a separate pregnancy outcomes monitoring ("basket") study. If a pregnancy occurs in a participant, study interventions must be discontinued immediately. The pregnant participant should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. The course of the pregnancy should be monitored either on this study or through a separate monitoring protocol, and then the subject removed from this study upon either completion of the pregnancy, enrollment on such a monitoring protocol, or if the subject refuses pregnancy monitoring.

At Screening, all participants (male and female) must agree to use a highly effective method of contraception during the duration of the study and for 30 days after the last dose of study drug (see **Section 5.2 Exclusion Criteria, item 11** for a description of acceptable methods).

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

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

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801 and to the manufacturer.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

We hypothesize that bitopertin is effective (response rate greater than 10%) in treating DBA (i.e., reduces transfusion requirements and/or increases hemoglobin levels relative to baseline). To that end, we have established the following endpoints:

- ***Primary endpoint:*** efficacy (response rate as defined below) during 8 months of therapy with bitopertin in subjects with Diamond-Blackfan anemia.
- ***Secondary endpoints:*** safety defined as the toxicity profile over 8 months of therapy; assessments of the impact of bitopertin upon rates of relapse and survival, clonal evolution, toxicities of extended therapy, the impact of treatment upon health-related quality of life, and the impact of bitopertin upon stem cell and erythroid cell dynamics and niche interactions.

9.2 Sample Size Determination

Because the efficacy of bitopertin in this patient population is unknown, we would like to reject the treatment as quickly as possible with a small number of patients if the treatment is not effective. We will use the Two-Stage Minimax Design outlined in Table 1 of Simon (56) with a response probability of 10% or less to terminate the treatment and the hypothesized actual response probability of 30% or more.. The sample size is determined by testing the null hypothesis H_0: $p \leq 10\%$ versus the alternative H_1: $p \geq 30\%$ at a significance level of 0.05 and a power of 0.8.

At the first stage, we will accrue 15 subjects, and the alternative hypothesis will be rejected if no more than 1 subject responds (criteria below, see section **9.4.2.1**) to the treatment within 8 months. If 2 or more subjects respond to the treatment at 8 months at the first stage, then we will

accrue an additional 10 subjects, bringing the total number of subjects to $n = 25$. The null hypothesis of $p \leq 10\%$ will be accepted if the total number of responders at 8 months is 5 or less. These efficacy thresholds were selected as being clinically relevant for the avoidance of alternative therapies such as hematopoietic stem cell transplant.

9.3 Populations for Analyses

This study, including all primary and secondary objectives and endpoints, shall be analyzed as an intention-to-treat, inclusive of all patients enrolled with the exception of screening failures.

9.3.1 Evaluable for toxicity

All participants will be evaluable for toxicity from the time of their first treatment with bitopertin.

9.3.2 Evaluable for objective response

All participants reaching the primary endpoint (8 months) will be evaluable for objective response as evaluable disease is an inclusion criterion.

9.3.3 Evaluable Non-Target Disease Response

Not applicable

9.4 Statistical Analyses

9.4.1 General Approach

This is a single-arm pilot (phase I/II) study without a control arm as a statistical comparator, and as such, results will be primarily descriptive and qualitative. “Success” versus “failure” of the intervention (i.e., acceptance or rejection of the alternative hypothesis) will be solely based upon achieving a response rate threshold of 24% or greater (6 or more responses out of 25 patients eligible enrolled), as discussed above (see **Section 9.2, Sample Size Determination** for statistical discussion)

For quantitative results (specifically response rates), attempts will be made to examine predictive factors such as demographics and disease characteristics. In such cases, regression models (linear and/or logistic), contingency analyses (i.e., Fisher’s exact test or chi-squared (χ^2) test), non-parametric (Wilcoxon rank-sum/Mann-Whitney *U*), and parametric (ANOVA, Student’s *t*) will be used for comparing subgroups as appropriate.

The predictive analyses will include descriptive statistics on the proportions of responses (i.e., % subjects with treatment response) and the time to response. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypothesis testing will be evaluated using Binomial distributions.

The time to responses and the progression-free survival will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate, Kaplan-Meier curves, and Cox regression with age, gender and ethnicity as covariates, which take consideration of both death without the event of interest as a competing risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. Graphical tools will be used to display the appropriate estimates (i.e., estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their

corresponding 95% confidence intervals. Exploratory subgroup analysis will be used to compare the possible different response rates among male, female and different age categories.

9.4.2 Primary Endpoints

Primary efficacy endpoint will be the proportion of drug responders during 8 months of treatment. Best response (robust or not) will be noted, regardless of when it is achieved; however, for protocol purposes, primary response will be assessed on a rolling basis beginning with drug initiation up to and including the 8-month primary endpoint assessment. Time-to-response will also be measured according to the time from bitopertin initiation to the first time the patient met criteria for response.

9.4.2.1 Response Definitions

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

- increase in pre-transfusion ("nadir") hemoglobin by >1.5 G/dL from baseline, *and/or*
- a reduction in the units of PRBC transfusions by at least 50% from baseline during the eight consecutive weeks prior to time of assessment (rolling basis or primary endpoint).

Robust response to treatment will be defined as both:

- stable hemoglobin (two consecutive measurements at least 2 weeks apart) greater than 10 g/dL, *and*
- transfusion independence for the previous 8 weeks

9.4.2.2 Analysis of the Primary Endpoints

Baseline "nadir" hemoglobin level is defined as the average pre-transfusion hemoglobin over the 8 weeks preceding initiation of drug. Baseline transfusion rate is the number (units, see below) of transfusions received over the same 8 weeks preceding initiation of drug. Pre-transfusion "nadir" is 4 days or less prior to a transfusion. Note, the 8-week baseline is prior to initiation of drug, not study enrollment, and drug initiation should not occur until the principal investigator has confirmed that the study subject has been on a stable transfusion frequency to ensure an accurate assessment of baseline transfusion requirements. Timing of all study events will be relative to the first day of drug ("Day/Week 0").

Changing standards of care have led to using higher thresholds for transfusion that may obscure a response based solely upon either transfusion rates or un-transfused hemoglobin levels. Therefore, to capture changes in hemoglobin production masked by ongoing transfusions, the pre-transfusion hemoglobin nadir will be used. As this can be variable, an average level defined over 8 weeks prior to the start of drug will be compared to a comparable average over the 8 weeks leading up to the response assessment. As robust response requires transfusion independence for 8 weeks prior to determination, averages will not be used to make that determination, but instead two hemoglobin levels separated by at least 2 weeks and greater than 10 g/dL will be required.

For sub-analyses, ***transient response*** to treatment (robust or otherwise) is defined as any response meeting the respective criteria above within the first 8 months (during any 8-week window) that is subsequently lost and not regained prior to the 8-month primary endpoint assessment. While note will be made of such patients, for the purposes of primary response analysis, these patients will be considered ***responders*** along with patients who continue to meet the above response criteria at the 8-month primary endpoint.

Transfusion Units: This trial will enroll only adult patients, and "units" will be measured in bags of packed red blood cells transfused. Although true pediatric patients (those under 18 years of age) will not be enrolled in this study, weight-based parameters are provided in the case of any ambiguity for young adults where weight-based transfusions might be used depending upon local institutional practices. In pediatric patients, the administration of red blood cell transfusions is typically weight based, typically 10-15 mL/kg, reaching a maximum of one bag of packed erythrocytes (300-350 mL). For protocol purposes, a "transfusion unit" for small adult patients administered blood on a weight basis will be considered to be 15 mL/kg of packed erythrocytes. Where ambiguity exists, the clinical record shall be reviewed to determine the intent of the ordering physician as to whether dosing was per-bag or on a weight basis. It will be the responsibility of the PI to adjudicate any remaining ambiguity.

Transfusions administered for non-disease indications (e.g., trauma, acute blood loss, perioperative hemostasis, *etc.*) will be noted, but not included in statistical and response considerations.

9.4.3 Analysis of the Secondary and Tertiary/Exploratory Endpoint(s)

Secondary objectives and endpoints will include:

Examining the safety and tolerability of bitopertin in treating DBA (see **9.4.4**) including:

- Safety as demonstrated by toxicity profile assessed at 8 months using CTCAE criteria
- Tolerability as indicated by the maximally tolerated dose identified during intra-patient dose escalation prior to the primary endpoint.

Subgroup analysis of primary response including:

- Response rates after 12 weeks at the maximally tolerated dose (i.e.: for those reaching an MTD prior to the formal start of maintenance at 20 weeks)
- Time to response (from start of bitopertin and from reaching MTD)
- Best response, regardless of response status at primary endpoint

Examine the effects of long-term treatment of Diamond-Blackfan anemia with bitopertin:

- Relapse during the extension phase
- Clonal evolution, according to cytogenetic, mutational or flow cytometric markers
- Toxicities with extended duration of therapy
- The impact of treatment and treatment response on quality of life.

Tertiary and exploratory endpoints will include:

Evaluate the impact of bitopertin on stem cell and erythroid cell dynamics in DBA:

- Changes in reticulocyte hemoglobin by time and dose level
- Iron loading as measured by ferritin trends at each evaluation, and by iron staining of bone marrow biopsies at baseline and 8-month evaluations (and during extension at the PI's discretion)
- Evaluation of reactive oxygen species HSPCs (ROS flow cytometry assay at baseline and 8 months), and at interval evaluations during extended access at the PI's discretion
- Evaluation of global transcriptome in HSPCs (single cell RNA seq at baseline and 8 months)

- The impact of bitopertin on reconstitution of the stem cell compartment (multicolor flow cytometry of bone marrow cells) and erythroid compartment (multicolor flow cytometry and CFU-E/BFU-E assays) at baseline and 8-month evaluations, and at interval evaluations during extended access at the PI's discretion

Examine the pharmacology of bitopertin in DBA:

- Pharmacokinetic and pharmacodynamic studies of bitopertin during dose escalation and at major timepoints

9.4.4 Safety Analyses

As noted above (**Section 8.4.4, Classification of an Adverse Event**), events will be classified and graded according to the CTCAE version 5.0, including a determination of the relationship with the study intervention. The primary safety endpoint will be the cumulative toxicity profile at the primary 8-month endpoint. Secondary analyses will include toxicities at each dose level and at the subjects' different maximal dose levels. Safety will also be monitored for all subjects enrolling in extended access.

9.4.4.1 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, including the extension phase, does not substantially exceed an anticipated rate. The following specified TRSAEs will be considered for early stopping of the study unless they can clearly be determined to be unrelated to the study drug:

- Grade III or higher psychiatric, neurologic, or ophthalmologic AE *requiring interruption of study drug*
- Any death
- Any Grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities

We anticipate the rate of these specified TRSAEs within the 24-week study period to be 20% or less. Following Geller *et al.* (57), 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 9** summarizes the threshold numbers for stopping the study.

Table 9: Threshold numbers for stopping the study

Number of subjects in the experiment	Stop if the number of subjects who have developed any of the specified TRSAE's reaches:
≤ 5	3
≤ 9	4
≤ 12	5
≤ 16	6
≤ 20	7
≤ 24	8
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" per 100,000) which were stopped using the above stopping rule. **Table 10** summarizes the proportion of stopped studies under a number of scenarios for p:

Table 10: Predicted rates of stopping under various scenarios

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

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

9.4.5 Baseline Descriptive Statistics

Not applicable.

9.4.6 Planned Interim Analyses

As noted in sections **1.2**, **5.7.1**, and **9.2**, this study will be conducted in two stages, with an interim futility analysis after 15 patients have been enrolled and have reached the primary endpoint. Continuation to the second stage and enrollment of the remaining 10 patients requires at least 2 patients experience a response at the primary endpoint (see §**9.2** for statistical considerations).

9.4.7 Sub-Group Analyses

Sub-group analyses are not planned as the study group is too small and is expected to be too heterogenous with regards to various demographic categories based upon prior interventional trials with this disease and of similar sizes.

9.4.8 Tabulation of individual Participant Data

Individual participant data will be listed by time point.

9.4.9 Exploratory Analyses

No exploratory analyses are planned for this study.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent/Assent Procedures and Documentation

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

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

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

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

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

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

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.

10.1.2 Consent for minors when they reach the age of majority

Not applicable as minors will not be consented to this trial.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH staff will be obtained as detailed above with following additional protections:

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

10.1.4 Consent of Subjects who are, or become, decisionally impaired

The PI will ensure that study investigators will identify an appropriate LAR consistent with requirements of Policy 403 and will obtain consent from the LAR as outlined in the consent process in Section **10.1.1** before initiating research interventions.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor (NHLBI OCD) and regulatory authorities (FDA). If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants (see **Section 9.4.4.1, Stopping Rules**)
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and, as applicable, the Food and Drug Administration (FDA).

10.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Clinical Trials Database (CTDB), a 21 CFR Part 11-compliant data capture system provided by the National Institute of Child Health and Human Development. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by CTDB research staff will be secured and password protected.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future use of Stored Specimens and Data

As noted above (see section **8.2.6**) samples collected for research shall be cataloged in the Biospecimen Inventory (BSI) Management system and stored securely. We may share specimens and data with other researchers for future use.

Following analyses of biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB. Any future research use of identifiable biospecimens not defined in the research protocol will occur only after IRB review and approval.

10.5 Safety Oversight

Safety oversight will be under the direction of the National Heart, Lung, and Blood Institute (NHLBI) Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including clinical trial design and execution, standard-of-care medical practices, and experimental pharmacology. Members of the DSMB are independent from the study conduct and free of conflict of interest. The DSMB will meet regularly to assess safety and efficacy of the study. The DSMB will operate under the rules of an approved charter and will provide its input to National Heart, Lung, and Blood Institute the NHLBI Clinical Director.

10.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with

International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

10.7 Quality Assurance and Quality Control

NHLBI will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the study team for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and Good Laboratory Practices (GLP).

The principal investigator will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff under the supervision of the principal investigator (who, for this single-center trial, is also the principal investigator). The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Those source documents (i.e., labs, imaging results, notes, *etc*.) not already a part of the medical record (CRIS) will be reviewed by the PI and entered (electronically scanned) into CRIS.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Clinical Trials Database (CTDB), a 21 CFR Part 11-compliant data capture system provided by the National Institute of Child Health and Human Development. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be

destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to Office of the Clinical Director, National Heart, Lung, and Blood Institute. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- **Major deviations:** Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- **Minor deviations:** Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 Publication and Data Sharing Policy

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the ***2023 NIH Data Management and Sharing Policy*** and ***Policy on the Dissemination of NIH-Funded Clinical Trial Information*** and the ***Clinical Trials Registration and Results Information Submission*** rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

Research data will be deposited in BioData Catalyst (BDC) at the time of study publication or closure, whichever comes first. Genomic data will be deposited in dbGap. Both BDC and dbGap are controlled access repositories and in order to access the data, an investigator must have an approved Data Access Request (DAR) through dbGaP. Data will be available for access indefinitely.

10.10.2 Genomic Data Sharing Compliance

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data. Genomic data will be deposited in dbGap.

10.11 Collaborative Agreements

10.11.1 Collaborative Research and Development Agreements

This study is supported under a Collaborative Research and Development Agreement (CRADA) between the National Institutes of Health and Disc Medicine.

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

Haley Howell
Disc Medicine, Clinical Development Operations
321 Arsenal Street, Suite 101
Watertown, MA 02472
hhowell@discmedicine.com

10.11.2 Material Transfer Agreements

Between NHLBI (David Young, MD, Ph.D.) and Dr. Jan Abkowitz at University of Washington. Upon execution of the Material Transfer Agreement (MTA), coded peripheral blood or marrow samples from this protocol may be sent to the University of Washington. The goal of this collaboration is to identify the defect in red blood cell development for each patient, matching these results with disease-causing mutation (if known) and drug response, the final goal of which is to understand the underlying cause of response or lack thereof. The NIH research team will receive research results that can be linked to identifiers as part of this research collaboration.

10.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Heart, Lung, and Blood Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
δ-ALA	δ-aminolevulinic acid
ALAS2	aminolevulinic acid synthase 2
ARC	Absolute reticulocyte count
BDC	BioData Catalyst
BSI	Biospecimen Inventory
CBC	Complete blood count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COC	Certificate of Confidentiality
CRF	Case Report Form
C-SSRS	Columbia-Suicide Severity Rating Scale

CTDB	Clinical Trials Database
DBA	Diamond-Blackfan Anemia
DLM	(NIH) Department of Laboratory Medicine
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FECH	ferrochelatase
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
HRQL	Health-related quality of life
HSCT	Hematopoietic Stem Cell Transplant
IBMFS	Inherited Bone Marrow Failure Syndrome
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
IRBO	(Office of) IRB Operations
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
KSP	Key Study Personnel
MED	Minimal effective dose
MTD	Maximum (maximally) tolerated dose
NCT	National Clinical Trial
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OCD	Obsessive-Compulsive Disorder
OHRP	Office for Human Research Protections
PHQ-8	Patient Health Questionnaire-8
PI	Principal Investigator
PK/PD	Pharmacokinetic-pharmacodynamic
PPIX	protoporphyrin IX
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

REFERENCES

1. Boria I, Quarello P, Avondo F, Garelli E, Aspesi A, Carando A, et al. A new database for ribosomal protein genes which are mutated in Diamond-Blackfan Anemia. Hum Mutat. 2008 Nov;29(11):E263-70.

2. Ulirsch JC, Verboon JM, Kazerounian S, Guo MH, Yuan D, Ludwig LS, et al. The Genetic Landscape of Diamond-Blackfan Anemia. Am J Hum Genet. 2018 Dec 6;103(6):930-47.

3. Gazda HT, Zhong R, Long L, Niewiadomska E, Lipton JM, Ploszynska A, et al. RNA and protein evidence for haplo-insufficiency in Diamond-Blackfan anaemia patients with RPS19 mutations. Br J Haematol. 2004 Oct;127(1):105-13.

4. Da Costa L, Narla A, Mohandas N. An update on the pathogenesis and diagnosis of Diamond-Blackfan anemia. F1000Res. 2018;7.

5. Doty RT, Phelps SR, Shadle C, Sanchez-Bonilla M, Keel SB, Abkowitz JL. Coordinate expression of heme and globin is essential for effective erythropoiesis. J Clin Invest. 2015 Dec;125(12):4681-91.

6. Yang Z, Keel SB, Shimamura A, Liu L, Gerds AT, Li HY, et al. Delayed globin synthesis leads to excess heme and the macrocytic anemia of Diamond Blackfan anemia and del(5q) myelodysplastic syndrome. Sci Transl Med. 2016 May 11;8(338):338ra67.

7. Giri N, Kang E, Tisdale JF, Follman D, Rivera M, Schwartz GN, et al. Clinical and laboratory evidence for a trilineage haematopoietic defect in patients with refractory Diamond-Blackfan anaemia. Br J Haematol. 2000 Jan;108(1):167-75.

8. Doty RT, Fan X, Young DJ, Liang J, Singh K, Pakbaz Z, et al. Studies of a mosaic patient with DBA and chimeric mice reveal erythroid cell-extrinsic contributions to erythropoiesis. Blood. 2022 Jun 9;139(23):3439-49.

9. Rey MA, Duffy SP, Brown JK, Kennedy JA, Dick JE, Dror Y, et al. Enhanced alternative splicing of the FLVCR1 gene in Diamond Blackfan anemia disrupts FLVCR1 expression and function that are critical for erythropoiesis. Haematologica. 2008 Nov;93(11):1617-26.

10. Quigley JG, Yang Z, Worthington MT, Phillips JD, Sabo KM, Sabath DE, et al. Identification of a human heme exporter that is essential for erythropoiesis. Cell. 2004 Sep 17;118(6):757-66.

11. Keel SB, Doty RT, Yang Z, Quigley JG, Chen J, Knoblaugh S, et al. A heme export protein is required for red blood cell differentiation and iron homeostasis. Science. 2008 Feb 8;319(5864):825-8.

12. Doty RT, Yan X, Lausted C, Munday AD, Yang Z, Yi D, et al. Single-cell analyses demonstrate that a heme-GATA1 feedback loop regulates red cell differentiation. Blood. 2019 Jan 31;133(5):457-69.

13. Rio S, Gastou M, Karboul N, Derman R, Suriyun T, Manceau H, et al. Regulation of globin-heme balance in Diamond-Blackfan anemia by HSP70/GATA1. Blood. 2019 Mar 21;133(12):1358-70.

14. Willig TN, Draptchinskaia N, Dianzani I, Ball S, Niemeyer C, Ramenghi U, et al. Mutations in ribosomal protein S19 gene and diamond blackfan anemia: wide variations in phenotypic expression. Blood. 1999 Dec 15;94(12):4294-306.

15. Quarello P, Garelli E, Carando A, Brusco A, Calabrese R, Dufour C, et al. Diamond-Blackfan anemia: genotype-phenotype correlations in Italian patients with RPL5 and RPL11 mutations. Haematologica. 2010 Feb;95(2):206-13.

16. Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, et al. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. Am J Hum Genet. 2008 Dec;83(6):769-80.

17. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood. 2012 Apr 19;119(16):3815-9.

18. Vlachos A, Rosenberg PS, Atsidaftos E, Kang J, Onel K, Sharaf RN, et al. Increased risk of colon cancer and osteogenic sarcoma in Diamond-Blackfan anemia. Blood. 2018 Nov 15;132(20):2205-8.

19. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008 Sep;142(6):859-76.

20. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood. 2010 Nov 11;116(19):3715-23.

21. Mantovani LF, Santos FPS, Perini GF, Nascimento CMB, Silva LP, Wroclawski CK, et al. Hepatic and cardiac and iron overload detected by T2* magnetic resonance (MRI) in patients with myelodisplastic syndrome: A cross-sectional study. Leuk Res. 2019 Jan;76:53-7.

22. Buzzetti E, Petta S, Manuguerra R, Luong TV, Cabibi D, Corradini E, et al. Evaluating the association of serum ferritin and hepatic iron with disease severity in non-alcoholic fatty liver disease. Liver Int. 2019 Jul;39(7):1325-34.

23. Jarisch A, Salzmann-Manrique E, Cario H, Grosse R, Soerensen J, Fischer R, et al. Serum ferritin is not a reliable predictor to determine iron overload in thalassemia major patients post-hematopoietic stem cell transplantation. Eur J Haematol. 2018 Dec;101(6):791-7.

24. Wood JC, Zhang P, Rienhoff H, Abi-Saab W, Neufeld EJ. Liver MRI is more precise than liver biopsy for assessing total body iron balance: a comparison of MRI relaxometry with simulated liver biopsy results. Magn Reson Imaging. 2015 Jul;33(6):761-7.

25. Emond MJ, Bronner MP, Carlson TH, Lin M, Labbe RF, Kowdley KV. Quantitative study of the variability of hepatic iron concentrations. Clin Chem. 1999 Mar;45(3):340-6.

26. Menacho K, Abdel-Gadir A, Moon JC, Fernandes JL. T2* Mapping Techniques: Iron Overload Assessment and Other Potential Clinical Applications. Magn Reson Imaging Clin N Am. 2019 Aug;27(3):439-51.

27. Ruefer A, Bapst C, Benz R, Bremerich J, Cantoni N, Infanti L, et al. Role of liver magnetic resonance imaging in hyperferritinaemia and the diagnosis of iron overload. Swiss Med Wkly. 2017;147:w14550.

28. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, et al. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation. 2013 Jul 16;128(3):281-308.

29. Cancado R, Watman NP, Lobo C, Chona Z, Manzur F, Traina F, et al. Assessment of liver and cardiac iron overload using MRI in patients with chronic anemias in Latin American countries: results from ASIMILA study. Hematology. 2018 Oct;23(9):676-82.

30. Wahidiyat PA, Liauw F, Sekarsari D, Putriasih SA, Berdoukas V, Pennell DJ. Evaluation of cardiac and hepatic iron overload in thalassemia major patients with T2* magnetic resonance imaging. Hematology. 2017 Sep;22(8):501-7.

31. Jackson LH, Vlachodimitropoulou E, Shangaris P, Roberts TA, Ryan TM, Campbell-Washburn AE, et al. Non-invasive MRI biomarkers for the early assessment of iron overload in a humanized mouse model of beta-thalassemia. Sci Rep. 2017 Feb 27;7:43439.

32. Berdoukas V, Nord A, Carson S, Puliyel M, Hofstra T, Wood J, et al. Tissue iron evaluation in chronically transfused children shows significant levels of iron loading at a very young age. Am J Hematol. 2013 Nov;88(11):E283-5.

33. Henter JI, Karlen J. Fatal agranulocytosis after deferiprone therapy in a child with Diamond-Blackfan anemia. Blood. 2007 Jun 15;109(12):5157-9.

34. Hoffbrand AV, Bartlett AN, Veys PA, O'Connor NT, Kontoghiorghes GJ. Agranulocytosis and thrombocytopenia in patient with Blackfan-Diamond anaemia during oral chelator trial. Lancet. 1989 Aug 19;2(8660):457.

35. Bugarski-Kirola D, Blaettler T, Arango C, Fleischhacker WW, Garibaldi G, Wang A, et al. Bitopertin in Negative Symptoms of Schizophrenia-Results From the Phase III FlashLyte and DayLyte Studies. Biol Psychiatry. 2017 Jul 1;82(1):8-16.

36. Bugarski-Kirola D, Iwata N, Sameljak S, Reid C, Blaettler T, Millar L, et al. Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics: results from three phase 3, randomised, double-blind, parallel-group, placebo-controlled, multicentre studies in the SearchLyte clinical trial programme. Lancet Psychiatry. 2016 Dec;3(12):1115-28.

37. Bugarski-Kirola D, Wang A, Abi-Saab D, Blattler T. A phase II/III trial of bitopertin monotherapy compared with placebo in patients with an acute exacerbation of schizophrenia - results from the CandleLyte study. Eur Neuropsychopharmacol. 2014 Jul;24(7):1024-36.

38. Hirayasu Y, Sato S, Takahashi H, Iida S, Shuto N, Yoshida S, et al. A double-blind randomized study assessing safety and efficacy following one-year adjunctive treatment with bitopertin, a glycine reuptake inhibitor, in Japanese patients with schizophrenia. BMC Psychiatry. 2016 Mar 15;16:66.

39. Hirayasu Y, Sato SI, Shuto N, Nakano M, Higuchi T. Efficacy and Safety of Bitopertin in Patients with Schizophrenia and Predominant Negative Symptoms: Subgroup Analysis of Japanese Patients from the Global Randomized Phase 2 Trial. Psychiatry Investig. 2017 Jan;14(1):63-73.

40. Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, et al. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. JAMA Psychiatry. 2014 Jun;71(6):637-46.

41. Pinard E, Alanine A, Alberati D, Bender M, Borroni E, Bourdeaux P, et al. Selective GlyT1 inhibitors: discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. J Med Chem. 2010 Jun 24;53(12):4603-14.

42. Alberati D, Moreau JL, Lengyel J, Hauser N, Mory R, Borroni E, et al. Glycine reuptake inhibitor RG1678: a pharmacologic characterization of an investigational agent for the treatment of schizophrenia. Neuropharmacology. 2012 Feb;62(2):1152-61.

43. Kim KM, Kingsmore SF, Han H, Yang-Feng TL, Godinot N, Seldin MF, et al. Cloning of the human glycine transporter type 1: molecular and pharmacological characterization of novel isoform variants and chromosomal localization of the gene in the human and mouse genomes. Mol Pharmacol. 1994 Apr;45(4):608-17.

44. Garcia-Santos D, Schranzhofer M, Bergeron R, Sheftel AD, Ponka P. Extracellular glycine is necessary for optimal hemoglobinization of erythroid cells. Haematologica. 2017 Aug;102(8):1314-23.

45. Felipe A, Vinas O, Remesar X. Changes in glycine and leucine transport during red cell maturation in the rat. Biosci Rep. 1990 Apr;10(2):209-16.

46. King PA, Gunn RB. Na- and Cl-dependent glycine transport in human red blood cells and ghosts. A study of the binding of substrates to the outward-facing carrier. J Gen Physiol. 1989 Feb;93(2):321-42.

47. Ponka P. Tissue-specific regulation of iron metabolism and heme synthesis: distinct control mechanisms in erythroid cells. Blood. 1997 Jan 1;89(1):1-25.

48. Matte A, Federti E, Winter M, Koerner A, Harmeier A, Mazer N, et al. Bitopertin, a selective oral GLYT1 inhibitor, improves anemia in a mouse model of beta-thalassemia. JCI Insight. 2019 Nov 14;4(22).

49. Taher AT, Viprakasit V, Cappellini MD, Kraus D, Cech P, Volz D, et al. Haematological effects of oral administration of bitopertin, a glycine transport inhibitor, in patients with non-transfusion-dependent beta-thalassaemia. Br J Haematol. 2021 Jul;194(2):474-7.

50. Hofmann C, Banken L, Hahn M, Swearingen D, Nagel S, Martin-Facklam M. Evaluation of the effects of bitopertin (RG1678) on cardiac repolarization: a thorough corrected QT study in healthy male volunteers. Clin Ther. 2012 Oct;34(10):2061-71.

51. Disc Medicine. Investigators's Brochure: DISC-1459 (RO4917838, Bitopertin). version 1.0 ed2022.

52. Garelli E, Quarello P, Giorgio E, Carando A, Menegatti E, Mancini C, et al. Spontaneous remission in a Diamond-Blackfan anaemia patient due to a revertant uniparental disomy ablating a de novo RPS19 mutation. Br J Haematol. 2019 Jun;185(5):994-8.

53. Venugopal P, Moore S, Lawrence DM, George AJ, Hannan RD, Bray SC, et al. Self-reverting mutations partially correct the blood phenotype in a Diamond Blackfan anemia patient. Haematologica. 2017 Dec;102(12):e506-e9.

54. Jongmans MCJ, Diets IJ, Quarello P, Garelli E, Kuiper RP, Pfundt R. Somatic reversion events point towards RPL4 as a novel disease gene in a condition resembling Diamond-Blackfan anemia. Haematologica. 2018 Dec;103(12):e607-e9.

55. Errichiello E, Vetro A, Mina T, Wischmeijer A, Berrino E, Carella M, et al. Whole exome sequencing in the differential diagnosis of Diamond-Blackfan anemia: Clinical and molecular study of three patients with novel RPL5 and mosaic RPS19 mutations. Blood Cells Mol Dis. 2017 May;64:38-44.

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

57. Geller NL, Follman D, Leifer DS, Carter SL. Design of early trials in stem cell transplantation: a hybrid frequentist-Bayesian approach. In: Geller N, editor. Advances in clinical trial biostatistics. Boca Raton, FL: CRC Press; 2003. p. 41-52.

APPENDIX A: Handling, Processing, and Shipment of Pharmacokinetic Samples

Pharmacokinetic (PK) samples will be collected as a free-flowing, venipuncture sample into a 4 mL EDTA tube ("purple top", "lavender top") with the time and date drawn noted. Samples from the local institution/lab will be shipped, refrigerated to arrive within 24-48 hours of collection.

Upon receipt at the NIH, the plasma will be clarified by centrifugation at 1,500 x *g* (RCF) for 10 minutes, aliquoted into 0.5 – 1 mL aliquots, and stored at –70ºC until shipment to the processing center. All samples will be recorded in BSI (or appropriate database, see section **8.2.6**)

Plasma Tube Label Guidelines

Aliquot (plasma tubes) should be labeled with the following, at a minimum:

- Protocol ID
- Subject ID
- Study Timepoint (i.e., Day, Visit)
- Sample Type (i.e., PK)
- Unique sample ID (i.e., barcode)
- Indication of Primary or Back-up

Shipment Instructions

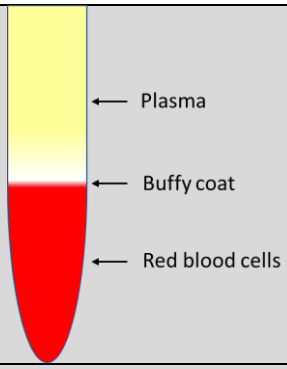
An electronic manifest (excel spreadsheet) is required for all samples shipped to Resolian Bioanalytics. A separate manifest must be created for each shipment which, at a minimum, must include the following column headings:

- Subject/participant ID
- Date collected
- Study part
- Day
- Timepoint
- Sample type (e.g., PK)

The electronic manifest should be created prior to shipment and emailed to each of the following: **samples@resolian.com**, **lgrant@resolian.com**, **mli@resolian.com**, **hmangus@discmedicine.com**, and **gmensing@discmedicine.com**

Samples should be shipped overnight on dry ice to the address below. Each shipment should include a hard copy of the manifest. Resolian is not open for sample receipt on the weekends, and most major public holidays. Please plan to ship only on Monday through Wednesday.

Resolian Bioanalytics
17 Lee Boulevard
Malvern, PA 19355

Step	Process	Visual Aid	Comments
1	Collect whole blood into a 4 mL K_2EDTA tube.		
2	Note the exact start time of blood draw.		
3	Transfer samples to the processing laboratory (if processing laboratory is different to collection site).		Samples must be kept on wet ice until centrifugation.
4	After receipt, centrifuge at 1500 RCF (g) for 10 minutes (room temperature).		If the sample has haemolysed, it should be documented in the source documents/inventory log and recorded as a laboratory-related protocol deviation.
5	Using an appropriate pipette, plasma should be divided into 2 x 2 mL pre-labelled polypropylene screw-cap tubes. Tube labels must include the following designations. 1. Primary 2. Back-Up		Primary and back-up aliquots should contain approximately 0.5 - 1 mL whole blood each. If the total yield is < 2 mL, aliquot as follows: • Primary: 1 mL • Backup: remaining plasma See label guidelines below.
6	Store the samples at or below -70°C, within 30 minutes of blood collection, until shipment to Resolian Bioanalytics.		If samples are unable to be immediately transferred to the freezer, it is acceptable to freeze the samples on dry ice for up to 4 hours prior to transferring to the freezer. Ensure a shipment manifest accompanies each shipment to Resolian Bioanalytics.