Phase I/II, Open-Label Study to Determine Safety of Trifluoperazine (TFP) in Adults with Red Blood Cell Transfusion-Dependent Diamond Blackfan Anemia

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
ALT	Alanine aminotransferase
ARC	Absolute reticulocyte count
AST	Aspartate aminotransferase
BFU-E	Burst-forming unit - erythroid
BL	Baseline
CFU-E	Colony-forming unit - erythroid
CNS	Central nervous system
CR	Complete response
CRF	Case report form
DBA	Diamond Blackfan anemia
DBAR	Diamond Blackfan Anemia Registry of North America
EoS	End of Study
EPO	Erythropoietin
FDA	Food and Drug Administration
FIH	First in human
GCP	Good Clinical Practice
Hb	Hemoglobin
HLA	Human leukocyte antigen
IA	Interim Analysis
IP	Intraperitoneal
MTD	Maximum tolerated dose
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NMS	Neuroleptic malignant syndrome
NSUH	North Shore University Hospital
PHI	Protected health information
PK	Pharmacokinetic
PO	Orally
PoC	Proof of Concept
PR	Partial response
RBC	Red blood cell

Abbreviation	Definition
SAE	Serious adverse event
SCT	Stem cell transplantation
shRNA	Small hairpin ribonucleic acid
S/T	Safety/Tolerability
TFP	Trifluoperazine
ULN	Upper limit of normal
UPIN	Unique patient identification number
USP	United States Pharmacopeia

Study Summary

Title	Phase I/II, Open-Label Study to Determine Safety of Trifluoperazine (TFP) in Adults with Red Blood Cell Transfusion-Dependent Diamond Blackfan Anemia
Short Title	TFP in DBA
Protocol Number	17-0748
Phase	Phase I/II
Methodology	Open-Label, Non-Randomized
Study Duration	18-24 months
Study Center(s)	Single-center/Additional centers may be recruited if enrollment is slow
Objectives	To determine the safety and maximum tolerated dose (MTD) of TFP in red blood cell transfusion-dependent adults with Diamond Blackfan anemia
Number of Subjects	minimum 3, maximum 24
Diagnosis and Main Inclusion Criteria	Transfusion Dependent Adults Diagnosed with Diamond Blackfan Anemia
Study Product, Dose, Route, Regimen	Trifluoperazine (TFP)1mg, 2mg, 5mg, or 10mg once daily by mouth
Duration of administration	Maximum of 21 days
Reference therapy	Red Cell Transfusion
Statistical Methodology	N/A- dose escalation study for safety/tolerability

1 Previous Study History

Has this study ever been reviewed and rejected/disapproved by another IRB prior to submission to this IRB?

☒ No ☐ Yes – if yes, please explain:

2 Brief Summary of Research

Diamond Blackfan anemia (DBA) is a rare inherited pure red cell aplasia. The two main non-stem cell transplant therapeutic options are corticosteroids and red blood cell (RBC) transfusions. About 80% of DBA patients initially respond to corticosteroids, however, half of the patients cannot continue due to side effects or loss of response. These patients are then typically dependent on RBC transfusions throughout life. Each of these treatments is fraught with many side effects and significant morbidity and mortality are potential consequences of hematopoietic stem cell transplantation (SCT). The majority of individuals with DBA have mutations in genes encoding structural proteins of the small or large ribosomal subunit leading to deficiency of the particular ribosomal protein (RP). Studies in zebrafish with RP deficiencies confirmed the association between RP defects, p53 activation and the appearance of a DBA phenotype. Using the RP deficient zebrafish embryo model, high throughput drug screens have demonstrated a strong hematologic response to several calmodulin inhibitors. One of these chemicals is trifluoperazine (TFP). TFP treatment of a mouse model of DBA also increased the red blood cell count and the hemoglobin (Hb) levels in the mice. TFP is a FDA-approved typical antipsychotic agent that has been available since 1958 with a well-known safety profile. In the United States, TFP is approved for the short-term treatment of generalized non-psychotic anxiety; treatment or prevention of nausea and vomiting of various causes; and, management of psychotic disorders.

In this study we aim to determine the safety and tolerability of TFP in adult subjects with DBA, as a prelude to a possible proof of concept (PoC) trial (as a separate study). TFP's expected dose-limiting toxicity is primarily neurologic (extrapyramidal) when used long-term at typical anti-psychotic doses (range 10-50 mg daily). Non-neurologic adverse effects in

subjects with DBA have not been investigated. We will perform a dose escalation study to define the safety and tolerability of lower doses of this agent in subjects with DBA.

This class of drugs has not been tested specifically in subjects with DBA prior to this study. To mitigate the potential risks of administering TFP to this new population, we will (1) start dosing at dose levels well below those prescribed for psychosis over the past several decades, (2) dose escalate to a maximum of 10 mg daily (the lowest dose typically prescribed for psychosis), and (3) perform weekly safety monitoring. Given the positive signal in DBA animal models and the 60-year clinical experience with higher doses of TFP, this drug warrants a trial in humans to assess tolerability in DBA. If tolerated, this trial will support either a PoC trial of low-dose TFP in DBA, or the advancement of a chemically modified TFP-like drug (to alleviate the neurologic toxicity) for the treatment of DBA.

This is a dose escalation safety and tolerability study to evaluate the presence of TFP-related adverse events in DBA subjects, and to determine the maximum tolerated dose (MTD) of TFP in DBA. The dose escalation strategy is described in Section 3.5 Dose Rationale and Risk/Benefits.

Some first-in-human (FIH) trials may implement an enrollment restriction rule such that two subjects are dosed in a given cohort, and a third subject is not dosed until both of the first two subjects have demonstrated tolerability. For this trial, we have elected not to use such an enrollment restriction design for the following reasons:

a. This is not a FIH trial: TFP has been in wide clinical use for 60 years.

b. Our dose levels (1 mg daily, up to max 10 mg daily) are all well within and below the safe range of clinical use. Doses in the 2-6 mg daily range have been shown to have a side effect profile similar to placebo in patients with anxiety (Mendels, 1986). A randomized clinical trial comparing TFP 15 mg/day vs. placebo in schizophrenic subjects revealed no difference in the rates of severe adverse effects, Parkinsonism or dystonia compared to placebo (Koch, 2014). Doses for psychosis range from 10 mg daily up to 40-50 mg daily, whereas we will dose at 1, 2, 5, and 10 mg daily only.

c. We will monitor for safety, specifically for any hematologic toxicity and for extrapyramidal/central nervous system (CNS) effects. Dose escalation criteria and actions, as well as subject and cohort stopping rules are detailed in Section 6 Study Design.

3 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and Institutional research policies and procedures.

3.1 Background

Diamond Blackfan Anemia

Diamond Blackfan anemia is a rare inherited pure red cell aplasia. Over the past 20 years, mutations have been described in genes encoding both the small and large ribosome associated proteins (Vlachos, 2014; Draptchinskaia, 1999; Gazda, 2006; Cmejla, 2007; Farrar, 2008; Gazda 2008; Doherty 2010). There is wide variability in clinical and biologic features, familial history, and therapeutic responses. Currently standard therapy includes corticosteroids, RBC transfusions or SCT (Vlachos, 2001a). Approximately 80% of subjects have an initial response to corticosteroids. Of these subjects, approximately half will fail to achieve a taper to a tolerable steroid dose or have intolerable side effects and will need to discontinue steroid therapy. These subjects and the 20% who do not respond initially to such therapy must be maintained on a chronic RBC transfusion with iron chelation. Chronic transfusions have considerable treatment-related morbidity, most notably iron overload-related organ failure.

SCT has proven to be effective therapy for a select group of subjects. Allogeneic SCT with a histocompatible donor has been performed successfully in a number of subjects. The Diamond Blackfan Anemia Registry of North America (DBAR) experience has been published (Vlachos, 2001b; Lipton 2006). However, because of remissions (sustained physiologically acceptable erythropoiesis for ≥6 months without any medication or transfusion) in up to 20% of subjects with DBA, SCT, regardless of donor type, is not consistently offered to corticosteroid-dependent or transfusion-dependent subjects with DBA and no other complications precluding corticosteroid or transfusion use. For many subjects, the lack of a suitable donor does not permit SCT as a therapeutic option, as human leukocyte antigen (HLA)-matched unrelated donor transplants have been associated with mortality and morbidity.

Historically, the lack of truly effective and safe therapy has prompted the investigation of a number of other agents, including high-dose corticosteroids, immunosuppressive therapy (cyclosporine A and antithymocyte globulin), intravenous immunoglobulin, high-dose erythropoietin, interleukin-3, metoclopramide and others; however, these modalities have achieved, at best, anecdotal success (Vlachos, 2008).

3.2 Investigational Agent - Trifluoperazine

3.2.1 Description

TFP is a piperazine phenothiazine antipsychotic with 5 mg orally equivalent to 100 mg of oral chlorpromazine, the prototype antipsychotic. TFP is a typical antipsychotic of the phenothiazine chemical class that has been used for the treatment of psychiatric subjects since 1958 (Rudy, 1958). TFP acts as a dopamine antagonist with antipsychotic and antiemetic activities. TFP blocks CNS postsynaptic mesolimbic dopaminergic D1 and D2 receptors and depresses the release of hypothalamic and hypophyseal hormones and the reticular activating system, thus affecting basal metabolism, body temperature, wakefulness, vasomotor tone, and emesis. In the United States, trifluoperazine hydrochloride tablets, USP, are approved for the short-term treatment of generalized non-psychotic anxiety; treatment or prevention of nausea and vomiting of various causes; and management of psychotic disorders. In part because of its side effect profile, TFP has been largely replaced by second and third generation anti-psychotic medications. It is associated with an increased risk of death in elderly subjects treated for dementia-related psychosis. Nevertheless, TFP remains commercially available worldwide and the risks attendant to its use are well understood and generally manageable.

3.2.2 Pharmacokinetics

Trifluoperazine may be administered orally or intramuscularly. This study will only use the oral route of administration. Following administration of the drug, antipsychotic effects are gradual, with considerable individual subject variation, and peak effects may not occur for 6 weeks to 6 months. TFP is readily absorbed from the gastrointestinal tract and is subject to first-pass metabolism in the gut wall. It is metabolized in the liver and excreted in the urine and feces in the form of active and inactive metabolites. TFP is extensively bound to plasma proteins. The drug crosses the blood-brain barrier and its metabolites also cross the placental barrier and are excreted in milk (MHRA, PAR, 2006).

The single-dose pharmacokinetics (PK) of TFP were determined in 5 healthy adult male volunteers who received TFP 5 mg orally after an overnight fast (Midha, 1983). Peak TFP concentrations varied widely (range 0.53 to 3.09 ng/mL^{-1}) and were reached at 2.8 ± 0.5 hours post-dose. The area under the plasma concentration-time curve also differed widely among subjects (range 5.9 to 17.6 $ng/mL^{-1}{\cdot}h$; mean 11.7 ± 2.2 $ng/mL^{-1}{\cdot}h$), suggesting large individual

differences in the extent of pre-systemic TFP elimination. The mean apparent terminal elimination half-life (t1/2) of TFP was 12.5 ± 1.4 hours.

More relevant to its function as a potential medication in DBA, TFP also serves as an inhibitor of calmodulin, a multi-functional, intermediate calcium-binding messenger protein.

3.3 Preclinical Data

3.3.1 In Vivo and In Vitro Activity in DBA Models

It has been demonstrated that zebrafish embryos with mutations in the *rps29* gene have an erythroid defect, similar to that reported in subjects with DBA (Taylor, 2012). Furthermore, zebrafish embryos also have endothelial defects as observed by decreased flk1 expression in the intersegmental vessels at 24 hours post-fertilization. Consistent with other animal models of RP mutations, the p53 pathway is activated in the rps29$^{-/-}$ embryo, and p53 knockdown in the mutant zebrafish rescues the hematopoietic and endothelial defects (Taylor, 2012).

In order to identify compounds with the ability to rescue DBA phenotypes *in vivo*, 600 chemicals were screened for their ability to rescue flk intersegmental vessels and reverse the erythroid defect in rps29$^{-/-}$ zebrafish embryos. Findings revealed that several structurally diverse calmodulin inhibitors successfully rescued flk staining in the vasculature and Hb levels in the mutant embryo.

TFP was also tested in a murine model of DBA in which mice were transplanted with bone marrow from mice harboring a knock-in allele expressing a doxycycline-inducible rps19 shRNA (Montague, 2014). Following engraftment of the transplanted cells, recipient mice were administered doxycycline in drinking water to induce the rps19 deficiency, p53-induced p21 expression, and severe anemia. Diseased mice then received an intraperitoneal (IP) injection of 5 mg/kg TFP every other day, and after two weeks, bone marrow was collected for quantitative polymerase change reaction (qPCR) analysis and blood was analyzed for RBC counts and Hb levels. TFP treatment of rps19-deficient mice decreased p21 levels, indicating that TFP was hitting the expected target, and significantly increased both RBC counts and Hb levels compared to vehicle-treated controls (Macari, 2015). Importantly, TFP reduced p53 activity only in the presence of the stress-induced state of rps19 deficiency, while basal levels of p53 in wild-type mice actually increased modestly. Furthermore, the levels of p53 activity in the rps19-deficient mice treated with TFP were not lower than those in wild type mice. TFP was well tolerated in this model, indicating that the TFP dose resulting in improved anemia did not cause gross toxicity.

3.3.2 In Vitro Activity of TFP in Human CD34^{+} Cells

In vitro studies in normal human $CD34^+$ cells transduced with small hairpin ribonucleic acid (shRNA) against RPS19 and in primary $CD34^+$ bone marrow cells from subjects with DBA with *RPS19* and *RPS29* mutations confirmed the association of RP deficiency with upregulation of p53 and rescue of the ensuing DBA phenotype with TFP. (Macari, 2016)

The hypocellularity of bone marrow in subjects with DBA presents a challenge for obtaining adequate material for testing drugs, necessitating an alternative strategy. Consequently, human $CD34^+$ cells were transduced with shRNA against RPS19 and erythroid differentiation was evaluated in a two-stage process adapted from Zu *et al*. (Zu, 2013). During the expansion phase, RPS19 knockdown increased p53 protein and p53 target gene, p21 and inhibited erythroid differentiation, demonstrated by a 30% reduction in $CD71^+$ cells at Day 12. Treatment of these cells with TFP during the differentiation phase rescued $CD71^+$ erythroid precursor cells to that of control and reduced expression of p21 (Macari, 2015). In addition, TFP treatment specifically decreased p53 in RPS19-deficient cells, but not in control cells. This demonstrates that the effect of TFP on p53 activity is specific to RP deficient cells and that TFP restores p53 to normal levels.

Primary $CD34^+$ cells from bone marrow samples of two subjects with DBA (*RPS19* R94X and *RPS29* I31F) were treated *in vitro* with a single dose of TFP and improved erythroid differentiation was observed (Macari, unpublished data).

In summary, multiple lines of evidence demonstrate that TFP, the compound identified in a zebrafish chemical screen, restores erythropoiesis in models of DBA, providing a strong rationale for clinical studies in subjects with DBA. Furthermore, TFP appears to lower elevated p53 activity levels in the diseased state with little effect on basal levels.

3.4 Clinical Data to Date

There are no available clinical research data on TFP in subjects with DBA.

A randomized, double-blind, placebo-controlled 4-week trial of low dose TFP (2–6 mg/day) in 415 subjects with generalized anxiety disorder showed that the side effect profile of TFP and

placebo were similar. Anxiety was also significantly improved in the TFP treatment arm (Mendels, 1986).

A randomized clinical trial comparing TFP 15 mg/day vs. placebo in schizophrenic subjects revealed no difference in the rates of severe adverse effects, Parkinsonism or dystonia compared to placebo. Subjects in the TFP group did experience higher rates of akathisia than did the placebo group. However, significantly more placebo arm subjects left the study early due to poor efficacy, in comparison to TFP-treated subjects (Koch, 2014).

3.5 Dose Rationale and Risk/Benefits

In an *in vivo* mouse model of DBA, TFP at a dose of 5 mg/kg intraperitoneally every other day reduced apparent p53 activity and raised the RBC count and Hb level after 2 weeks of treatment (Macari, 2016). The murine dose scales allometrically to a daily dose of 12–15 mg of TFP in humans. As the 5 mg/kg dose was the only dose tested in the mouse study, and daily administration of TFP may be more efficacious than every other day dosing, it is possible that a daily dose of 5 mg or less in humans may be efficacious. Given the potential margin of error in the allometric calculation, a maximum dose of 10 mg daily may be tested in this trial.

The starting dose of TFP in subjects with DBA (1 mg) is lower than the starting dose in psychiatric indications, and the maximum TFP dose to be administered to subjects with DBA will not exceed 10 mg. As side effects of TFP have been shown to be dose-/plasma level-related, it is anticipated that the more severe side effects associated with TFP, including extrapyramidal symptoms, will be less likely to occur at doses ≤10 mg. The two trials cited in section 3.4 Clinical Data to Date also support the likely tolerability of the proposed dose range in this trial (Mendels, 1986; Koch, 2014).

3.5.1 Dose Escalation

Up to 4 cohort dose levels of TFP will be studied: 1, 2, 5, and 10 mg/day for up to 21 days each, as tolerated. Cohort A will start at 1 mg/day, and the dose will escalate to 2 mg/day in Cohort B, 5 mg/day in Cohort C, and 10 mg/day in Cohort D. At the start of each cohort, 3 subjects will be enrolled, and an interim analysis (IA) for safety and tolerability will be

conducted after all 3 subjects have completed 21 days of treatment (See Section 6 Study Design for details). The 21-day time frame is based on the t1/2 from the healthy volunteer 5 mg single dose TFP study (Midha, 1983), showing that TFP's elimination t1/2 is approximately 12-14 hours. Therefore, steady state is reached approximately 3 days after initiating therapy, and 21 days of therapy provides at least 18 days of exposure at steady state (the 1 and 2 mg doses likely have shorter t1/2 and so steady state will be reached even sooner in the first 2 cohorts).

Whereas some FIH trials may use an enrollment restriction rule such that only two subjects are dosed at a time, this trial will not use such an enrollment restriction design. Please see Section 2 Brief Summary of Research for the supporting rationale.

3.5.2 Dose Modifications

Dose of TFP will be discontinued in case of sustained increase of hemoglobin ≥ 2 gm/dL from the previous dose except those attributed to RBC transfusions. Dose should be held in the event of an AE ≥ Grade 3 until resolution (to baseline) or ≤ Grade 1. If AE is not resolved in 7 days then TFP will be discontinued. The guidelines for dose modifications are listed below.

Dose Modification Guidelines

NCI Toxicity-Grade	Event	Action
	Hb ≥ 12 gm/dL (not associated with RBC transfusion)	Discontinue *
≥ Grade 3	Any adverse event	Dose delay **

* Subject will discontinue the study drug.

** Delay dose of TFP for up to 7 days or until AE severity improves to ≤ Grade 1 or to Baseline status.

4 Study Objectives

Primary Objective

- To evaluate the safety profile of a 21-day course of TFP at doses up to 10 mg/day in RBC transfusion-dependent adults with DBA

5 Resources Available to Conduct the Human Research

Adrianna Vlachos, MD is the PI and sponsor of this study and the Director of the Diamond Blackfan Anemia Registry (DBAR) of North America. The DBAR is housed at the Feinstein Institute for Medical Research. There are over 750 subjects with Diamond Blackfan anemia enrolled in the DBAR. Because of this, the investigator has access to the specific subject population required for this study. Subjects/parents of subjects and their physicians contact the DBAR periodically to inquire about available studies. Also, the DBA Foundation (DBAF), the family support group, has access to most of the DBA families. The DBAF is highly supportive of the research and they also encourage the families to contact the DBAR for any available studies. We have prepared an announcement to inform the subjects of the study as we have for other studies in the past. This has worked well previously, and we do not expect having any issues in recruiting subjects.

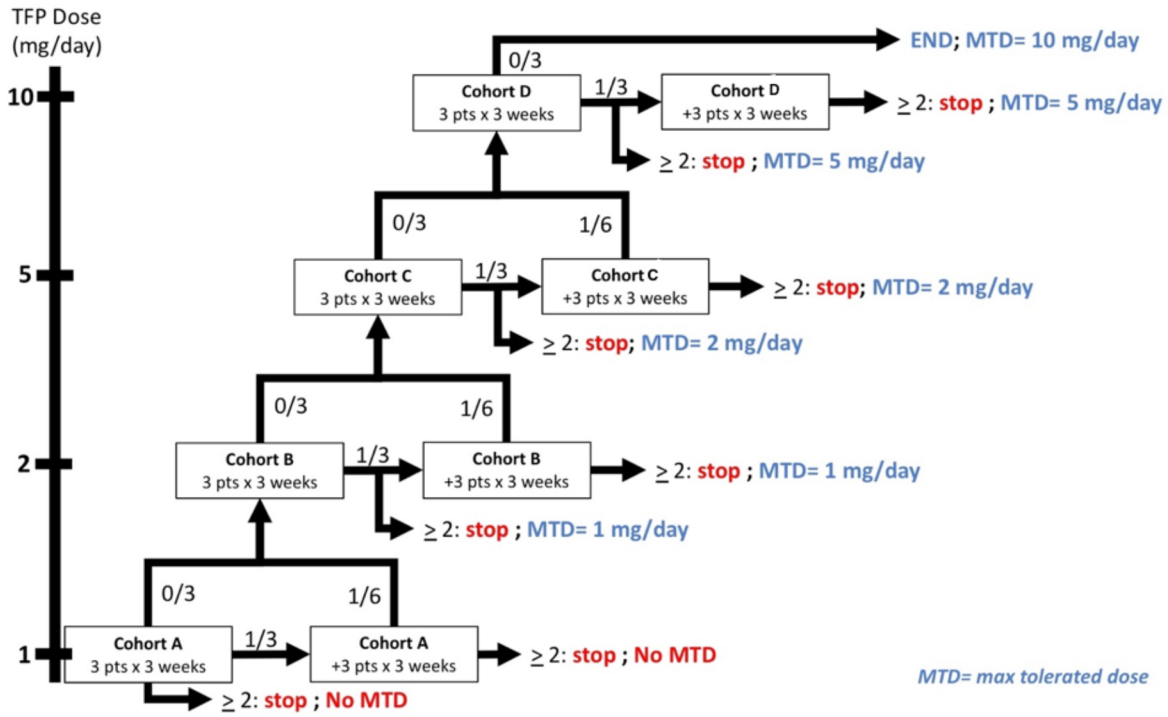
With regards to the trial logistics, the protocol is reviewed at the site initiation visit with all study personnel, along with the trial-related duties and functions.

6 Study Design

6.1 General Design

This study is a multi-center, Phase I/II, dose escalation, open-label study to determine the safety of TFP in adult subjects with DBA and RBC-transfusion-dependence. A 3+3 dose escalation regimen will be used. The maximum time on study drug will be 21 days with a 1 week post-treatment follow-up period. It is expected that up to 24 subjects can be recruited and complete the study within 12-18 months. The dose escalation study design is shown in Figure 1:

Figure 1. Dose escalation study design



1. Cohort A: Three subjects will receive TFP 1 mg PO daily.
    - If there is no toxicity ≥ Grade 3 at the end of the 21 days, Cohort B will start.
    - If 1/3 subjects in Cohort A demonstrates toxicity ≥ Grade 3, an additional 3 subjects will be enrolled in Cohort A:
        - If 2 or more of the 6 subjects in Cohort A demonstrate toxicity ≥ Grade 3, the trial will be stopped; no MTD will be declared.
        - If < 2 of the 6 subjects in Cohort A demonstrate toxicity ≥ Grade 3 within 21 days of starting therapy, Cohort B will start.
2. Cohort B: Three subjects will receive TFP 2 mg PO daily.
    - If there is no toxicity ≥ Grade 3 at the end of the 21 days, Cohort C will start.
    - If 1/3 subjects in Cohort B demonstrates toxicity ≥ Grade 3, an additional 3 subjects will be enrolled in Cohort B:

- If 2 or more of the 6 subjects in Cohort B demonstrate toxicity ≥ Grade 3, the study will be stopped, and 1 mg/day will be declared the MTD.
- If < 2 of the 6 subjects in Cohort B demonstrate toxicity ≥ Grade 3 within 21 days of starting therapy, Cohort C will start.

3. Cohort C: Three subjects will receive TFP 5 mg PO daily.
   - If there is no toxicity ≥ Grade 3 at the end of the 21 days, Cohort D will start.
   - If 1/3 subjects in Cohort C demonstrates toxicity ≥ Grade 3, an additional 3 subjects will be enrolled in Cohort C:
     - If 2 or more of the 6 subjects in Cohort C demonstrate toxicity ≥ Grade 3, the study will be stopped, and 2 mg/day will be declared the MTD.
     - If < 2 of the 6 subjects in Cohort C demonstrate toxicity ≥ Grade 3 within 21 days of starting therapy, cohort D will start.
4. Cohort D: Three subjects will receive TFP 10 mg PO daily.
     - If 0/3 subjects in Cohort D demonstrates toxicity ≥ Grade 3, the study will be stopped, and 10 mg/day will be declared the MTD.
     - If 1/3 subjects in Cohort D demonstrates toxicity ≥ Grade 3, an additional 3 subjects will be enrolled in Cohort D.
     - If 2 or more of the 6 subjects in Cohort D demonstrate toxicity ≥ Grade 3, the study will be stopped, and 5 mg/day will be declared the MTD.
     - If <2 of the 6 subjects in Cohort D demonstrate toxicity ≥ Grade 3 within 21 days of starting therapy, 10mg/day will be declared the MTD.

Each subject will undergo safety assessments as per the required schedule while on the study drug. All dosed subjects will be followed for an additional 1 week after discontinuing study drug (post-study safety follow-up). There will be no more than 6 subjects enrolled at any particular time.

Treatment will be discontinued for any subject if their Hb is > 12 gm/dL, not associated with RBC transfusions.

6.2 Primary Study Endpoints

- Safety profile: No adverse events greater than Grade 3-4
- Safety: Treatment-emergent adverse events (AEs)

6.3 Secondary Study Endpoints

- Pharmacodynamic markers of TFP target engagement

6.4 Primary Safety Endpoints

The primary safety endpoint is to evaluate the safety profile of TFP for red blood cell transfusion-dependent adults with DBA.

7 Subject Selection and Withdrawal

7.1 Inclusion Criteria

In order to be eligible to participate subjects must meet all of the following criteria:

1. Men and women age: ≥18 years and <65 years of age.
2. Weight: ≥45 kilograms.
3. DBA diagnosed according to the DBA criteria (Vlachos, 2008) (Appendix B).
4. RBC transfusion-dependence (defined as 2 units packed RBCs per 28 days averaged over 84 days [12 weeks] prior to study entry) (Gale, 2011) (Appendix C).
5. Calculated creatinine clearance ≥ 30 mL/min (Appendix D).
6. Karnofsky performance status scale score ≥ 70 (Appendix E).
7. Female subjects of childbearing potential participating in the study are to use highly effective methods of birth control (abstinence, oral contraceptives, barrier method with spermicide or surgical sterilization) during study participation and for 1 week following the last dose of TFP (a total of 4 weeks of the study). Female subjects of childbearing potential must have a negative serum β-human chorionic gonadotropin pregnancy test within 3 days prior to the start of TFP given on Day 1. Subjects must be counseled concerning measures to be used to prevent pregnancy and potential toxicities prior to the first dose of TFP. A female subject of childbearing potential is a sexually mature woman who has not undergone a hysterectomy or bilateral oophorectomy; or who has not been postmenopausal for at least 24 consecutive months (i.e., who has had menses at some time in the preceding 24 months).
8. Male subjects must agree to use a latex condom during any sexual contact with females of childbearing potential while participating in the study and for 1 week following the last dose of TFP, even if he has undergone a successful vasectomy (a total of 4 weeks of the study). Male subjects must be counseled concerning measures to be used to prevent pregnancy and potential toxicities prior to the first dose of TFP.
9. Agreement to adhere to the study visit schedule, understand and comply with all protocol requirements.
10. The subjects are capable of giving informed consent.

7.2 Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible to participate in the study:

1. Liver: aspartate aminotransferase (AST) > 5 x the upper limit of normal (ULN), alanine aminotransferase (ALT) >5 x ULN, or bilirubin > 5 x ULN.
2. Heart disease (New York Heart Association classification of ≥ 3; Appendix F).
3. History of angina.
4. Uncontrolled hypertension. If hypertension is considered clinically stable, systolic blood pressure must be <150 mmHg or diastolic blood pressure must be <100 mmHg.
5. Subjects currently responsive to corticosteroids for treatment of DBA.
6. Treatment with another investigational drug or device <56 days pre-study entry. Subjects who participated in an earlier cohort of this trial may participate in a subsequent cohort if (a) they meet all eligibility criteria, (b) did not experience any adverse effects ≥ Grade 3 while on TFP, and (c) discontinued TFP ≥ 6 days (10 half-lives) prior to day 1 of the subsequent cohort.
7. Pregnant or lactating females.
8. Any history of severe allergic reaction requiring the use of epinephrine.
9. Known hypersensitivity to the study drug or other phenothiazines.
10. History or presence of extrapyramidal signs.
11. History of cancer.

7.3 Vulnerable Populations

No vulnerable populations are specifically being targeted for this study.

☐ *Children or viable neonate*
☐ *Cognitively impaired*
☐ *Pregnant Women, Fetuses or neonates of uncertain viability or nonviable*
☐ *Prisoners*
☐ *NSLIJ Employees, residents, fellows, etc*
☐ *poor/uninsured*
☐ *Students*
☐ *Minorities*
☐ *Elderly*

☐ *Healthy Controls*

7.4 Subject Recruitment and Screening

Adrianna Vlachos, MD, is the PI and the Director and Jeffrey Lipton, MD, PhD is a Co-Investigator and the Co-Director of the Diamond Blackfan Anemia Registry of North America, respectively. The DBAR is housed at the Feinstein Institute for Medical Research. There are over 750 subjects with Diamond Blackfan anemia enrolled in this Registry. Because of this, the investigators have access to the specific subject population required for this study. Subjects contact the DBAR periodically to inquire about available studies. Also the DBA Foundation (DBAF) (the family group) has access to most of the DBA families. The DBAF is highly supportive of the research and they also encourage the families to contact the DBAR for any available studies. We have prepared a study announcement (Appendix G) to inform the subjects of the study as we have for other studies in the past. This will be posted on the DBAF website (mostly visited by subjects/families) and our DBAR website (often visited by physicians as well as subjects/families) and has worked well previously.

Once informed consent is obtained from the subject, the following screening requirements will be performed:

1- Physical examination (including height at 1st visit only, weight, vital signs, blood pressure and neurological examination)
2- Hematology evaluation including RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and platelet count
3- Blood chemistry including blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], and alanine aminotransferase [ALT])
4- If female, serum pregnancy test
5- Documentation of transfusion history for > 3 months before initiation of treatment

7.5 Consent Process

Informed consent will be obtained by the study PI or authorized co-investigators after they have fully explained the study. The study will be presented to all eligible subjects and explained in terms that are appropriate for each subject. After a full discussion and the

opportunity to ask questions, subjects will be consented. The subject may take as much time as needed to decide on his/her participation in the study, and may consent after the initial contact is made. The consent will be obtained either in the outpatient area at a regular office visit or via mail, after the study is discussed on a telephone call.

The subjects with DBA who may be eligible for this study may live in any state in the United States. Since travel may be difficult, it is often necessary to screen the subject for eligibility before they travel for the study. Therefore the consent is mailed to the subject. Once the subject has the consent in hand, the consent will be reviewed with him/her over the phone in its entirety. Questions can be asked and answered and the subject can determine the appropriateness of the study for him/herself. Once consent is signed and received by the PI, then the screening procedure can begin. Eligibility can therefore be determined prior to the subject's travel and confirmed upon subject's arrival to the office visit. A standard informed consent form will be utilized for this study. Subjects may have family members, their local physicians and any other chosen individuals review the consent with them prior to their signing. For subjects who sign consent remotely, the study PI or authorized co-investigator will review the study and the consent form again when the subject comes for the in-person physical examination and to begin the study. At that time an enrollment note will document the continued willingness of the subject to participate in the study.

The study is 4 weeks in duration and subjects may withdraw their consent at any time. Subjects will be seen weekly for the actual study drug timeframe of the study and will be given the opportunity to withdraw consent at any time.

This study does not involve subjects less than 18 years of age.

This study does not involve cognitively impaired adults.

Non-English speaking subjects with DBA are eligible for this study. Our institution provides a translator service through Pacific Interpreters, Inc. Any language can be accommodated. The translator will be used to explain the consent and give the subject an opportunity to ask questions and have them answered. The subject will be given a short form of the consent to sign.

7.6 Early Withdrawal of Subjects

7.6.1 When and How to Withdraw Subjects

The following reasons are considered sufficient for withdrawing a subject from the study drug and/or from the study:

- Sustained hemoglobin ≥ 12 mg/dL (not influenced by transfusions).
- Any toxicity ≥ Grade 3 (Appendix H).
- Adverse event that, in the judgment of the investigator, may cause severe or permanent harm or that rules out continuation of treatment.
- Hypersensitivity reaction to TFP.
- Withdrawal of consent.
- Death.
- Lost to follow-up.
- Pattern of significant noncompliance.

The reason for subject withdrawal must be documented in the subject chart / source records.

7.6.2 Data Collection and Follow-up for Withdrawn Subjects

Subjects who discontinue from the Treatment Period early will still continue to the Follow-Up Period for 1 week from the last dose of TFP. For any subjects lost to follow-up, attempts will be made by phone to obtain permission to record at least survival data up to the end of study follow-up period.

8 Study Drug

8.1 Description

TFP is a commercially-available phenothiazine tranquilizer with potent anti-psychotic, anti-anxiolytic and antiemetic activity.

TFP is commercially available in the United States in a variety of formulations, including the following:

1 mg tablets are lavender, film-coated, round, unscored tablets debossed with 1 on one side of the tablet and GG51 on the other side. They are available as follows: NDC 0781-8028-01, in bottles of 100 tablets.

5 mg tablets are lavender, film-coated, round, unscored tablets debossed with 5 on one side of the tablet and GG55 on the other side. They are available as follows: NDC 0781-8034-01, in bottles of 100 tablets.

8.2 Treatment Regimen

Trifluoperazine will be given orally once daily as 1 mg, 2 mg (two 1mg tablets), 5 mg, or 10 mg (two 5mg tablets). The treatment duration will be up to 21 days (3 weeks).

8.3 Method for Assigning Subjects to Treatment Groups (RANDOMIZATION)

There is no randomization in this study. All subjects who qualify for the study will be enrolled into the study and receive up to 3 weeks of TFP. A unique multi-digit subject identification number will be manually assigned by the site staff to each subject who signs consent.

8.4 Preparation and Administration of Study Drug

The staff in the pharmacy will fill out the following information on the Trifluoperazine Drug Accountability Log upon distribution to the subject:

- Date
- Visit
- Subject Number
- Subject Initials
- Dose
- Number of pills dispensed
- Lot Number

The site personnel will confirm the lot number for TFP and the number of pills dispensed and record this information on the Trifluoperazine Drug Accountability Log and in the pharmacy records.

8.5 Subject Compliance Monitoring

The study team will assess compliance with the study treatment by report at each study visit. The subject may be discontinued by the study PI at any time for non-compliance with the study treatment regimen.

8.6 Prior and Concomitant Therapy

All concomitant medications will be collected. Prior corticosteroid therapy will be collected as well. All concomitant medications except for steroids are permitted during the study.

8.7 Packaging

TFP is to be dispensed in a tight, light-resistant container, as defined in the USP, using a child-resistant closure. The subject will receive a 21 day supply of 1 mg or 5 mg tablets. The label for TFP will include the institution name, address and telephone number, the protocol number, drug name, dosage form and strength, amount of drug per container, lot number, expiration date, medication identification, dosing instructions, storage conditions, and required caution statements and/or regulatory statements as applicable. Additional information may be included on the label as applicable per local regulations.

8.8 Blinding of Study Drug/Device

There is no blinding of the study drug in this study.

8.9 Receiving, Storage, Dispensing and Return

8.9.1 Receipt of Drug Supplies/Device

Upon receipt of the study drug, an inventory must be performed and a Drug Receipt Log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in

the study files. The investigator must notify study sponsor of any damaged or unusable study drug that were supplied to the investigator's site.

8.9.2 Storage

TFP tablets are to be stored at 20° to 25°C (68° to 77°F) in the study pharmacy.

8.9.3 Dispensing of Study Drug

The subject will be dispensed a 21 day supply of 1 mg or 5 mg tablets based on Dose Level 1, 2, or 3 and 4, respectively. The subject will receive study drug once during this study.

8.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

9 Study Procedures (Screening and Visits 1-4)

Please see Appendix A for a Schedule of Study Assessments.

9.1 Screening

The Screening Period is from Day -28 to Day -1 prior to the subject starting the study drug on Day 1 of the study. If consent was not previously obtained by mail then the PI will obtain informed consent at a Screening visit. If the consent was obtained by mail then the consent will be reviewed again with the subject at the initial visit. The subject will be then be assessed for inclusion and exclusion criteria with a review of his/her medical record, including prior treatments, procedures, and transfusion history (for the previous 12 weeks at least). A physical examination including height at 1st visit, weight, vital signs, blood pressure and neurological exam will be performed on the subject. Karnofsky performance status will be assessed. Blood work will be performed for a complete blood count (CBC; RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and

platelet count), serum chemistry panel for hepatic and renal function (blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], and alanine aminotransferase [ALT]). If the subject is a female, then menstrual status will be assessed and a serum pregnancy test will also be performed. If all the criteria are evaluated and subject is confirmed to start the study, Screening and Visit 1 may be combined into one visit and the Screening blood work can be used as the Visit 1 blood work if within ± 1 day.

9.2 Visit 1

Visit 1 will be Day 1 of the study and will be the day that the subject starts the study drug. If Screening assessments were completed the day before then the subject's physical examination and blood work results can be used for Visit 1 as well. A physical examination including height at 1st visit, weight, vital signs, blood pressure and neurological exam will be performed on the subject. Karnofsky performance status will be assessed. Blood work will be performed for a complete blood count (CBC; RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and platelet count), serum chemistry panel for hepatic and renal function (blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], and alanine aminotransferase [ALT]). If the subject is a female, then menstrual status will be assessed and a serum pregnancy test will also be performed. Blood for pharmacodynamic marker analysis will be collected, if applicable (Appendix J). All concomitant therapies will be documented. The subject will be dispensed a 21-day supply of the study drug once all evaluable criteria are met and will start the study drug on Day 1. Visit 1 should occur within 7 days following a blood transfusion.

9.3 Visit 2

Visit 2 will be Day 11 (± 1 day) of the study. The subject will undergo full safety analysis, including a physical examination (including weight, vital signs, blood pressure and neurological exam). Karnofsky performance status will be assessed. Blood work will be performed for a complete blood count (CBC; RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and platelet count), serum chemistry panel for hepatic and renal function (blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], and alanine aminotransferase [ALT]), and

pharmacodynamic marker analysis, if applicable (Appendix J). If the subject is a female, menstrual status will be assessed and a serum pregnancy test will also be performed. Transfusion history will be documented. The subject will be questioned and evaluated for any adverse events. All concomitant therapies will be documented.

9.4 Visit 3

Visit 3 will be Day 22 (± 2 days) of the study. The subject will undergo full safety analysis, including a physical examination (including weight, vital signs, blood pressure and neurological exam). Karnofsky performance status will be assessed. Blood work will be performed for a complete blood count (CBC; RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and platelet count), serum chemistry panel for hepatic and renal function (blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], and alanine aminotransferase [ALT]), and pharmacodynamic marker analysis, if applicable (Appendix J). If the subject is a female, menstrual status will be assessed. Transfusion history will be documented. The subject will be questioned and evaluated for any adverse events. All concomitant therapies will be documented.

9.5 Visit 4

Visit 4 will be Day 29 (± 2 days) of the study (Follow-up Period). The subject will undergo post-treatment safety assessment including a physical examination and blood work (CBC and chemistry panel). Transfusion history will be documented. The subject will be questioned and evaluated for any adverse events. This visit may be done virtually through a system that can visually assess the subject for neurologic issues. Blood work may be done at a local laboratory.

10 Risks to Subjects

Adverse reactions with TFP include drowsiness, dizziness, skin reactions, rash, dry mouth, insomnia, amenorrhea, fatigue, muscular weakness, anorexia, lactation, blurred vision, and neuromuscular (extrapyramidal) reactions. Details regarding extrapyramidal and other adverse reactions with TFP follow:

- Neuromuscular (Extrapyramidal) Reactions: These symptoms are characterized by motor restlessness. They may be of the dystonic type, or they may resemble Parkinsonism (described below).
- Motor Restlessness: Symptoms may include agitation or jitteriness and sometimes insomnia. These symptoms often disappear spontaneously.
- Dystonia (Class Effect): Symptoms of dystonia, prolonged abnormal contractions of muscle groups, may occur in susceptible individuals during the first few days of treatment. Dystonic symptoms include spasm of the neck muscles, sometimes progressing to tightness of the throat, swallowing difficulty, difficulty breathing, and/or protrusion of the tongue. While these symptoms can occur at low doses, they occur more frequently at higher doses of first generation antipsychotic drugs.
- Pseudoparkinsonism: Symptoms may include mask-like facies, drooling, tremors, pill-rolling motion, cogwheel rigidity, and shuffling gait.
- Tardive Dyskinesia: Tardive dyskinesia is a rare syndrome characterized by rhythmical involuntary movements of the tongue, face, mouth, or jaw (e.g., protrusion of tongue, puffing of cheeks, puckering of mouth, chewing movements). These symptoms may be accompanied by involuntary movements of extremities. In rare instances, these involuntary movements of the extremities are the only manifestations of tardive dyskinesia. Symptoms may persist for months or years and, while they gradually disappear in some subjects, they may be irreversible in others. The risk appears to be greatest in elderly subjects (especially women) on prolonged, high-dose therapy. There is no known effective treatment for tardive dyskinesia. It has been reported that fine vermicular movements of the tongue may be an early sign of the syndrome and if the medication is stopped at that time the syndrome may not develop.
- Adverse Reactions Reported with TFP or Other Phenothiazine Derivatives: Adverse effects with different phenothiazines vary in type, frequency, and mechanism of

occurrence, i.e. some are dose-related, while others involve individual subject sensitivity. Some adverse effects may be more likely to occur, or occur with greater intensity, in subjects with special medical problems, e.g., subjects with mitral insufficiency or pheochromocytoma have experienced hypotension following recommended doses of certain phenothiazines.

- Neuroleptic malignant syndrome (NMS) has been reported in association with antipsychotic drugs. Cardinal features of NMS are hyperpyrexia, muscle rigidity, altered mental status (including catatonic signs), and evidence of autonomic instability (irregular pulse or blood pressure). Additional signs may include elevated creatine phosphokinase, myoglobinuria (rhabdomyolysis), and acute renal failure. NMS is potentially fatal and requires symptomatic treatment and immediate discontinuation of neuroleptic treatment.
- Not all of the following adverse reactions have been observed with every phenothiazine derivative, but they have been reported with one or more and should be borne in mind when drugs of this class are administered: extrapyramidal symptoms (opisthotonos, oculogyric crisis, hyperreflexia, dystonia, akathisia, dyskinesia, parkinsonism) some of which have lasted months and even years–particularly in elderly subjects with previous brain damage; grand mal and petit mal convulsions, particularly in subjects with electroencephalogram abnormalities or history of such disorders; altered cerebrospinal fluid proteins; cerebral edema; intensification and prolongation of the action of central nervous system depressants (opiates, analgesics, antihistamines, barbiturates, alcohol), atropine, heat, organophosphorus insecticides; autonomic reactions (dryness of mouth, nasal congestion, headache, nausea, constipation, obstipation, adynamic ileus, ejaculatory disorders/impotence, priapism, atonic colon, urinary retention, miosis and mydriasis); reactivation of psychotic processes, catatonic-like states; hypotension (sometimes fatal); cardiac arrest; blood dyscrasias (pancytopenia, thrombocytopenic purpura, leukopenia, agranulocytosis, eosinophilia, hemolytic anemia, aplastic anemia); liver damage (jaundice, biliary stasis); endocrine disturbances (hyperglycemia, hypoglycemia, glycosuria, lactation, galactorrhea, gynecomastia, menstrual irregularities, false-positive pregnancy tests); skin disorders (photosensitivity, itching, erythema, urticaria, eczema up to exfoliative

dermatitis); other allergic reactions (asthma, laryngeal edema, angioneurotic edema, anaphylactoid reactions); peripheral edema; reversed epinephrine effect; hyperpyrexia; increased appetite; increased weight; a systemic lupus erythematosus-like syndrome; pigmentary retinopathy; with prolonged administration of substantial doses, skin pigmentation, epithelial keratopathy, and lenticular and corneal deposits.

- Electrocardiogram changes, particularly nonspecific, usually reversible Q and T wave distortions, have been observed in some subjects receiving phenothiazine antipsychotics.

There have been occasional reports of sudden death in subjects receiving phenothiazines. In some cases, the cause appeared to be cardiac arrest or asphyxia due to failure of the cough reflex.

The effects of TFP on fertility or a fetus are not known. The side effects of TFP on newborns are also not known, therefore breastfeeding will not be allowed on the study. The subject will be removed from the study if she becomes pregnant during the study.

Adult subjects with DBA who are on chronic transfusion therapy may have iron overload despite iron chelation or due to non-compliance. Iron overload can lead to liver dysfunction (liver function test elevations initially and eventual fibrosis and cirrhosis), cardiac dysfunction (arrhythmias and eventual heart failure), and endocrine dysfunction (hypothyroidism, diabetes mellitus, growth hormone deficiency, hypogonadism, premature ovarian failure, etc.). If such dysfunction is documented but is adequately addressed, then these subjects will be eligible for this study. If any of these potential side effects occur for the first time while on the subject is on the study drug, the event will be treated as an adverse event (AE).

Subjects with DBA have RBC aplasia, thus necessitating transfusion therapy. As this is a bone marrow failure syndrome these subjects also can have other cytopenias including neutropenia and thrombocytopenia, and often pancytopenia (not necessarily consistent with aplastic anemia). The eligibility criteria for entry into the study allow mild abnormalities in all the cell lines, but subjects with severe cytopenia are ineligible.

In the present study, TFP will be investigated in subjects with DBA over a lower dose range than is generally employed in subjects with schizophrenia. The maximum anticipated daily dose will be 10 mg. In contrast, the usual initial adult dose of TFP for schizophrenia is 2 to 5 mg orally 2 times a day with a maintenance dose of 15 to 20 mg/day and a maximum dose of 40 mg/day. Therefore, more severe adverse reactions with TFP may be less likely to occur in the current study.

The only alternative to this treatment is not to participate in this trial. The subject will continue on transfusion therapy during this trial and be transfused every 3-4 weeks when the hemoglobin is less than 8 gm/dL as prior.

Given the serious potential side effects of chronic, often life-long transfusion therapy and the continuous need for chelation therapy, the risks of this short, 3-week study (plus 1 week follow-up) are reasonable for this subject population. The preliminary data from the zebrafish and mice models and information gained from this study will be proof-of-principle of the use of this drug in the treatment of DBA. If no significant toxicity to this drug is noted, the drug will be chemically altered as to not cross the blood-brain barrier and potentially be a viable treatment for DBA, without the untoward neurological side effects.

11 Potential Benefit to Subjects

This is a safety study but the possible benefits from participation in this study include an improvement in the anemia of DBA and a decrease in the amount of transfusions needed by the subject. Since the study drug will be used for only 21 days, the risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. If the study drug is safe then it would introduce a new class of therapeutic agents to this rare population.

12 Research Related Harm/Injury

You will receive medical care and treatment as needed from Northwell Health. However, you will be responsible for the costs of such medical treatment, directly or through your medical insurance or other forms of medical coverage. No money will be given to you for participation in the study.

13 Provisions to Protect Privacy Interests of Subjects

There are over 750 subjects with Diamond Blackfan anemia enrolled in this Registry. Because of this, the investigators have access to the specific subject population required for this study. Subjects contact the DBAR periodically to inquire about available studies. Also the announcement (Appendix G) informs potential subjects of the study and we anticipate the subjects calling us if they are interested in the study. The consent will be mailed to the subject or handed to the subject at an office visit.

Once consented, subjects will be assigned a unique patient identification number (UPIN) that is used for entering data into the database. Protected health information (PHI) collected includes: name, address, contact numbers, email, and date of birth. Hard copies of study documents will be stored in a locked fireproof cabinet. Any research data that will be emailed will be encrypted.

14 Statistical Plan

14.1 Sample Size Determination

This is a 4-Cohort, 3+3 study, and will therefore enroll a minimum of 3 patients and a maximum of 24 patients. Sample size power to detect a given effect does not apply.

14.2 Statistical Methods

All subjects who received at least one dose of study drug will be included for safety analysis. Demographics and baseline characteristics will be summarized by dose levels.

Primary Endpoint(s)

- All AEs (including non-serious and serious) will be summarized. Key laboratory results will be summarized using CTCAE grade.

Secondary Endpoint(s):

- Not applicable.

14.3 Subject Population for Analysis

There is no randomization for this study. All enrolled subjects will be included in the study analysis for the primary endpoint.

15 Safety and Adverse Events (Appendix H)

15.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity) of a pre-existing condition should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the case report forms (CRF) rather than the individual signs or symptoms of the diagnosis or syndrome.

Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

A serious adverse event (SAE) is one that at any dose (including overdose):

- Results in death
- Is life-threatening[1]
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity[2]
- Results in a congenital anomaly or birth defect
- Is an important medical event[3]

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above.

An overdose, accidental or intentional, whether or not it is associated with an AE, or abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. If an overdose is associated with an AE, the overdose and adverse event should be reported as separate terms.

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or other appropriate tests and procedures.

Adverse Event Reporting Period

All AEs will be recorded by the Investigator from the time the subject signs informed consent to 7 days after the last dose (completion of the 4th week). AEs and SAEs will be recorded on the AE page of the CRF and in the subject's source documents. All SAEs must be reported within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form.

Preexisting Condition

Any worsening (i.e., any clinically significant adverse change in the frequency or intensity) of a pre-existing condition should be considered an AE.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an AE.

Post-study Adverse Event

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1 "Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

2 "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

3 Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

15.2 Recording of Adverse Events (Appendix H)

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Toxicity will be scored using the NCI CTCAE, Version 5.0. A copy of the CTCAE, Version 5.0, can be downloaded from https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50. All appropriate treatment areas should have access to a copy of the CTCAE, Version 5.0. All adverse clinical experiences, whether observed by the investigator or reported by the subject, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the subject's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug and for its seriousness.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.
The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the subject's outcome.

15.3 Reporting of Serious Adverse Events

15.3.1 Study Sponsor Notification by Investigator

All SAEs must be reported within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form. The investigator will keep a copy of this SAE form on file at the study site. Report SAEs by phone and facsimile to:

[Adrianna Vlachos, MD	516-562-1504	516-562-1599

At the time of the initial report, the following information should be provided:

- Study identifier
- Study center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the investigator must provide further information on the SAE in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the study sponsor.

15.3.2 IRB Notification by Investigator

The Principal Investigator is required to notify his/her Institutional Review Board (IRB) of a SAE according to institutional policy. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

15.3.3 FDA Notification by Sponsor

SAEs that are **unlisted/unexpected, and at least possibly associated to the drug,** and that have not previously been reported in the Investigators' brochure, or reference safety information document should be reported promptly to the FDA by telephone or by fax.

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

15.4 Stopping Rules

The stopping rules are as follows:

Scenario 1: The occurrence of extrapyramidal symptoms, symptoms suggestive of tardive dyskinesia (including vermicular movements of the tongue), neuroleptic malignant syndrome or any SAE or treatment-emergent AE ≥ Grade 3 felt to be causally related to TFP in a single subject:

- Rule: Treatment will be immediately stopped in that subject and the subject will be discontinued from the study.

Scenario 2: The occurrence of the extrapyramidal symptoms, symptoms suggestive of tardive dyskinesia (including vermicular movements of the tongue), neuroleptic malignant syndrome or the same SAE or treatment-emergent AE ≥ Grade 3 felt to be causally related to TFP in more than 1 subject:

- Rules:
  a. Treatment will be immediately stopped in those subjects and those subjects will be discontinued from the study.
  b. If the 2 or more subjects are in Cohort A, the trial will be stopped; no MTD will be declared.
  c. If the 2 or more subjects are in Cohort B, the study will be stopped, and 1 mg/day will be declared the MTD.

d. If the 2 or more subjects are in Cohort C, the study will be stopped, and 2 mg/day will be declared the MTD.

e. If the 2 or more subjects are in Cohort D, the study will be stopped, and 5 mg/day will be declared the MTD.

Scenario 3: Hemoglobin > 12 mg/dL in a subject sustained for more than 7 days (not associated with RBC transfusion)

- Rule: The subject will be considered a complete responder, treatment will be discontinued, and the duration of the effect will be monitored until day 29 after start of treatment.

In addition to the stopping rules, a subject may be discontinued by the investigator at any time for failure to comply with protocol requirements, in the event that the investigator feels that continuation in the study poses a risk to the health of the subject, or if the subject withdraws consent.

The reason for treatment discontinuation must be documented in the subject chart / source records.

The trial will be stopped, as noted above, if more than one subject experiences the occurrence of the extrapyramidal symptoms, symptoms suggestive of tardive dyskinesia, neuroleptic malignant syndrome, the same SAE or treatment-emergent AE ≥ Grade 3 felt to be causally related to TFP at the lowest dose level.

15.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety monitoring plan (see Section 17 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of SAEs.

15.6 Data and Safety Monitoring

The medical monitor will serve as the monitoring entity for this study. The monitor will be a qualified physician, other than the Principal Investigator, who is not associated with the protocol, and will monitor the study to protect the safety and well-being of the subjects. The medical monitor plays a role in reviewing serious adverse events and unanticipated problems.

The physician will not be involved with the conduct of the study, but will serve as a hematology consultant.

For this study Dr. Lawrence Wolfe has been selected as the Medical Monitor. He has served in this role in a prior DBA clinical study. He is the Deputy Chief, Operations and Safety, in the Division of Hematology/Oncology and Stem Cell Transplantation at Cohen Children's Medical Center and as such meets the criteria stated above.

Summary data related to the study will be provided to the medical monitor in written form. The following materials will be provided: a report of all deaths, a summary of cumulative adverse event data with intervention causality, a summary of assessment of conduct of the study (e.g. site enrollment, subject enrollment (target vs. actual), protocol compliance per site, informed consent records, treatment received), and a summary of assessment to evaluate factors that may impact on the safety of study subjects.

Adverse Event (AE) Data: The frequencies of each type of AE will be tabulated and the incidence rate will computed as the percentage of subjects experiencing that AE at some time during the course of the trial. These intervals will be used by the medical monitor for determining whether a particular AE or class of AEs should be "flagged" for safety concerns.

Severe AE/Major Protocol Deviation: Should any major or serious adverse event or protocol deviation occur that requires rapid action, the Sponsor-Investigator will notify the IRB, and FDA by telephone, and in writing within 24 hours.

Meeting Schedule: The medical monitor will review the data quarterly, or as necessary for major infractions of protocol or SAEs. Meetings may be by telephone.

Reports from the Medical Monitor: After the Medical Monitor meets, a written summary of the meeting will be provided to the Sponsor-Investigator. The summary will include:

- Date that the meeting took place
- Summary of cumulative adverse event data
- Recommendations

Distribution of Report: The Sponsor-Investigator will distribute the report to the Northwell IRB and all co-investigators, and the FDA.

16 Data Handling and Record Keeping

16.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why

- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

16.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

16.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

16.4 Records Retention

It is the Investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

17 Study Monitoring, Auditing, and Inspecting

17.1 Study Monitoring Plan

This study will be monitored according to the study monitoring plan in Appendix I. The Investigator will allocate adequate time for such monitoring activities. The Investigator will also

ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

17.2 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The Investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an Investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

18 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The Investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

19 Study Finances

19.1 Funding Source

Funding for this research study is provided by CAMP4 Therapeutics and the National Institutes of Health National Heart, Lung, and Blood Institute. Study related travel costs will be offset by the National Organization of Rare Diseases (NORD) so that subjects will not incur any additional costs as a result of participating in this trial.

19.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Northwell Health investigators will follow the University conflict of interest policy.

20 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

21 References

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22 Appendices

22.1 Appendix A: Study Assessment Schedule

	Screening Period	Treatment Period			Follow-Up
	Screening	Visit 1	Visit 2	Visit 3	Visit 4
	Day -28 to Day -1	Day 1[a] (±1d)	Day 11 (±1d)	Day 22 (±2d)	Day 29 (±2d)
Obtain subject informed consent form	☒	-	-	-	-
Assess inclusion/ exclusion criteria	☒	-	-	-	-
Obtain medical subject history	☒	-	-	-	-
Record prior treatments and procedures (including transfusion history)	☒	-	-	-	-
Physical examination (including height at 1st visit only, weight, vital signs, blood pressure and neurological examination)	☒	☒	☒	☒	☒
Karnofsky performance status (Appendix E)	☒	☒	☒	☒	-
Hematology [b,*]	☒	☒	☒	☒	☒
Blood chemistry, hepatic function, kidney function [c,*]	☒	☒	☒	☒	☒
Blood for pharmacodynamic disease marker analysis (Appendix J)[d]	-	☒	☒	☒	-
Menstrual status	☒	☒	☒	☒	-
Pregnancy test [e,*]	☒	☒	☒	-	-
Transfusion assessment [f]	☒	☒	☒	☒	☒
Adverse events	-	☒	☒	☒	☒
Concomitant therapies /procedures	-	☒	☒	☒	-
Provide study drug (21-day supply)	-	☒	-	-	-

22.1 Appendix A continued

*Historical clinical laboratory evaluations performed for medical evaluation within 28 days are acceptable for study entry.

a: All Day 1 assessments are to be done prior to TFP administration. TFP should be initiated within 7 days following a blood transfusion.

b: Hematology laboratory evaluations (RBC count, hemoglobin, hematocrit, absolute reticulocyte count, white blood cell count, absolute neutrophil count, and platelet count) will be collected at Screening and at subsequent visits during the Treatment and Follow-Up Periods, as referenced in the schedule above. Any laboratory evaluations may be repeated more frequently if clinically indicated.

c: Serum chemistry laboratory evaluations (blood urea nitrogen, creatinine, bilirubin [total and direct], aspartate aminotransferase [AST], alanine aminotransferase [ALT]) will be collected at Screening and at subsequent visits during the Treatment and Follow-Up Periods, as referenced in the schedule above. Any laboratory evaluations may be repeated more frequently if clinically indicated.

d: Blood for pharmacodynamic disease marker analysis will be shipped to supporting company (see Appendix F).

e: Females of childbearing potential: A female of childbearing potential is a sexually mature woman who has not undergone a hysterectomy or who has not been postmenopausal for at least 24 consecutive months (i.e., who has had menses at some time in the preceding 24 months). A serum pregnancy test (which must be negative) must be performed not more than 3 days from the start of TFP administration (Study Day 1). Subjects must agree to use highly effective methods of birth control during study participation and for at least 1 week following the last dose of TFP. Subjects must be counseled concerning measures to be used to prevent pregnancy and potential toxicities prior to the first dose of TFP. Serum pregnancy tests will be performed according to the above schedule during study participation.

f: Subjects must have documented transfusion history for > 12 weeks before inclusion/initiation of treatment.

22.2 Appendix B: Diamond Blackfan Anemia Diagnostic Criteria

Diagnostic Criteria

- Age less than 1 year
- Macrocytic anemia with no other significant cytopenias
- Reticulocytopenia
- Normal marrow cellularity with a paucity of erythroid precursors

Supporting Criteria

Major

- Ribosomal protein gene mutation described in "classical" DBA
- Positive family history

Minor

- Elevated erythrocyte adenosine deaminase activity
- Congenital anomalies described in "classical" DBA
- Elevated fetal hemoglobin
- No evidence of another inherited bone marrow failure syndrome

A diagnosis of "classical" DBA is made if all the diagnostic criteria are met. When there is a positive family history, an otherwise normal individual should be considered as having "non-classical" DBA if a mutation shared by affected family members is present. Anyone suspected of having DBA, but with insufficient diagnostic criteria, should be considered as having non-classical DBA if a reported mutation is present. A subject can be assigned as having a "probable" diagnosis, with a decreasing degree of certitude if:

- 3 diagnostic criteria are present along with a positive family history;
- 2 diagnostic criteria and 3 minor supporting criteria are present; or,
- positive family history and 3 minor supporting criteria are evident, even in the absence of diagnostic criteria.

22.3 Appendix C: Definitions of RBC-Transfusion-Dependence and -Independence

	RBC transfusions	**Hb (gm/dL)**	**Surveillance Interval**
RBC transfusion dependence	≥ 10 cc/kg/month Or 2 units/month for subjects >50 kg	< 8	6 months
RBC transfusion independence	None	≥ 9	6 months

22.4 Appendix D: Cockcroft-Gault Estimation of Creatinine Clearance

Cockcroft-Gault estimation of creatinine clearance (CrCl) (Cockcroft, 1976; Luke 1990):

$$\text{CrCl (mL/min)} = \frac{(140 - \text{age}) \times (\text{weight, kg})}{72 \times (\text{serum creatinine, mg/dL})}$$

(**Males**)

$$\text{CrCl (mL/min)} = \frac{(140 - \text{age}) \times (\text{weight, kg})}{72 \times (\text{serum creatinine, mg/dL})} \times 0.85$$

(**Females**)

22.5 Appendix E: Karnofsky Performance Status Scale

The Karnofsky Performance Scale Index allows subjects to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual subjects.

%	Criterion
100	Normal; no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled; hospitalization is indicated though death is not imminent
20	Very sick; hospitalization is necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

References:

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22.6 Appendix F: New York Heart Association – Classification of Heart Failure

Class	Symptoms
Class 1	No limitation of activities. No symptoms from ordinary activities
Class 2	Mild limitation of activity. Comfortable with rest or mild exertion
Class 3	Marked limitation of activity and be comfortable only at rest
Class 4	Complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest

22.7 Appendix G: Study Announcement

The Feinstein Institute for Medical Research and Cohen Children's Medical Center Announce a Clinical Trial of Trifluoperazine for Transfusion-Dependent Adult Patients with Diamond Blackfan Anemia

Dear DBA Patients and Families:

We are pleased to announce the opening of:
"Phase I/II, Open-Label Study to Determine Safety of Trifluoperazine (TFP) in Adults with Red Blood Cell Transfusion-Dependent Diamond Blackfan Anemia"

Diamond Blackfan Anemia (DBA) is a rare pure red cell anemia resulting from a failure of the bone marrow to make red blood cells. The anemia may present in infancy, in childhood, or occasionally in adulthood. For some patients a genetic cause for DBA can be found but for others no known genetic mutation is known at this time. Some patients have little or no anemia after treatment with steroids, whereas others may need continuous red blood cell transfusions or steroid therapy for a long time, sometimes for life. Steroids may control DBA in many patients at first, but after some time, the steroids may not work as well anymore. Unfortunately red cell transfusions have many possible negative side effects, such as iron overload. Iron overload can occur in the liver, heart, and endocrine organs which can cause more complicated health problems such as liver or heart failure, diabetes, thyroid issues, gonadal dysfunction, etc.

Purpose of this study
The purpose of this study is to find an alternative treatment for patients with DBA who are dependent upon transfusions. This study will use a medication called trifluoperazine (TFP) and determine if it is safe for the treatment of DBA in adult patients who require chronic blood transfusions. This is a research study because TFP has never been tested in patients with DBA. TFP is a drug that has been approved by the Food and Drug Administration (FDA) for the treatment of psychiatric disorders. TFP has not been previously approved by the FDA for treatment of DBA. In this research study the safety of TFP is being tested at different dose levels in patients with DBA. A total of 24 patients with DBA may be enrolled on this study.

About the study drug, Trifluoperazine or TFP
TFP is a typical oral antipsychotic medication that has been used for the treatment of psychiatric patients since 1958. In the United States, TFP is approved for the short-term treatment of anxiety; treatment or prevention of nausea and vomiting of various causes; and management of psychotic disorders. In part because of its side effect profile, TFP has been largely replaced by second and third generation anti-psychotic medications. Nevertheless, TFP remains commercially available worldwide and the risks associated with its use are well understood and generally manageable.

Description of procedure
The study is 29 days long and consists of taking the study drug TFP for 21 days and being monitored for an additional 8 days. The patient will have a total of 4 visits over the period of 29 days. A review of the patient's history, physical examination, and laboratory blood work will be

done in order to determine if the patient is eligible to participate. While the patient is taking the TFP, he or she will need to be seen at The Feinstein Institutes for Medical Research or the participating institution 3 times over the first 21 days and virtually for the last visit. During the first 21 days the patient will be taking TFP once a day, orally as a tablet, and will continue the red blood cell transfusions as routinely scheduled, every 3-4 weeks.

The following are some of the basic inclusion and exclusion criteria for this pilot trial.

Inclusion Criteria

- Diagnostic and supporting criteria for the diagnosis of DBA
- Transfusion dependence every 3-4 weeks
- Age greater than or equal to 18 years and less than 65 years
- Weight greater than or equal to 45 kilograms or 99 lbs
- Negative pregnancy test
- Signed informed consent

Exclusion Criteria

- Have a known allergy to TFP or other phenothiazines (class of drugs including TFP)
- Evidence of kidney or liver dysfunction
- Evidence of heart disease or history of angina
- Uncontrolled high blood pressure
- Pregnancy, or plans to become pregnant during duration of trial
- History of cancer

Please refer to the website link: http://www.clinicaltrials.gov and enter the search term "Diamond-Blackfan Anemia and trifluoperazine" for full eligibility criteria and additional trial information.

CONTACT INFORMATION

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Email: eatsidaf@northwell.edu

Also, feel free to contact Dr. Vlachos by email: avlachos@northwell.edu.

22.8 Appendix H: Side Effects Grading and Reporting

Side effects will be graded according to the NCI-CTCAE (version 5).

Link for NCI-CTCAE version 5:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

22.9 Appendix I: Clinical Trial Services Study Monitoring Plan

Services Provided to: Adrianna Vlachos, MD
Regulatory Sponsor (IND#TBD)
Feinstein Institutes for Medical Research
Pediatric Hematology/Oncology
350 Community Drive
Manhasset, NY 11030

Services Provided for: Phase I/II, Open-Label Study to Determine Safety of Trifluoperazine (TFP) in Adults with Red Blood Cell Transfusion-Dependent Diamond Blackfan Anemia

Performance Sites: Feinstein Institutes for Medical Research/Cohen Children's Medical Center

Services Provided by: The Office of Research Compliance
Regulatory Affairs Division
Northwell Health
3333 New Hyde Park Rd., Suite 317
New Hyde Park, NY 11042

Scope of Services

The Office of Research Compliance (ORC) will provide clinical research monitoring services as outlined in the Risk Based Monitoring Plan for all subjects to be enrolled in this protocol at the above referenced performance site to include the general areas:

- Good Clinical Practice Regulatory Review
- Subject Case Review of Critical Study Data and Processes Including:
    - Case Report Form Completion and Source Document Verification
    - Protocol and Informed Consent Compliance
- Protocol Drug Accountability

In addition the ORC will provide regulatory consultative services to the research team in addition to training in Good Clinical Practice (GCP) requirements for all participating site personnel.

Review Schedule

The ORC agrees to provide primarily remote monitoring services for all performance sites including the following:

- Site Initiation: Prior to study start-up for GCP and protocol overview
- Routine monitoring: Occurs annually with the frequency outlined in the monitoring plan as long as the site continues to enroll subjects. However, additional visits may be performed for targeted reviews as required.
- Final monitoring: Study close out

Post-monitoring activities include follow-up with site for additional information required to complete monitoring, resolution of issues and report writing.

Reporting

Regular monitoring reports will be generated by the ORC after each review and will be provided to the respective performance site and Sponsor-Investigator and Data Coordinator. Resolution of queries and outstanding issues or concerns will be the responsibility of the individual performance site, Sponsor-Investigator, and Data Coordinator.

The Northwell Sponsor-Investigator will be responsible for reporting incidents of IRB non-compliance to the Northwell Human Research Protection Program (HRPP) (in compliance with regulations on the protection of human subjects and institutional policy and procedures) and responsible for securing compliance at all trials sites.

22.10 Appendix J: Blood for Pharmacodynamic Disease MarkerAnalysis

Descriptive pilot laboratory investigations will be performed to explore assay feasibility for TFP target engagement. Samples from each subject will be collected to study the pharmacodynamic effect of the drug TFP. Ten mL whole blood will be collected in EDTA (lavender) tubes prior to start of drug administration and at each visit of treatment for a total of 3 samples per subject. The sample will be used to isolate CD34+ cells (precursors for erythrocytes). Total protein and mRNA will be isolated and used to analyze disease markers.

In addition whole blood will be collected in a 3ml EDTA (lavender top) tube to measure the drug amount in serum, to understand the relationship between drug amount and the exploratory physiological impact being monitored. This sample will need to undergo centrifugation prior to shipment of the plasma as per the laboratory manual..

These samples should be shipped right after collection by FedEx First Overnight Delivery to:

Attn: Gavin Whissell
CAMP4 Therapeutics
One Kendall Square
Suite B14301
Cambridge, MA 02139
Tel: (617)766-3221
The specimens must be labelled with the subject's study ID and study week, as well as date collected.