Master Protocol

The Use of Novel Therapies to Reconstitute Blood Cell Production and Promote Organ Performance, using Bone Marrow Failure as a Model: A Pilot, Phase I/II Study of the Amino Acid Leucine in the Treatment of Patients with Transfusion-Dependent Diamond Blackfan Anemia

Proposal 09196004

Lead Principal Investigator
Adrianna Vlachos, MD
Associate Professor of Pediatrics
Hofstra North Shore-LIJ School of Medicine
Cohen Children's Medical Center of New York
Hematology/Oncology and Stem Cell Transplantation
The Feinstein Institute for Medical Research
269-01 76th Avenue, Rm. 255
New Hyde Park, NY
Phone: (516) 562-1506 Fax: (516) 562-1599
E-mail: avlachos@nshs.edu

Study Team Members
Monica Bessler, MD, PhD
George Buchanan, MD
Steven R Ellis, PhD
Bertil Glader, MD, PhD
Helge Hartung, MD
Jeffrey M. Lipton, MD, PhD
Zora Rogers, MD
Colin Sieff, MD

Central Study Coordinator
Eva Atsidaftos, MA, CCRA
The Feinstein Institute for Medical Research
350 Community Drive, Rm. 3146
Manhasset, NY 11030
Phone: 516-562-1505
Fax: 516-562-1599
E-mail: eatsidaf@nshs.edu

Protocol revised dates: v.2, 12/3/12; v.3, 6/7/13; v.4 8/27/13, v. 5.0 10/03/2013, v. 6.0 10/1/14

This study is being sponsored by the Department of Defense (DOD) Telemedicine and Advanced Technology Research Center (TATRC). The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

The Use of Novel Therapies to Reconstitute Blood Cell Production and Promote Organ Performance, using Bone Marrow Failure as a Model:

A Pilot, Phase I/II Study of the Amino Acid Leucine in the Treatment of Patients with Transfusion-Dependent Diamond Blackfan Anemia

Signature Page

Medical Coordinating Center Principal Investigator Approval.

Signature: _____ Date: _____
Adrianna Vlachos, MD

Sub Site Principal Investigator Approval:

Signature: _____Date: _____

Please fax this page back to Primary Coordinating Site, prior to site initiation.
Attn: Eva Atsidaftos
F) 516-562-1599

TABLE OF CONTENTS **PAGE**

1.0 STUDY TEAM

Lead Principal Investigator
Adrianna Vlachos, MD
Hofstra North Shore-LIJ School of Medicine
Cohen Children's Medical Center of New York
Hematology/Oncology and Stem Cell Transplantation
The Feinstein Institute for Medical Research
269-01 76th Avenue
New Hyde Park, NY
Phone: 516-562-1504
Fax: 516-562-1599
E-mail: avlachos@nshs.edu

Study Team Members
Monica Bessler, MD, PhD
Children's Hospital of Philadelphia
3615 Civic Center Blvd.
Philadelphia, PA 19104
Phone: 267-426-8782
E-mail: besslerm@email.chop.edu

George Buchanan, MD
University of Texas Southwestern Medical Center
Dept. of Pediatrics
5323 Harry Hines Blvd.
Dallas, TX 75235-9063
Phone: 214-648-8594
Fax: 214-648-3122
E-mail: George.buchanan@utsouthwestern.edu

Steven R Ellis, PhD
University of Louisville
Dept. of Biochemistry and Molecular Biology
Phone: 502-852-5222
Fax: 502-852-6222
E-mail: srellis@louisville.edu

Bertil Glader, MD, PhD
Stanford University
1000 Welch Rd. Ste. 300
Palo Alto, CA 94305
Phone: 650-723-5535
Fax#: 650-723-5231
E-mail: bglader@stanford.edu

Helge Hartung, MD
Children's Hospital of Philadelphia
34th Street and Civic Center Blvd

Philadelphia, PA 19104
Phone: 267-425-2126
Fax: 215-590-3992
E-mail: hartungh1@email.chop.edu

Jeffrey M. Lipton, MD, PhD
Director, Hematology/Oncology and Stem Cell Transplantation
Cohen Children's Medical Center of NY
269-01 76th Avenue, Room 255
New Hyde Park, NY 11040
Phone: 718-470-3703
Fax: 718-343-4642
E-mail: jlipton@nshs.edu

Zora Rogers, MD
University of Texas Southwestern Medical Center
Dept. of Pediatrics
5323 Harry Hines Blvd.
Dallas, TX 75235-9063
Phone: 214-648-8594
Fax: 214-648-3122
E-mail: zora.rogers@utsouthwestern.edu

Colin A. Sieff, MB.BCH., FRCPath,
Director, Bone Marrow Failure Program,
Attending in Hematology,
Boston Children's Hospital,
300 Longwood Ave.,
Boston, MA 02115
Phone: 617-919-4241 or 617- 355-8246
Fax: 617-730-0934
E-mail: c.sieff@childrens.harvard.edu

Study Coordinator
Eva Atsidaftos, MA, CCRA
The Feinstein Institute for Medical Research
350 Community Drive, Rm. 3146
Manhasset, NY 11030
Phone: 516-562-1505
Fax: 516-562-1599
E-mail: eatsidaf@nshs.edu

2.0 STUDY TEAM ROLES AND RESPONSIBILITIES

The Study Team consists of the Lead Principal Investigator (Adrianna Vlachos, MD - Cohen Children's Medical Center of New York, New Hyde Park, NY and The Feinstein Institute for Medical Research, Manhasset, NY) and five Co-Investigators from the four Diamond Blackfan Anemia Resource Centers (Jeffrey M. Lipton, MD, PhD - Cohen Children's Medical Center of New York, New Hyde Park, NY; George Buchanan, MD and Zora Rogers, MD - University of Texas Southwestern Medical Center, Dallas, TX; Bertil Glader, MD, PhD - Stanford University, Palo Alto, CA; Colin Sieff, MD - Dana Farber Cancer Institute, Boston, MA) established in cooperation with the Centers for Disease Control and Prevention. This protocol was initially discussed at the annual Diamond Blackfan Anemia International Consensus Conference and three more Co-Investigators were added to the Study Team at that time Steven Ellis, PhD is also a Study Team member. He is a researcher in ribosome biology and will be providing assistance in the scientific nature of this study. He has been involved in providing the background for the study design.

The lead PI will be responsible for submitting documents to the Institutional Review Board of the primary institution and the USAMRMC ORP HRPO, verifying IRB approvals at all sub-sites, recruiting DBA patients, verifying eligibility, consenting patients to the study, overseeing adherence to the protocol specifics, monitoring for drug toxicity and safety, evaluating feasibility and outcome and analyzing data and reporting results after the study is complete.

The Coordinating Center will be at The Feinstein Institute for Medical Research (FIMR) and the Central Study Coordinator will also be at the lead PI's institution. The Study Coordinator will be responsible for assisting the lead PI in the above-mentioned delegated roles. In addition the Central Study Coordinator will be responsible for assisting participating institutions in IRB submission of the protocol, drug shipment to the participating physicians/institutions, collecting Case Report Forms (CRFs) from all sites and assisting with their completion, as well as tracking protocol compliance at the participating sites. She will also assist in the analysis of the data.

All Study Team members, except for Steven Ellis, PhD, will be recruiting patients on to the study from their surrounding areas and, as such, will be engaged in human subjects' research. They will serve as local site PIs. Thus, they will be responsible for obtaining IRB approval and the conduct of the study at their institutions. They will also be responsible for submission of the required CRFs to the Central Study Coordinator. The Study Team will also review the data at completion of the study and assist in the analysis. They will also serve as authors in reporting of the data.

The funding for this study allows for up to 17 institutions to enroll 50 subjects. Aside for the PI's and Co-I's institutions, other collaborating institutions will be added once they are identified by their request to the Central Study Coordinator to enroll subjects. A designee at these participating institutions will be added as Co-Investigator and will be responsible for subject recruitment, obtaining IRB approval and completion and submission of CRFs to the Central Study Coordinator.

Site Information: The Feinstein Institute for Medical Research will be the main location of research activity. Subjects will also be enrolled at the participating institutions and data will be submitted to The FIMR.

Type of Research: Drug

3.0 STUDY ABSTRACT

Diamond Blackfan anemia (DBA) is a rare inherited pure red cell aplasia. Over the past ten years mutations have been described in genes encoding both the small and large ribosome associated proteins. There is wide variability in clinical and biologic features, familial history, and therapeutic responses. Currently standard therapy includes corticosteroids, red cell transfusions or stem cell transplantation. Approximately 80% of patients have an initial response to corticosteroids. Of these patients approximately half will fail to achieve a taper to a tolerable steroid dose and will need to discontinue steroid therapy. These patients and the 20% who do not respond initially to steroid therapy must be maintained on a chronic red cell transfusion/iron chelation program. Chronic transfusion/chelation therapy has considerable treatment-related morbidity, most notably iron overload related organ dysfunction.

The branched chain amino acids (BCAA) are leucine, isoleucine, and valine. These amino acids are "essential" as they are not stored and must be supplied by a dietary source. These amino acids are utilized by muscle for energy and are the only amino acids that are not metabolized by the liver. Branched-chain amino acid therapy has been used in patients with hepatic encephalopathy, pre-operative care and post-operative recovery, burns, post-trauma, cirrhosis, phenylketonuria, renal insufficiency, sepsis, and spinocerebellar degeneration; however their role is not clear. Leucine is one of three BCAA that have been studied extensively over the years for the ability to promote muscle mass when given orally. Dietary supplementation with branched chain amino acids has been used by athletes to stimulate muscle mass and increase endurance and, in clinical settings, to reverse muscle deterioration. Leucine is sold over-the-counter as a nutritional supplement and L-Leucine has been Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act.

Leucine has been administered successfully in one patient reported in the literature with DBA. A complete response was associated with its administration. In unpublished data 5 more patients have been placed on a leucine trial with partial responses in 4 of the 5 patients without any toxicity. The hypothesis for the molecular basis for the possible use of the amino acid leucine as a therapeutic agent for DBA is based upon the observations in skeletal muscle that leucine supplementation can upregulate components of the translational machinery. These components include ribosomal proteins, whose haploinsufficiency appears to be the underlying cause of DBA. This pilot study will test the feasibility of administration of leucine in a larger group of patients with DBA. Subjects will also be monitored for clinical hematologic response. Leucine levels and total amino acid profiles will be ascertained in an attempt to correlate response with baseline levels. Responders will be evaluated with regard to phenotypic characteristics that might distinguish them prospectively from other patients.

4.0 OBJECTIVES

4.1 Primary

4.1.1 To determine the feasibility of administering the amino acid leucine and to determine the pharmacokinetics of leucine administration in patients with Diamond Blackfan anemia (DBA) who are red cell transfusion-dependent.

4.1.2 To determine the efficacy of leucine to produce a hematologic response in transfusion-dependent DBA patients.
4.1.3 To determine the safety of leucine administration in transfusion-dependent DBA patients.

4.2 Secondary
4.2.1 To determine if patients with DBA are deficient in branched chain amino acids.

5.0 BACKGROUND

5.1 Diamond Blackfan anemia (DBA)

Diamond Blackfan anemia is a rare inherited pure red cell aplasia. Over the past ten years mutations have been described in genes encoding both the small and large ribosome associated proteins (1-6). There is wide variability in clinical and biologic features, familial history, and therapeutic responses. Currently standard therapy includes corticosteroids, red cell transfusions or stem cell transplantation (7, 8). Approximately 80% of patients have an initial response to corticosteroids. Often patients require chronic, potentially life-long treatment, which is fraught with a variety of short-term and long-term complications including osteoporosis, Cushingoid features, diabetes, hypertension, severe growth retardation, aseptic necrosis, glaucoma, and cataracts. Twenty percent of patients are initially unresponsive to corticosteroid treatment and about half of the initial responders cannot be tapered to a safe and effective dose. Other patients may become refractory to corticosteroids after a period of responsiveness. These patients must be maintained on a chronic red cell transfusion/iron chelation program. Long-term red cell transfusions will result in iron overload and iron chelation therapy is required. With inadequate chelation, iron overload will inevitably lead to organ damage, especially of the heart and liver, and may result in death.

The alternative to corticosteroids or red blood cell transfusions and chelation is hematopoietic stem cell transplantation. Hematopoietic allogeneic stem cell transplant with a histocompatible donor has been proven to be effective therapy and has been performed successfully for a select group of patients. The DBAR experience has been recently updated (9, 10). However, because of remissions (sustained physiologically acceptable erythropoiesis for $\geq$ 6 months) in up to 20% of DBA patients, stem cell transplantation, regardless of donor type, is not consistently offered to corticosteroid-dependent or transfusion-dependent DBA patients. For many patients, the lack of a suitable donor does not allow stem cell transplantation as a therapeutic option. In addition, HLA-matched unrelated donor transplants have been associated with mortality and morbidity.

5.2 Review of Experimental Treatment Modalities

Historically the lack of truly effective and safe therapy has prompted the investigation of a number of other agents. These modalities have achieved only anecdotal success.

5.2.1 High-dose Corticosteroids

Several small published trials have evaluated the efficacy of high-dose corticosteroids in those patients failing conventional therapy (11-13). In total, 10 of 29 patients have become transfusion independent after either intravenous methylprednisolone or oral prednisone/intravenous methylprednisolone. One patient had a partial response and 5 patients had a transient response. Varied responses at different doses have been reported, and the cumulative data suggest that extremely high doses may be necessary. Responses were not always durable upon

discontinuation of therapy and death from infection and the long-term side effects of high-dose steroids have justifiably led to the abandonment of this approach.

5.2.2 Cyclosporine A and Antithymocyte Globulin

Cyclosporine A (CSA) has been studied in DBA patients, but has demonstrated limited success. By combining several reports (14-21), a total of 30 patients tried CSA alone (10 patients) or in combination with steroids (20 patients). Only two of the CSA alone patients had a sustained response. Half of the patients treated with CSA plus steroid had a response but it was transient and failed once steroids were discontinued. Antithymocyte globulin (ATG) has also had very limited use in DBA. A few case reports of patients with acquired pure red cell aplasia have demonstrated mixed responses (22-24). A Phase II NIH-sponsored protocol [98-H-0144] combining CSA and ATG closed due to poor responses.

5.2.3 Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) has been used in very few cases and has had minimal success in patients who were refractory to conventional-dose corticosteroids. In one case, a patient was able to achieve transfusion independence, although continued to require monthly IVIG infusions in order to sustain a remission (25). In a few other cases, only limited therapeutic benefit was observed (26-28). This modality is not recommended.

5.2.4 Interleukin-3

Interleukin-3 (IL-3) has been studied in a number of trials. An increase in erythroid burst forming unit colonies and an improved response to erythropoietin with the addition of IL-3 to *in vitro* cultures of bone marrow from DBA patients (29) prompted the investigation of IL-3 in clinical trials. Over 100 patients have been treated with an approximate 10% response (30-35). In one report two of the patients who responded, required discontinuation of therapy due to deep venous thrombosis. Others required discontinuation of therapy because of side effects (urticaria, wheezing, local reactions). The reason for the disparity between the laboratory results and the clinical trials is unclear. IL-3 is no longer available for clinical use.

5.2.5 Metoclopramide

Metoclopramide has also shown to be effective in DBA. Abkowitz *et al.* (36), described a 33% hematologic response rate in a small group of patients with DBA using metoclopramide, an inexpensive, commonly used dopamine antagonist that induces the release of prolactin from the pituitary gland, thereby increasing prolactin levels. It was proposed that prolactin likely improves erythropoiesis by stimulating cells in the microenvironment of erythroblasts. Unfortunately other studies in the US and Europe did not confirm these responses but showed a 10% response rate (37, 38).

In summary none of the agents described have been shown to be unequivocally effective and some carry significant treatment related toxicity. Thus a search for effective agents based upon the emergence of new scientific data is ongoing.

6.0 DRUG INFORMATION

The branched chain amino acids (BCAA) are leucine, isoleucine, and valine (39, 40). These amino acids are utilized by muscle for energy and are the only amino acids that are not

metabolized by the liver. Branched chain amino acid therapy has been used in patients with hepatic encephalopathy, bronchitis, preoperative care, cirrhosis, phenylketonuria, renal insufficiency, sepsis, sleep disorders, and spinocerebellar degeneration; however their role is not clear. Leucine is one of three BCAA that have been studied extensively over the years for the ability to promote muscle mass when given orally. Dietary supplementation with branched chain amino acids has been used by athletes to stimulate muscle mass and increase endurance and in clinical settings to reverse muscle deterioration (41). These latter clinical settings include patients undergoing post-operative recovery, in burn wards, post-trauma and cancer therapy. The potential benefit of BCAA enrichment, especially leucine, is still not clear however some small controlled studies have shown improved nitrogen balance and decreases in postoperative morbidity (42). Research on BCAA supplementation as an adjunct therapy in these clinical settings continues.

Branched chain amino acid products vary widely in their composition, ranging from 200-300mg of each BCAA daily to 2-5 grams of each daily. The total BCAA requirement for young adults and children 6-10 years of age is 147 mg/kg/day with increased doses for neonates and infants (46). While there is little indication that supplementation with BCAA produce significant results in healthy individuals, there are a number of targeted clinical uses. BCAA may support liver health in patients with liver disease such as cirrhosis and hepatic encephalopathy (47, 48). Also, patients suffering from amyotrophic lateral sclerosis may show improvement after using BCAA (49). BCAA may help support health and recovery in patients who have experienced trauma, extreme physical stress, kidney failure, and burns (50). BCAA may aid in recovery after surgery (51).

6.1 Leucine Preparation

Manufacturer: Ajinomoto Aminoscience LLC
Leucine is an amino acid available in many over-the-counter nutritional supplements. Leucine is a white or almost white, odorless crystalline powder or shiny flakes. Leucine can be administered orally or intravenously and can be supplied as tablet, capsule, powder, or solution. This pilot study will only utilize commercially available, pharmaceutical grade L-leucine from Ajinomoto Aminoscience LLC, Raleigh, NC. The leucine will be supplied in capsular form as 250 milligrams per capsule with excipient approved for use being vegetable cellulose.

6.2 Pharmacokinetics

6.2.1 Distribution Sites

Amino acids are widely distributed throughout the tissues of the body (54).

6.2.2 Metabolism Sites

Metabolism sites include all body tissues. Factors that influence the metabolism of amino acid include injury or stress which increases metabolism and liver dysfunction which decreases metabolism. Sepsis increases amino acid metabolism, while renal dysfunction decreases metabolism (55).

6.3 Mechanism of Action/Pharmacology

Various mechanisms of action for branched chain amino acids have been proposed. During exercise, supplementation appears to exert an anabolic and protein-sparing response. The

branched chain amino acids also appear to be a preferred energy substrate for muscles, at least in cirrhotic patients. The administration of branched chain amino acid resulted in an anabolic effect on protein metabolism during recovery following exercise in human subjects.

6.4 Contraindications
Hypersensitivity to branched chain amino acids, anuria, and inborn errors of amino acid metabolism, especially those that involve branched chain amino acid metabolism, such as maple syrup urine disease and isovaleric acidemia are contraindications.

6.5 Adverse Effects
Toxicity from orally administered leucine is extremely low. Ingestion of even large quantities produces only minor gastrointestinal symptoms such as nausea and/or vomiting. No consistent evidence of toxicity has been linked to leucine supplements. In healthy subjects, no adverse events have been reported by subjects consuming oral leucine for a 6-week period (56). In another study, healthy elderly men were supplemented with 2.5 g of leucine at each main meal for a period of 3 months without any reported adverse effects (44). Scarna et al. (45) has reported that no adverse effects are associated with repeated oral administration of up to 60 g/day of BCAAs (providing 24 g/day of leucine) in patients with bipolar disorder. The body of evidence on oral administration of leucine in humans indicates that it can be consumed in considerable amounts without adverse effects. There are no Dose-Limiting Toxicities known with oral Leucine. There is also no defined Maximum Tolerated Dose and no Recommended Phase II Dose reported for this supplement.

Thrombophlebitis, thrombocytopenia, hyperammonemia, nausea, flushing, and fever have been reported with administration of intravenous amino acids, but not with oral supplementation. Leucine aggravates a rare genetic condition called Maple Syrup Urine Disease and can cause delirium and life-threatening neurologic symptoms in these patients. It has been thought that higher levels of leucine taken over a prolonged period of time may contribute to pellagra. Pellagra is defined as a disease that results from a lack of niacin (vitamin B3) and tryptophan (another essential amino acid) but also rarely from an excess of leucine. Its symptoms include diarrhea, dermatitis (dry skin rash), dementia and even death in its extreme form. There have been reports of severe neurologic damage and death when given intravenously (IV) in high doses, which will not be done in this study.

6.6 Drug Interactions
No drug interaction data are available.

6.7 Pregnancy/Lactation
Excessive leucine administration interferes with embryo development in rats. Scientific evidence for the safe use of branched chain amino acids during pregnancy and lactation is not available as there are no human data.

6.8 Overdosage
Toxicity from orally administered amino acids is extremely low. A minimum toxic dose has not been established.

6.9 Leucine Levels

Normal reference blood levels of leucine (57) by age are:
Premature neonate, 1 day: 0.92 ± 0.33 mg/dL (70 ± 25 micromol/L)
Neonate, 1 day: 0.62-1.43 mg/dL (47-109 micromol/L)
Infants 1 to 3 months: 1.36 ± 0.39 mg/dL (104 ± 30 micromol/L)
Infants 9 to 24 months: 0.59-2.03 mg/dL (45-155 micromol/L)
Children 3 to 10 years: 0.73-2.33 mg/dL (56-178 micromol/L)
Children 6 to 18 years: 1.03-2.28 mg/dL (79-174 micromol/L)
Adults: 0.98-2.29 mg/dL (75-175 micromol/L)

7.0 SCIENTIFIC AND CLINICAL RATIONALE AND SIGNIFICANCE

The molecular basis for the use of the amino acid leucine as a therapeutic agent for Diamond Blackfan anemia is based upon the observations in skeletal muscle that leucine supplementation can upregulate components of the translational machinery (53). These components include ribosomal proteins, whose haploinsufficiency appears to be the underlying cause of DBA (5). Successful administration of leucine has been reported in the literature in one patient with DBA (52). A complete response was observed after approximately 6 months of use. In unpublished data reported by the same investigator at conferences 5 more patients were placed on the proposed dose of leucine for this trial (2100 mg/M^2/day in 3 divided doses, well below the highest reported safe dose in humans reported in the GRAS Exemption Claim) with partial responses in 4 of the 5 patients – 2 patients were able to reduce their steroid dose and 2 patients were able to lengthen their time between transfusions; 1 patient did not have a demonstrated difference in transfusion frequency. No toxicity was reported in these patients (personal communication, Pospisilova). This pilot study will test the feasibility of administration of leucine to DBA patients who are transfusion-dependent. The patients will be monitored for hematologic response. Although there is no pathophysiologic reason to expect incremental toxicity to leucine in patients with DBA, patients will be carefully monitored for side effects. Leucine levels and total amino acid profiles will be ascertained in an attempt to correlate response with baseline levels. Where available, responders will also be evaluated with regard to phenotypic and genotypic characteristics that might distinguish them prospectively from other patients.

8.0 STUDY DESIGN

This study will be conducted as a pilot, phase I/II, multi-center clinical trial.

8.1 Primary Endpoints

1) To determine the feasibility of administering the amino acid leucine and to determine the pharmacokinetics of leucine administration in patients with Diamond Blackfan anemia (DBA) who are red cell transfusion-dependent.
2) To determine the safety of leucine administration in transfusion-dependent DBA patients.
3) To determine the efficacy of leucine to produce a hematologic response in transfusion-dependent DBA patients.

8.2 Secondary Endpoint

4) To determine if patients with DBA are deficient in branched chain amino acids.

Since the previous reported patients experienced their responses at 6 months, the total length of this study will be 12- 15 months. The patients will have a screening visit and follow-up visits. Open-label, pharmaceutical-grade leucine capsules will be used and supplied to the subjects for consistency in leucine dosing and preparation. Thus, **oral leucine will be given for 9 months at 700 milligrams/M^2/dose three times daily.**

9.0 PATIENT ELIGIBILITY

All clinical and laboratory studies to determine patient eligibility must be performed within 30 days prior to study enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than thirty days at the start of therapy. Laboratory tests need not be repeated if therapy starts within thirty days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are >30 days old, then the following laboratory evaluations must be re-checked prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol.
Important note: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility of a subject enrolled on this trial must be available in the patient's medical or research record which will serve as the source document for verification at the time of audit.

9.1 Inclusion Criteria

9.1.1 Transfusion dependent DBA age 2 years and older.
9.1.1.1 Transfusion dependence is defined as ≥10 cc/kg or if over 60 kg 2 units of RBC per 28 days averaged over 84 days (3 months) prior to study entry.
9.1.1.2 Diagnostic and supporting criteria for the diagnosis of DBA as published in British Journal of Haematology (8) and described in table below.

Table. Diagnosis of Diamond Blackfan Anemia

Diagnostic criteria

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

Supporting criteria

- Major
  - Ribosomal protein (*RP*) gene or *GATA1* mutation identified
  - Positive family history
- Minor
  - Elevated erythrocyte adenosine deaminase activity
  - Presence of congenital anomaly(ies)
  - 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

sporadic, non-classical DBA if a reported mutation is present. A patient 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, a positive family history and 3 minor supporting criteria are evident, even in the absence of diagnostic criteria. Steroid responsiveness is not considered a diagnostic criterion and corticosteroids should not be administered until a diagnosis is made.

If the first three diagnostic criteria are present, but there is no paucity of red cell precursors in the bone marrow and no supporting criteria, the diagnosis of DBA cannot be made. A bone marrow evaluation should be repeated at a later date as red cell marrow hypoplasia may develop after anemia and reticulocytopenia are observed.

The criteria used for diagnosis must be delineated (Appendix A). Prior to enrollment the principal investigator will determine if the patient meets the diagnostic criteria for inclusion in the study.

9.1.2 The study is restricted to patients who are receiving no DBA specific therapy other than red blood cell transfusions. Patient will remain on his/her transfusion schedule for the duration of this study without any other DBA medications such as therapeutic corticosteroids, anabolic steroids, cyclosporine, etc. to be taken simultaneously or added during the course of this study. Patients must be off of therapeutic corticosteroids or any immunosuppressive therapy for 30 days prior to study enrollment. Hydrocortisone for treatment of adrenal insufficiency will be permitted. Iron chelation medications will be allowed as standard of care for transfused patients.

9.1.3 Patient should have adequate renal function defined as:
- Creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m2 OR
- A serum creatinine based on age/gender as follows:

Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.[35]

9.1.4 Patient should have adequate liver function defined as:
- Total Bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and
- SGPT (ALT) < 5 x upper limit of normal (ULN) for age
- Serum albumin ≥ 2 g/dL

9.1.5 Negative pregnancy test if subject is a menstruating female and documentation of agreement to use adequate contraception for at least 28 days before starting the study, while taking the leucine, and for at least 3 months after the last dose of study medication .

9.1.6 Signed informed consent.

9.1.7 Registration in the Diamond Blackfan Anemia Registry (DBAR) may be offered but is not required for study enrollment.

9.2 Exclusion Criteria
9.2.1 Known hypersensitivity to branched chain amino acids
9.2.2 Diagnosis of an inborn error of amino acid metabolism disorder
9.2.3 Prior hematopoietic stem cell transplantation
9.2.4 Pregnancy, or plans to become pregnant during duration of trial

10.0 SUBJECT RECRUITMENT
Patients with Diamond Blackfan anemia are generally cared for at larger academic centers because of the rarity of the disease and the need for transfusion therapy in about half of the patients. The PI of this protocol is a known clinical researcher in the DBA field. Dr. Vlachos directs the Comprehensive Diamond Blackfan Anemia Clinical Care Center and the Diamond Blackfan Anemia Surveillance and Awareness Program housed at the Steven and Alexandra Cohen Children's Medical Center of New York. Through these efforts many physicians and patients/parents of patients often contact Dr. Vlachos and the DBA Study Coordinator Eva Atsidaftos for information about DBA. Contact is made via telephone, fax and/or e-mail. Recruitment for this study can occur via three methods:

1) The PI and Co-Investigators' institutions will be recruiting patients by word of mouth as they have multiple DBA patients whom they care for that are eligible for enrollment. Many of these patients have already shown interest in this study.

2) Other physicians caring for DBA patients throughout the country that are interested in the study may directly contact the PI and/or the Study Coordinator. The protocol and consent will be sent to these physicians. IRB approval will be obtained at these institutions. The patient will be enrolled onto the study if they meet the eligibility requirements.

3) Subjects/Parents of subjects may contact the PI or Study Coordinator directly. The patient will be referred to a local institution that has an IRB-approved protocol. If one does not exist, the patient will be instructed to have his/her physician contact the PI and/or Study Coordinator to obtain the protocol. The PI and/or Study Coordinator will assist the physician to obtain IRB approval and enroll the patient if the patient is eligible.

This study will be announced via the Diamond Blackfan Anemia Foundation website (www.dbafoundation.org), the DBA Yahoo Group chat room, the Daniella Maria Arturi Foundation website (www.DMAF.org), the DBAR website (www.dbar.org), and the Diamond Blackfan Anemia Surveillance and Awareness Program Facebook site. As the DBA community is a close-knit one with an excellent network, recruitment is expected to be accomplished rapidly through word of mouth.

This study is funded for 50 subjects. We expect to recruit 50-60 subjects of which the first 50 eligible patients will be enrolled. Our target population is the DBA patient community, aged 2

years and older, males and females alike. This disease is noted in all racial and ethnic groups so all are included. The consent will be available in English only at this time. For non-English speaking subjects, a certified oral interpreter service will be used for obtaining informed consent.

11.0 TREATMENT OF SUBJECTS

This study will be a multi-institution study. Institutions must enroll patients directly on this protocol. Patients will also be offered enrollment on the Diamond Blackfan Anemia Registry (DBAR - see section 11.3).

11.1 Informed Consent, Subject Enrollment and Eligibility Screening

The process of informed consent, eligibility screening and enrollment is as follows:

a. The local PI or his/her designee calls the Central Study Coordinator to verify that the study is still accruing subjects.
b. The local PI will obtain informed consent from the subject or his/her parents/legal guardian for enrollment into the study.
c. Once informed consent is signed, the local PI will call/e-mail the Coordinating Center and a participant identification number will be assigned by the Central Study Coordinator.
d. Screening of the patient for eligibility will proceed with completion of the Eligibility Screening Checklist which will be forwarded to the Coordinating Center.
e. The local PI will be informed of the patient's eligibility by fax and/or e-mail within 24 hours of receipt of the Eligibility Screening Checklist or by the next business day.
f. Participant enrollment will then proceed with assignment of the leucine dose. The Enrollment Form will be submitted by local PI as required.
g. This process will be repeated until 50 subjects are enrolled. Patients found to be not eligible after screening will be deemed 'screen failures'.

11.1.1 Current Study Status

Local investigators or their designee must contact the Primary Institution (1-516-562-1505) to determine if the study is currently open for accrual. This is to ensure that no more than 50 subjects are enrolled on the study. Once 50 subjects are enrolled, excluding the 'screen failures', a memo will be sent out to all participating institutions that accrual has been reached.

11.1.2 Informed Consent/Assent (Appendix B)

A signed informed consent document will be obtained prior to entry onto this study. The consent documents may be mailed or e-mailed for potential subjects to review, however the actual consent process will take place in person. The principal investigator and/or sub investigator will obtain consent after the patient and/or patient's parent/legal guardian are informed of the investigational nature and research objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts as well as potential alternative therapies. The local PI and his/her designees will be well versed in this study. There is no urgency in the start of this medication for DBA patients. Patients and their guardians will be allotted ample time to review the study risks/benefits before signing consent. It will be allowable to have those patients not treated at the consenting institution to take the consent home to discuss with their treating hematologist, only after discussing the study and going through the consent process with appropriate documentation. If they decide to enroll, they may mail the consent back to the

coordinating center. It will not be uncommon to discuss the study with the patient and consent them at their next transfusion visit 3-4 weeks later. Any and all questions will be answered regarding the study and the required tasks. The informed consent and assent will be signed according to institutional guidelines. Documentation of the informed consent will be maintained in the subject's research chart.

11.1.2.1 Consent will be obtained from the subject himself/herself, or, in the case of a minor, from the parent or legal guardian. Subjects ages 7-11 and 12-17 (or as determined by the local IRB) who are able to read and understand an assent will be asked to sign a simplified document, along with their parent or guardian signing the standard consent form. Minors who turn 18 while still active in the study will be consented as adults. These may be mailed, as a face to face reconsent may not be feasible for those treated at outside facilities. A copy will be given to the subject/subject's legal guardian by the person obtaining the consent.

11.1.2.2 The original consent form will be kept in the subject's chart at the subject's local institution and will be available for review if needed by a monitoring body.

11.1.2.3 If at any time during study participation new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled subject and re-consent obtained as appropriate. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

11.1.3 Subject Identification

Once the subject/parent of patient/legal guardian has signed consent, the local institution will call the Coordinating Center (1-516-562-1505) to obtain participant identification (ID) number from the Central Study Coordinator. A confidential copy will be sent to the local institution with the subject's ID for confirmation. A participant identification number will be assigned to identify the subject to that investigator and institution and will be used in all future communications with the site. This is to assure that no two possible subjects are enrolled simultaneously.

11.1.3.1 *Participant Identification Number (PIN)*

Each subject registered will be assigned a unique participant identification number. This PIN will consist of two parts: the first two digits will identify the local institution who is registering the subject, and the three last digits will number the subjects from that institution consecutively. The funding allows for a total of 17 possible institutions so the first two digits will range from 01 (the Coordinating Center) to 17. For example, PIN 01-001 will indicate the first subject on study, which in this case would have been enrolled by the Coordinating Center. Each potential patient will be assigned an ID once the consent has been signed. If the subject does not meet the eligibility criteria once screening occurs then that number will be skipped.

11.1.4 Eligibility Screening

Diagnostic or laboratory studies performed exclusively to determine eligibility for this study must be done only after obtaining written informed consent. Eligibility is then verified by meeting the criteria listed in the Eligibility Screening Checklist (Appendix C). Studies or procedures that were performed for clinical indications (not exclusively for eligibility for this study) may be used for baseline values if the studies were done within thirty days of when the informed consent was obtained. Before the subject can be enrolled, the responsible institutional investigator must sign and date the completed Eligibility Screening Checklist. A signed copy of the Eligibility Screening Checklist will be faxed to the Coordinating Center (1-516-562-1599). The fax should be sent to the attention of the Central Study Coordinator and must include the

associated study number and participant identification number. The lead PI and Central Study Coordinator will review the Eligibility Screening Checklist. After review, the local investigator will be informed of eligibility status via a fax and/or e-mail within 24 hours, if received Monday through Thursday. If the form is received on Friday, eligibility will be confirmed by the next business day.

11.1.5 Study Enrollment
Subjects may be enrolled on the study once all eligibility requirements for the study have been met and confirmed. **Subjects who sign informed consent for the protocol and undergo screening for eligibility should not be started on the study drug until the screening is completed and they are deemed to meet all eligibility criteria.** Study enrollment is accomplished by completing the Enrollment Form (Appendix D). The Enrollment Form will be completed and faxed to the Coordinating Center (1-516-562-1599). The fax should be sent to the attention of the Central Study Coordinator and must include the associated study number and participant identification number.

11.1.6 Dose Assignment
The dose will be assigned by the local PI and Study Coordinator at the time of study enrollment based on the subject's body surface area. The prescribed amount of leucine will be rounded up to the nearest dosage tablet as indicated in the Leucine Dosage Calculation Table (Appendix E). A Leucine Dose Assignment Form (Appendix F) will be faxed to the participating institution from the Coordinating Center.

11.2 IRB Approval
Local IRB approval must be obtained at all participating sites in order to begin enrollment onto this protocol. Sites must submit copies of IRB approval to the Coordinating Center. IRB approval documents should be faxed to the Central Study Coordinator along with a cover sheet (Eva Atsidaftos at 1-516-562-1599), e-mailed (eatsidaf@nshs.edu) or mailed to the Coordinating Center.

11.3 Diamond Blackfan Anemia Registry (DBAR)
The Diamond Blackfan Anemia Registry is a demographic, laboratory and clinical database of patients with DBA in North America. Subjects screened for this study may be offered enrollment in the DBAR (through a separate consent and questionnaire) by their local investigator(s). Enrollment in the DBAR is not required for participation in this study.

12.0 DATA COLLECTION (Appendices D and G)
12.1 Enrollment Data (Appendix D)
12.1.1 Demographics required for shipping refills
12.1.2 Sex, race, date of birth, date of diagnosis (if available) (birth date to be collected in order to reduce error of mistaking one subject for another with the same age and initials)
12.1.3 Current and past medications
12.1.4 Transfusion history
12.1.5 Completion of DBAR consent and questionnaire – if the subject (or his/her parent/guardian) wants to enroll in the DBA Registry – which is not part of this protocol but will be offered to the subject under its own protocol/consent

12.2 Case Report Form Data (Appendix G)
The Case Report Form data is to be collected at baseline, at Weeks 2 and 4 and at Months 2, 3, 4, 5, 6, 7, 8, and 9 (or at time of transfusion, except where indicated otherwise). Each visit can be +/- 1 week of the requested time except week 2. Blood drawings can also be +/- 1 week of the requested time except week 2.

12.2.1 Physical Examination
12.2.1.1 Temperature, Pulse and Blood Pressure
12.2.1.2 Height (cm) at baseline, month 4 and month 9, weight (kg), and body surface area (M^2)

12.2.2 Laboratory Examinations
12.2.2.1 CBC with platelet count, differential, and red cell indices
12.2.2.2 Reticulocyte count
12.2.2.3 Percent Hemoglobin F at baseline and then every 3 months and at study completion
12.2.2.4 Hepatic transaminases, serum bilirubin, creatinine, and blood urea nitrogen
12.3.2.5 Serum ferritin at baseline and then every three months and at study completion
12.2.2.6 If female and of child-bearing potential, pregnancy test at baseline and every 3 months
12.2.2.7 Plasma leucine level as well as levels of isoleucine and valine and an amino acid profile at baseline. Plasma leucine levels are obtained for research only and will not be used for clinical decision making. This extra blood will be drawn at the same time as standard routine clinical blood draws. A standard 10cc's (2 teaspoons) will be taken as part of clinical care, with an additional 5 cc's (1 teaspoon) taken after Leucine treatment begins to monitor leucine levels in the blood. Plasma leucine levels (5 cc's) will also be drawn at 2 weeks, 4 weeks, then months 3, 6, and 9 months (These are the extra tests for this study protocol. All the others are standard of care for the enrolled subjects.) Subjects will fast for 4 hours and hold the morning dose of Leucine for evaluation of the Leucine level at these time points. Blood drawings can be $\pm$ 7 days of the requested time, except week 2.

Laboratory examinations 12.2.2.1, 12.2.2.2, 12.2.2.4 and 12.2.2.5 are standard of care for transfused DBA subjects who are on chelation medication – which the chronically transfused subjects eligible for this protocol will be receiving; 12.2.2.3, 12.2.2.6 and 12.2.2.7 are extra tests that are specific to the study protocol. The instructions for sample collection and shipping of blood for amino acid levels are delineated in Appendix H. The blood samples will be batched and shipped directly from the participating institution to Case Western Reserve University, Ohio where the tests will be performed. The costs for the mailing will be provided by the Coordinating Center through a FedEx account.

12.2.3 Other Specifics
12.3.3.1 Transfusion history (date and amount in ml and/or units q 3-4 weeks based on transfusions)
12.3.3.2 Adverse side effects
12.3.3.3 Hospitalizations (cause, dates)

12.4 Data Submission

Case Report Forms (Appendix G) will be completed by the local PI and/or his/her designee at each subject visit. Case Report forms will be faxed to the Coordinating Center (1-516-562-1599) where data will be stored on a secure database. These forms will be required to be submitted every 3 months, including at study completion (three times in total). The central Study Coordinator will perform data integrity monitoring visits of a minimum of 20% of the entered data through cross-checking and source documentation. Data will be analyzed with biostatistical support after completion of the study.

12.5 Data Storage

An institution or, when appropriate, an IRB shall prepare and maintain adequate documentation of IRB activities, including the following:
(1) Copies of all research proposals reviewed; scientific evaluations, if any, that accompany the proposals; approved sample consent documents; progress reports submitted by investigators; and reports of injuries to subjects.
(2) Copies of all correspondence between the IRB and the investigators.

With regard to the consent document, all sites must specify consent document storage and length of time, as well as destroyed/disposed specifics. Also research records and data storage and time destroyed/disposed, hard copies vs. electronic storage, research specimens storage and time destroyed/disposed, and any special storage conditions must be specified according to federal and institutional requirements.

Electronic and paper clinical research records will be retained for at least 7 years for adult subjects and 10 years for pediatric subjects after completion of the research study or at least 2 years after marketing approval by the FDA (or if not approved, until 2 years after shipment and delivery for investigational use is discontinued), whichever is longer. Electronic or paperless data sets should be kept on a secure server with adequate backup to assure record retention. Paper research records should be maintained in a secure office, in a secure file cabinet for the duration of the study and then archived.

All data collected from all health system sources will be maintained on a secure and password protected server and backed up every 24 hours. Study staff should have restricted access to directories and files on the server, according to project responsibilities. “Login/password security” should be used to control access to the network. Individuals with data entry permissions are able to add records only to the databases to which they have access.

A unique participant identification number will be assigned to each subject at the time of enrollment. In an effort to correlate information every unique PIN will be linked (coded) with the subject’s medical record information. The coded link between this unique identifier and the subject’s identifying information will be maintained in locked files. When information is shared internally or externally, investigators will only be provided with the subject’s unique PIN.

Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command, the North Shore-LIJ Health System, and other federal and state authorities as a part of their responsibility to protect human subjects in

research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

13.0 STUDY DRUG ADMINISTRATION

Each subject will receive standard dose Leucine therapy for 9 months:

● 700 mg/m^2/dose orally three times daily for a total of 2100 mg/m^2/day

13.1 Study Drug - Leucine

This clinical trial will only utilize commercially available Leucine from Ajinomoto Aminoscience LLC, Raleigh, NC. The Leucine will be supplied in capsule form in 250 mg/capsule concentration. The Coordinating Center will purchase the Leucine supply.

13.1.1 *Dispensing of Study Drug* Once the subject has signed consent and is enrolled on the study, the local PI will be faxed the Dose Assignment Form from the Coordinating Center. The participating center will complete the Supply Request (Appendix F) and fax it to the Coordinating Center at 516-562-1599. Once received the Coordinating Center's Study Pharmacy will be informed of the subject enrollment and will dispense a 3-month supply of the Leucine to the participating subject. The Leucine will be dispensed in 250 mg capsules, in child-proof capped containers, to be stored at room temperature. The shipment will be sent directly to the subject. Upon request, local investigators may dispense the study drug after showing they are able to store drug in a locked area and maintain a log of receiving and dispensing the study drug**13.1.2** *Dispensing of Study Drug to Subject*

The drug will be shipped directly to the subject from the coordinating site. The amount sent will be enough drug for a 3 month period.

13.1.3 *Medication Instructions*

The Leucine Dose Assignment Form (Appendix F) will be faxed/e-mailed to the local PI to be reviewed with the subject. Instructions for medication usage will be distributed to the subject and/or parent/guardian of the subject along with the dose. May be taken with or without food. Capsules can be mixed into food or dissolved in liquids. Total daily dose will be divided into 3 doses to be given AM, afternoon and PM.

13.1.4 *Concomitant Medications*

Concomitant medications will be documented on the Case Report Form (Appendix G).

13.2 Transfusion Therapy During Study Drug Administration

Subjects will receive 10-15 ml/kg/transfusion, with all volumes recorded on the Case Report Forms.

13.2.1 If the hemoglobin (Hb) is less than 8 gm/dL and there is no evidence of reticulocyte response (retic count < 0.5%), **the subject will be transfused as usual**.

13.2.2 If the Hb is greater than 8 gm/dL, the reticulocyte count is greater than 0.5% (or baseline) and the subject is clinically stable (without fever, respiratory illness, etc.), **the transfusion should be held**. The subject should be followed again in 1-2 weeks and reassessment will be made to see if a response is occurring.

13.2.3 If the Hb is between 7-8 gm/dL, the reticulocyte count is greater than 0.5% (or baseline), and the subject is clinically stable, then the subject can opt out of transfusion, based upon clinical considerations, for one week, **or** receive 10 ml/kg packed red blood cell transfusion so as not to

abort a possible response. If the subject continues to show a response then Hb can be checked every 2 weeks and transfusion held. If not, the subject will resume the transfusion schedule.

13.3 Schedule of Evaluations

Oral Leucine x 9 months

VISIT *	0	2wk	4wk	2mo	3mo	4mo	5mo	6mo	7mo	8mo	9mo
PE **	X	X	X	X	X	X	X	X	X	X	X
BP	X	X	X	X	X	X	X	X	X	X	X
Height	X					X					X
Weight	X	X	X	X	X	X	X	X	X	X	X
CBC with Retic ***	X	X	X	X	X	X	X	X	X	X	X
%Hb F	X				X			X			X
Chemistry**** Panel	X	X	X	X	X	X	X	X	X	X	X
Ferritin	X				X			X			X
Pregnancy test	X				X			X			
Leucine level	X	X	X		X			X			X

*Each visit can be ± 1 week of the requested time – except visit 2.

** PE includes Temperature, Pulse and Blood Pressure complete physical exam as clinically necessary *** CBC to include WBC, RBC, Hb, Hct, Plts, differential and reticulocyte count (%)

**** Chemistry Panel to includeUrea Nitrogen, Creatinine, AST, ALT, Bilirubin, total and direct,

13.4 Grading of Adverse Events

Adverse events (toxicities) will be graded according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE) (http://ctep.cancer.gov/reporting/ctc.html). Any suspected or confirmed dose-limiting toxicity should be reported immediately (within 24 hours) to the Study PI.

13.5 Dose Modifications for Adverse Events

13.5.1 Dose Reduction

There will be no drug reduction for the study drug.

13.5.2 Dose Escalation

There will be no drug escalation for the study drug.

13.5.3 Dose Discontinuation

In the event of any serious adverse event (SAE), the study drug will be discontinued until all symptoms resolve. If the SAE is deemed possibly related to the protocol therapy, the subject will be removed from the study and leucine will be permanently discontinued.

14.0 EVALUATION CRITERIA

14.1 On-Study Response Criteria - evaluated at 6 and 9 months of treatment

14.1.1 Complete Response (CR) – Hb > 9 gm/dL and weaned off of transfusion therapy

1a- Complete response lasting ≥ 6 months

14.1.2 Partial Response (PR) - Hb < 9 gm/dL with an increase in reticulocyte count to 1% and any transfusion interval increase
14.1.3 No Response (NR) - No change in transfusion needs, no change in Hb or reticulocyte count
14.1.4 Progression - An increased transfusion requirement with increased volume or increased interval

15.0 WITHDRAWAL AND OFF STUDY CRITERIA

15.1 Subject's choice: A subject may withdraw from the study at any time. The risk of withdrawing will be discussed, as will alternative treatment options. Once off study, subjects will be referred back to his or her referring physician. Alternative treatment will then be discussed with the primary hematologist.
15.2 PI Decision: Alternative treatment options will be discussed with the subject, who will then be referred back to his or her referring physician.
15.3 Discontinuation for Toxicity: Subjects will be discontinued from study for any Grade III/IV toxicity related to the study drug as determined by PI.
15.4 Discontinuation for Non-Adherence: Subjects will be discontinued from study if non-adherent to protocol.

16.0 BIOSTATISTICAL CONSIDERATIONS

Response to treatment (Complete, Partial, None, Progression) will be evaluated at 6 and 9 months. Time-to-achieve a complete or partial response will be examined as well.

16.1 General Study Objectives

16.1.1 To determine the feasibility of administering the amino acid leucine and to determine the pharmacokinetics of leucine administration in subjects with Diamond Blackfan anemia (DBA) who are red cell transfusion-dependent.
16.1.2 To determine the efficacy of leucine to produce a hematologic response in subjects with DBA.
16.1.3 To determine the side effects of leucine administration in DBA subjects (monthly).
16.1.4 To determine if subjects with DBA are deficient in branched chain amino acids (baseline).

16.2 Specific Statistical Aims of the Study (for the Data Analysis)

16.2.1 The primary objective of the study is to estimate the proportion of complete responders, partial responders or non-responders at 9 months. These proportions will also be estimated at 6 months.
16.2.2 To determine the time-to-first occurrence of a CR or PR. Subjects who do not achieve CR or PR by the end of the study at 9 months will be considered 'censored for CR (PR)'.
16.2.3 To catalogue the phenotypic characteristics of the responders and non-responders at 6 and 9 months.
16.2.4 To examine the frequency and severity of adverse events (AEs) that may arise from the study and to determine if these AEs are related to the study drug.

16.3 Endpoint Variables

The primary outcome is the type of response observed at 9 months (and 6 months). Response to treatment can be one of the following:

a. Complete response (CR): Hb > 9 gm/dL and transfusion-independence as defined in DBA
b. Partial response (PR): Hb < 9 gm/dL and increased reticulocyte count to greater than 1% and any increase in transfusion interval from baseline. (Baseline reticulocytes range from 0.1 to 0.5 and transfusions are usually performed every 3 weeks. An increase of reticulocyte counts to over 1 to 1.5% and any increase in transfusion interval will be considered a PR.)
c. No response (NR): no change in transfusion requirements and no significant change in Hb or reticulocytes (or any response that does not satisfy the conditions of either a PR or CR)
d. Progression: worsening of disease as defined by the need for more frequent transfusions

Secondary outcomes include time-to-occurrence of either a PR or CR, and safety parameters such as type, frequency, and severity of adverse events and relationship to leucine (according to the NCI CTCAE version 4.0).

16.4 Statistical Methods

This is a standard Phase I/II study with the goal of determining the feasibility, safety and efficacy of the use of leucine in transfusion-dependent DBA subjects. A maximum of 50 subjects will be enrolled into the study. The statistical approach will be primarily descriptive in nature.

For specific aim 1, the proportion of subjects achieving a specific response at 6 and 9 months (CR, PR, NR or progression) along with their corresponding 95% confidence intervals, will be calculated using standard methods of estimating proportions.

For specific aim 2, time-to-response (separately for CR and PR) will be analyzed using survival analysis techniques. Subjects who do not achieve CR or PR by the end of the study at 9 months will be considered 'censored for CR (PR)'. Kaplan-Meier curves will be generated and the log-rank test will be used for any comparisons with respect to time-to-CR (PR), if feasible.

For specific aim 3, the approach will be primarily descriptive. However, we may carry out some analyses if feasible. First, response status at 9 months (separately for CR and PR) will be cross-tabulated with certain subject phenotypes; a chi-square test or Fisher's exact test, as appropriate, will be used to determine if there is an association between response status and the certain phenotype. Second, time-to-response will be analyzed. Kaplan-Meier curves will be generated and the log-rank test will be used to compare phenotypes with respect to time-to-CR (PR), if feasible. Subjects who do not achieve CR or PR by the end of the study at 9 months will be considered 'censored for CR (PR)'.

For specific aim 4, the proportion of adverse events will be tabulated and their corresponding 95% confidence interval will be calculated.

16.5 Sample Size Considerations

The sample size for the Phase I/II study (i.e. a maximum of 50 subjects) is a sample size of convenience. The number of subjects in the proposed study is based on logistics/funding

provided by the Department of Defense Telemedicine and Advanced Technology Research Center (TATRC); it is not based on any formal power calculations. Results from this study may provide valuable information for quantifying the effects of leucine in this group of transfusion-dependent DBA subjects and may be useful in estimating the effect size necessary for appropriately designing future larger-scale Phase III clinical trials. However, in order to have some idea as to the precision of estimates that can be obtained from this study, when the sample size is 50, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation will be no wider than 50%±13.9% i.e. the 95% confidence interval will range from at most, 36.1% to 63.9%.

17.0 RESEARCH MONITOR REQUIREMENT

17.1 Per DOD Directive 3216.2, all GREATER THAN MINIMAL RISK STUDIES require a Research Monitor. This individual should be a qualified physician, other than the Principal Investigator, not associated with the protocol, able to provide medical care to research volunteers for conditions that may arise during the conduct of the study, and who will monitor the volunteers during the conduct of the study. The research monitor plays a role in reviewing monitoring plans and unanticipated problems.

17.2 The research monitor is required to review all unanticipated problems involving risk to subjects or others associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor will comment on the outcomes of the event or problem. The research monitor will also indicate whether he/she concurs with the details of the report provided by the site principal investigator.
17.3 The Research Monitor has been identified as:

Dr. Lawrence Wolfe,
at The Steven and Alexandra Cohen Children's Medical Center of New York
269-01 76th Avenue, Rm. 255
New Hyde Park, NY 11040
Phone: 718-470-3460
Fax: 718-343-4642
E-mail: lwolfe2@nshs.edu

18.0 SAFETY MONITORING

18.1 Reporting of Adverse Events and Unanticipated Problems

Information about all serious adverse events, which may be noted through history, investigator questioning, by physical examination, or by subject volunteering, will be reported to the Coordinating Center by fax (516-562-1599) on the Severe Adverse Event (SAE) Reporting Form (Appendix I) and the local IRB **within 24 hours** of learning of the occurrence of the event.

18.2 Clinical Research Monitoring Services

The Office of Research Compliance (ORC), located at The Feinstein Institute of Medical Research (FIMR) and part of the Coordinating Center will provide clinical research monitoring services.
18.2.1 These services include 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

18.2.2 The ORC agrees to provide primarily remote monitoring services for all performance sites according to the risk-based monitoring plan including the following:

- Site initiation: Prior to study start-up for good clinical practice (GCP) and protocol overview

-Follow-up monitoring: Within 2 weeks after the first subject is enrolled and then after every fifth subject is enrolled (for a minimum of 11 monitoring visits) in addition to targeted reviews as required

- Final monitoring: Study close out

18.2.3 Reporting: Regular monitoring reports will be generated by the ORC after each review and will be provided to the respective performance site and Study PI. Resolution of queries and outstanding issues or concerns will be the responsibility of the individual performance site and local PI. The lead PI will be responsible for reporting incidents of IRB non-compliance to the North Shore-LIJ Health System IRB and for securing compliance at all trial sites.

18.3 Reporting to the U.S. Army Medical Research and Materiel Command

18.3.1 The protocol will be conducted in accordance with the protocol submitted to and approved by the ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the ORP HRPO.

18.3.2 Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

18.3.3 Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

18.3.4 Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

18.3.5 A copy of the approved continuing review report and the IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

18.3.6 The knowledge of any pending compliance inspection/visit by the FDA, DHHS-OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO.

18.4 SAE Definitions An SAE is any untoward medical occurrence that:

18.4.1 Results in death

18.4.2 Is life-threatening (defined as an event in which the subject is at risk of death at the time of SAE; this does not refer to an event which hypothetically might have caused death if more severe)
18.4.3 Requires inpatient hospitalization or prolongation of existing hospitalization
18.4.4 Results in more frequent need for erythrocyte transfusions (more than pre-study)
18.4.5 Results in persistence or significant disability or incapacity
18.4.6 Results in overdose (defined as the intentional or accidental ingestion of study drug at any dose which is considered medically relevant and excessive)
18.4.7 Is medically significant (defined as the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above)
18.4.8 Any SAE occurring after the subject has signed the informed consent form, and until 12 weeks after the subject has stopped study participation, must be reported.

18.5 Assessment Terms for SAE and Causal Relationship to Study Drug
18. 5.1 DEFINITE means there is a reasonable causal relationship between study drug and the SAE. The event responds to withdrawal of drug and recurs with re-administration of the study drug (if clinically feasible).
18.5.2 PROBABLE means there is a reasonable causal relationship between study drug and the SAE. The SAE tapers or diminishes once study drug has been discontinued. Re-administration of the study drug not required. Relationship between study drug and SAE cannot be reasonably explained by the known characteristics of subject's clinical state.
18.5.3 POSSIBLE means there is a reasonable causal relationship between study drug and the SAE but the SAE could have been produced by subject's clinical state or by other means of treatment or medications concurrently administered to the subject.
18.5.4 UNLIKELY means there is a temporal relationship to study drug administration but no reasonable causal relationship exists between study drug and SAE. The SAE does not follow a reasonable sequence from administration of study drug. SAE could have been produced by subject's clinical state or by other means of treatment or medications concurrently administered to the subject.
18.5.5 UNRELATED means there is no temporal relationship between SAE and study drug administration (e.g., the SAE occurred too early, too late, or study drug not taken) or there is reasonable causal relationship between SAE and another drug, concurrent disease, or circumstance and the SAE. Subjects experiencing AEs that cause an interruption or discontinuation of study drug or those who experience AEs that are present at the end of study should receive appropriate follow-up care. Sites should report the outcome of any AE that caused permanent discontinuation of the study drug or that was present at the end of the study.

18.6 Grades of Severity
18.6.1 MILD An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
18.6.2 MODERATE An event that is sufficiently discomforting to interfere with normal everyday activities.
18.6.3 SEVERE An event that prevents normal everyday activities.

18.7 Non-Serious Adverse Event (AE) Reporting

18.7.1 An Adverse Event (AE) is *any* unexpected, non-serious event that is NOT an SAE. An AE is any untoward medical occurrence or worsening of a pre-existing medical condition in a subject who receives a study drug and which does not have a necessarily causal relationship with study treatment.
18.7.2 A "study drug" is defined as a pharmaceutical form of an active ingredient being tested in this study.
18.7.3 An AE, therefore, can be any unfavorable and unintentional sign (*including* a clinically significant abnormal laboratory finding, for example), symptom or disease associated with the use of the medicinal product, whether or not considered related to the medicinal product. The collection of Non-Serious AE information should begin at Day 1, initiation of study drug.
18.7.4 All identified AEs must be recorded on the data capture forms, including description of AE, date and time of onset and resolution; duration of AE if less than 24 hours, severity of event, investigator's opinion of the relationship of the study drug, study treatment action (if any), and outcome of AE (if known).

19.0 HUMAN SUBJECT PROTECTION

19.1 Rationale for Subject Selection

19.1.1 This study will be open to all subjects who fully meet the inclusion and exclusion criteria as described in section **9.0**. Epidemiologic data suggests that the gender will be approximately evenly split between males and females as the DBAR has reported the male: female ratio to be 1.03:1.00. DBA has been reported in nearly all ethnicities.
19.1.2 This study will be listed on:
- *www.clinicaltrials.gov* (NHLBI patient recruitment site)
- *www.dbar.org* (Diamond Blackfan Anemia Registry site)
- *www.dmaf.org* (Daniella Maria Arturi Foundation site)
- *www.dbafoundation.org* (Diamond Blackfan Anemia Foundation site)

19.2 Participation of Children

DBA is an extremely rare disorder with approximately 1,000 cases in the United States. An estimated 90% are diagnosed before one year of age. Consequently, it is vital to include children in this protocol because this age group is most likely to benefit from future intervention. The majority of subjects expected to be treated will be children as
a) this is a congenital disorder
b) 90% of DBA patients are anemic by 1 year of age
c) the mean age of the patients in the DBAR is 18 years, and
d) it is desirable to avoid the complications of chronic transfusion therapy as soon as possible.

19.3 Risks and Discomforts

19.3.1 Related to Leucine **(**See Section **6.0**)
19.3.2 Related to blood draws
Blood samples will be obtained during the screening and periodic visits for diagnostic tests to monitor the effectiveness of the therapy, detect possible side effects, and for research purposes. No major risk is associated with blood drawing. Minor complications may include bleeding, pain, and hematoma formation at the site.

19.4 Risks in Relation to Benefit for Adult and Pediatric Subjects

19.4.1 Benefits to the patients themselves could be a reduction or even absence of transfusion requirements. This could in the long run prevent morbidity and mortality from transfusion-associated viral agents and more importantly, from iron overload. The need for chronic chelation could also be avoided in responding subjects. Therefore, this research involves more than a minor increase over minimal risk to subjects with the prospect of direct benefit.
19.4.2 Risks in Relation to Benefit for Pediatric Subjects Specifically This study meets the DHHS regulation *§46.405* as follows: *a) the risk is justified by the anticipated benefit to the subjects* Pediatric subjects with a serious, chronic hematologic disease will be offered a possible alternative to transfusion therapy. *b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches* The benefits to the patients could be a reduction or even a discontinuation of transfusion requirements and/or improvement of anemia, resulting in improved quality of life. It may offer decreased morbidity and mortality from transfusion-associated viral agents and the complications of iron overload. The need for chronic iron chelation could also be avoided in responding subjects.
Adequate provisions are made for soliciting the assent of the children and permission of their parents or legal guardians. Therefore, for pediatric subjects, this research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects.

20.0 COMPLIANCE

The knowledge of any pending compliance inspection/visit by the FDA, DHHS-OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Records, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to ORP HRPO and according to institutional policy.

21.0 REFERENCES

1. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, Ball S, Tchernia G, Klar J, Matsson H, Tentler D, Mohandas N, Carlsson B, Dahl N. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* 1999; 21(2):169-75.
2. Gazda HT, Grabowska A, Merida-Long LB, Latawiec E, Schneider HE, Lipton JM, Vlachos A, Atsidaftos E, Ball SE, Orfali KA, Niewiadomska E, Da Costa L, Tchernia G, Niemeyer C, Meerpohl JJ, Stahl J, Schratt G, Glader B, Backer K, Wong C, Nathan DG, Beggs AH, Sieff CA. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2006; 79(6):1110-8.
3. Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. *Hum Mutat* 2007; 28(12):1178-82.
4. Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, Talbot CC Jr, Meltzer P, Esposito D, Beggs AH, Schneider HE, Grabowska A, Ball SE, Niewiadomska E, Sieff CA, Vlachos A, Atsidaftos E, Ellis SR, Lipton JM, Gazda HT, Arceci RJ. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. *Blood* 2008; 112(5):1582-92.
5. Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, Darras N, Hasman C, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Zaucha JM, Glader B, Niemeyer C, Meerpohl JJ, Atsidaftos E, Lipton JM, Gleizes PE, Beggs AH.

Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. *Am J Hum Genet* 2008; 83(6):769-80.
6. Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue M, Clinton C, Schneider H, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Glader B, Atsidaftos E, Lipton JM, Gleizes P-E, Gazda HT. Ribosomal protein genes S10 and S26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2010 Feb 12;86(2):222-8. Epub 2010 Jan 28. Erratum in: *Am J Hum Genet* 2010 Apr 9;86(4):655.
7. Vlachos A, Klein GW, Lipton JM.The Diamond Blackfan Anemia Registry: tool for investigating the epidemiology and biology of Diamond-Blackfan anemia. *J Pediatr Hematol Oncol* 2001; 23(6):377-82.
8. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, Meerpohl J, Karlsson S, Liu JM, Leblanc T, Paley C, Kang EM, Leder EJ, Atsidaftos E, Shimamura A, Bessler M, Glader B, Lipton JM; Participants of Sixth Annual Daniella Maria Arturi International Consensus Conference. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* 2008; 142:859-76.
9. Vlachos A, Federman N, Reyes-Haley C, Abramson J, Lipton JM. Hematopoietic stem cell transplantation for Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Bone Marrow Transplant* 2001; 27(4):381-86.
10. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond Blackfan anemia: an update from the Diamond Blackfan Anemia Registry. *Pediatr Blood Cancer* 2006; 46(5):558-64.
11. Ozsoylu S. Oral megadose methylprednisolone for the treatment of Diamond-Blackfan anemia. *Pediatr Hematol Oncol* 1994; 11:561-562, 567-568.
12. Bernini JC, Carrillo JM, Buchanan GR. High-dose intravenous methylprednisolone therapy for patients with Diamond-Blackfan anemia refractory to conventional doses of prednisone. *J Pediatr* 1995; 127(4):654-59.
13. Buchanan GR. Oral megadose methylprednisolone therapy for refractory Diamond-Blackfan anemia. International Diamond-Blackfan Anemia Study Group. *J Pediatr Hematol Oncol* 2001; 23:353-56.
14. Totterman TH, Nisell J, Killander A, Gahrton G, Lonnqvist B. Successful treatment of pure red-cell aplasia with cyclosporin. *Lancet* 1984; 2(8404):693.
15. Williams DL, Mageed AS, Findley H, Ragab AH. Cyclosporine in the treatment of red cell aplasia. *Am J Pediatr Hematol/Oncol* 1987; 9:314-16.
16. Seip M and Zanussi GF. Cyclosporine in steroid-resistant Diamond-Blackfan anaemia. *Acta Paediatr Scand* 1988; 77(3):464-66.
17. Leonard EM, Raefsky E, Griffith P, Kimball J, Nienhuis AW, Young NS. Cyclosporine therapy of aplastic anaemia, congenital and acquired red cell aplasia. *Br J Haematol* 1989; 72(2): 278-84.
18. Splain J and Berman BW. Cyclosporin A treatment for Diamond-Blackfan anemia. *Am J Hematol* 1992; 39(3):208-11.
19. Monteserin MC, Garcia Vela JA, Ona F, Lastra AM. Cyclosporin A for Diamond-Blackfan anemia: a new case. *Am J Hematol* 1993; 42:406-7.
20. Alessandri AJ, Rogers PC, Wadsworth LD, Davis JH. Diamond-blackfan anemia and cyclosporine therapy revisited. *J Pediatr Hematol Oncol* 2000; 22:176-79.

21. Bobey NA, Carcao M, Dror Y, Freedman MH, Dahl N, Woodman RC. Sustained cyclosporine-induced erythropoietic response in identical male twins with diamond-blackfan anemia. *J Pediatr Hematol Oncol* 2003; 25(11):914-18.
22. Harris SI and Weinberg JB. Treatment of red cell aplasia with antithymocyte globulin: repeated inductions of complete remissions in two patients. *Am J Hematol* 1985; 20(2):183-86.
23. Roychowdhury DF and Linker CA. Pure red cell aplasia complicating an ABO-compatible allogeneic bone marrow transplantation, treated successfully with antithymocyte globulin. *Bone Marrow Transplant* 1995; 16(3):471-72.
24. Abkowitz JL, Powell JS, Nakamura JM, Kadin ME, Adamson JW. Pure red cell aplasia: response to therapy with anti-thymocyte globulin. *Am J Hematol* 1986; 23(4):363-71.
25. McGuire WA, Yang HH, Bruno E, Brandt J, Briddell R, Coates TD, Hoffman R. Treatment of antibody-mediated pure red-cell aplasia with high-dose intravenous gamma globulin. *N Engl J Med* 1987; 317(16):1004-8.
26. Bejaoui M, Fitouri Z, Sfar MT, Lakhoua R. Failure of immunosuppressive therapy and high-dose intravenous immunoglobulins in four transfusion-dependent, steroid-unresponsive Blackfan-Diamond anemia patients. *Haematologica* 1993; 78(1):38-9.
27. Sumimoto S, Kawai M, Kasajima Y, Hamamoto T. Intravenous gamma-globulin therapy in Diamond-Blackfan anemia. *Acta Paediatr Jpn* 1992; 34(2):179-80.
28. Miceli Sopo S, Pesaresi MA, Pastore M, Stabile A. Intravenous immunoglobulin in Diamond-Blackfan anaemia. *Eur J Pediatr* 1990; 149(11):779-80.
29. Halperin DS, Estrov Z, Freedman MH. Diamond-Blackfan anemia: promotion of marrow erythropoiesis in vitro by recombinant interleukin-3. *Blood* 1989; 73(5):1168-74.
30. Dunbar CE, Smith DA, Kimball J, Garrison L, Nienhuis AW, Young NS. Treatment of Diamond-Blackfan anaemia with haematopoietic growth factors, granulocyte-macrophage colony stimulating factor and interleukin 3: sustained remissions following IL-3. *Br J Haematol* 1991; 79:316-21.
31. Gillio AP, Faulkner LB, Alter BP, Reilly L, Klafter R, Heller G, Young DC, Lipton JM, Moore MA, O'Reilly RJ. Treatment of Diamond-Blackfan anemia with recombinant human interleukin-3. *Blood* 1993; 82(3):744-51.
32. Gillio AP, Faulkner LB, Alter BP, Reilly L, Klafter R, Heller G, Young DC, Lipton JM, Moore MA, O'Reilly RJ. Successful treatment of Diamond-Blackfan anemia with interleukin 3. *Stem Cells* 1993; 11 Suppl 2:123-30.
33. Bastion Y, Bordigoni P, Debre M, Girault D, Leblanc T, Tchernia G, Ball S, McGuckin C, Gordon-Smith EC, Bekassy A, et al. Sustained response after recombinant interleukin-3 in diamond blackfan anemia. *Blood* 1994; 83:617-18.
34. Olivieri NF, Feig SA, Valentino L, Berriman AM, Shore R, Freedman MH. Failure of recombinant human interleukin-3 therapy to induce erythropoiesis in patients with refractory Diamond-Blackfan anemia. *Blood* 1994; 83(9):2444-50.
35. Ball SE, Tchernia G, Wranne L, Bastion Y, Bekassy NA, Bordigoni P, Debre M, Elinder G, Kamps WA, Lanning M, et al. Is there a role for interleukin-3 in Diamond-Blackfan anaemia? Results of a European multicentre study. *Br J Haematol* 1995; 91: 313-318.
36. Abkowitz JL, Schaison G, Boulad F, Brown DL, Buchanan GR, Johnson CA, Murray JC, Sabo KM. Response of Diamond-Blackfan anemia to metoclopramide: evidence for a role for prolactin in erythropoiesis. *Blood* 2002; 100: 2687-91.

37. Akiyama M, Yanagisawa T, Yuza Y, Yokoi K, Ariga M, Fujisawa K, Hoshi Y, Eto Y. Successful treatment of Diamond-Blackfan anemia with metoclopramide. *Am J Hematol* 2005; 78:295-298.
38. Leblanc TM, Da Costa L, Marie I, Demolis P, Tchernia G. Metoclopramide treatment in DBA patients: no complete response in a French prospective study. *Blood* 2007; 109:2266-2267.
39. AltMedDex® Summary: Branched Chain Amino Acids (BCAA) in Micromedex® Healthcare Series.
40. Lab Advisor™: Leucine Measurements in Micromedex® Healthcare Series.
41. Cynober L, Harris RA. Symposium on branched-chain amino acids: conference summary. *J Nutr* 2006; 136(1 Suppl):333S-336S.
42. Choudry HA, Pan M, Karinch AM, SoubaWW. Branched-Chain Amino Acid-Enriched Nutritional Support in Surgical and Cancer Patients. *J Nutr* 2006; 136:314S-8S.
43. Sakai R, Miura M, Amao M, Kodama R, Toue S, Noguchi Y, et al. Potential approaches to the assessment of amino acid adequacy in rats: a progress report [includes discussion]. *J Nutr* 2004; 134(6, Suppl.):165lS-l655S, discussion 1664S-I666S, 1667s-1672s.
44. Verhoeven S, Vanschoonbeek K, Verdijk LB, Koopman R, Wodzig WK, Dendale P et al. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. *Am J Clin Nutr* 2009 May; 89(5):1468-75.
45. Scarna A, Gijsman HJ, McTavish SF, Harmer CJ, Cowen PJ, Goodwin GM. Effects of a branched-chain amino acid drink in mania. *Br J Psychiatry* 2003; 182(3):210-213.
46. Pencharz PB, Ball RO. Amino Acid Needs for Early Growth and Development. *J Nutr* 2004; 134:1566S-8S.
47. Bernardini P, et al. Amino Acid Imbalance and Hepatic Encephalopathy. *Annu Rev Nutr* 1982; 2:419-54.
48. Poon RT, Yu WC, Fan ST, Wong J. Long-term oral branched chain amino acids in patients undergoing chemoembolization for hepatocellular carcinoma: a randomized trial. *Aliment Pharmacol Ther* 2004; 19:779-88.
49. Plaitakis A, et al. Pilot Trial of Branched-chain Amino acids in Amyotrophic Lateral Sclerosis. *Lancet* 1988; 1:1015-18.
50. Sax HC, et al. Clinical Use of Branched-chain Amino Acids in Liver Disease, Sepsis, Trauma, and Burns. *Arch Surg*1986; 121:358-66.
51. Braverman ER. The Healing Nutrients Within. New Canaan, CT: Keats Publishing, Inc. 1997;339.
52. Pospisilova D, Cmejlova J, Hak J, Adam T, Cmejla R. Successful treatment of a Diamond-Blackfan anemia patient with amino acid leucine. *Haematologica* 2007; 92(5):e66-67.
53. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. *J Nutr* 2006; 136(1 Suppl):227S-231S.
54. Gilman AG, Rall JW, Nies AD et al (eds): Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th ed. Pergamon Press, New York, NY, 1990.
55. AMA Department of Drugs: AMA Drug Evaluations, 6th ed. American Medical Association, Chicago, IL, 1986.
56. Crowe 2006
57. Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995.

58. Pass KA, Lane PA, Fernhoff PM, et al: US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). *J Pediatr* 2000; 137(4 Suppl):S1-46.
59. Henry JB: Clinical Diagnosis and Management by Laboratory Methods, 20th ed. Saunders, 2001.
60. Cmejlova J, Dolezalova L, Pospisilova D, Petrtylova K, Petrak J, Cmejla R. Translational efficiency in patients with Diamond-Blackfan anemia. *Haematologica* 2006; 91:1456-1464.
61. Hutson SM, Sweatt AJ, Lanoue KF. Branched-chain [corrected] amino acid metabolism: implications for establishing safe intakes. *J Nutr* 2005; 135(6 Suppl):1557S-64S.
62. Jellin JM, Gregory P, Batz F. Pharmacist's letter/Prescriber's Letter Natural Medicines Comprehensive Database, 3rd ed, Therapeutic Research Faculty, Stockton, CA, 2000