

PHASE II TRIAL OF PULSE BUTYRATE + ERYTHROPOIETIN IN BETA THALASSEMIA INTERMEDIA

PROTOCOL

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AGENT:

Arginine Butyrate
IND# 36,957

1. OBJECTIVES

1.1 The first objective is to test the hypothesis that treatment with pulsed butyrate can stimulate γ -globin chain production to a degree that decreases anemia and results in hematologic improvement in a significant proportion of patients with beta thalassemia intermedia.

1.2 The second objective is to test the hypothesis that treatment with EPO + pulsed butyrate will result in hematologic improvement in thalassemia intermedia patients.

1.3 The third objective is to determine whether any of the following correlate with improved hematologic response:

- 1) A proportional increase in γ -globin synthesis and mRNA and an improvement in non- α : α -globin chain imbalance by at least 10% over baseline
- 2) A decrease in hemolysis, as assayed by a decrease in LDH, compared to baseline levels
- 3) Whether any particular genotypes are more responsive than others to either therapy
- 4) Baseline Erythropoietin levels
- 5) Gender
- 6) Baseline reticulocyte counts (or % circulating nucleated erythroblasts/100 WBCs)

2. BACKGROUND

A large body of laboratory and clinical data from many studies have shown that as little as a 10% increase in expression of the endogenous fetal globin genes can decrease globin chain imbalance and improve anemia in beta thalassemia. Toward this end, three agents have been studied in small samples of patients and have increased HbF in a few thalassemia patients. The chemotherapeutic agent 5-Azacytidine has been administered to 5 patients, resulting in an increase in total hemoglobin of 1.5 to 3.1 g/dl above baseline. Treatment with sodium phenylbutyrate (SPB) increased total hemoglobin by a mean of 2.1 g/dl (range 1.2 to 2.8 g/dl) above baseline in 4/8 untransfused patients with thalassemia (Collins and Dover, 1995). The SPB study was important in showing that a non-chemotherapeutic agent could induce hematologic responses in beta thalassemia patients. Addition of hydroxyurea to SPB has been effective only in patients with Hb Lepore. Hydroxyurea alone has increased total hemoglobin by 0.4 to 1.8 g/dl in a few patients with thalassemia intermedia, especially HbF/thalassemia. Arginine butyrate has been studied in continuous and pulsed regimens, with the latter producing best results, resulting in a mean increase in total hemoglobin of 2.8 g/dl (range 1.5 - 4 g/dl) above baseline.

Butyrate acid analogues were first demonstrated to induce γ -globin gene expression 14 years ago when the γ globin gene was selectively expressed from a transfected $\gamma\beta\delta$ -globin gene complex by Partington et al. In experimental systems, butyrates were subsequently shown to prevent suppression of γ globin in human fetuses (infants of diabetic mothers) without causing any side effects, and to induce expression of the gamma globin genes in cultured human cells, in fetal sheep, in primates, and in transgenic mice by several laboratories. When applied to patients, continuous butyrate infusions induced γ globin, F-cells, F-reticulocytes, and HbF, but the effects declined with constant administration.

As Butyrate is also known to cause growth arrest in G1 of the cell cycle, a pulsed regimen of butyrate was designed to avoid its anti-proliferative effects and was studied in 11 patients with sickle cell anemia and 6 patients with beta thalassemia. Pulsed treatment resulted in an increase in HbF to a mean of 20% in two-thirds of the sickle cell patients and resulted in an average increase in total Hb of 2.8 gram/dl above baseline in 5/6 thalassemia patients in dose-ranging pilot studies.

Erythropoietin (EPO) is the hematopoietic growth factor that stimulates red blood cell production and prevents apoptosis (programmed cell death) of developing erythroblasts. Thalassemic erythroblasts have been shown to undergo apoptosis at an accelerated rate. When administered alone to a few (6) patients with beta thalassemia, and although the patients already had elevated endogenous levels of erythropoietin, an increase in hemoglobin and hematocrit resulted in one-third to one-half of patients in two different reports, without any improvement in globin chain balance, and, consequently an increase only in thalassemic red cells which do not transport oxygen well. **We hypothesize that prolongation of cell lifespan by EPO may provide a longer window during which a γ -globin inducer can act and that EPO and Butyrate should be synergistic.**

These studies should determine prospectively if pulsed arginine butyrate alone or pulsed butyrate with erythropoietin can significantly reduce the anemia of patients with beta thalassemia intermedia.

3.0 DRUG INFORMATION

3.1 Arginine Butyrate (IND-36,957) and Rhu-Erythropoietin-(Ortho)

a. Chemistry Butyric acid is a short chain fatty acid which is naturally present in foods such as dairy products. L-Arginine is an amino acid which is commonly used in hyperalimentation solutions. Rhu-Erythropoietin is a synthetic recombinant version of the growth factor.

b. Mechanism of Action: Butyrate induces γ -globin gene expression in cultured human cells and in animal models by stimulating activity from the promoter of the γ -globin gene. Erythropoietin can prolong red blood cell viability and stimulate red blood cell production.

c. Human Toxicity: Arginine butyrate has been given to approximately 50 adults and children with blood diseases and cancer without any serious side effects occurring at the doses proposed. Observed side effects have been headache, nausea, and anorexia and have been prevented with premedication with Tylenol and standard anti-emetics, or by decreasing the rate of infusion to 65 mg/kg/hour. Hypokalemia may occasionally occur and is preventable by giving potassium supplements. There is often a transient rise in BUN from conversion of Arginine to urea. Other uncommon side effects include: protein in the urine, due to excretion of the arginine.

Single episodes of low blood pressure, shortness of breath, muscle aches, chest and arm pain, insomnia and anxiety were experienced by five patients receiving high infusion rates for cancer. This drug also has an odor which is unpleasant. One patient reported a temporary loss of appetite. One patient had a mild increase in a liver enzymes which did not cause any symptoms and was reversed when the drug was stopped. One patient who received an overdose at another hospital had a seizure, but recovered completely.

Erythropoietin has been associated with high blood pressure, seizures, and thromboses in patients with chronic renal failure and congestive heart failure. (These will be exclusion criteria for this study.) Other side effects which have occurred in 11-38% of patients include fever, fatigue, headache, cough, diarrhea, nausea, shortness of breath, and bone pain.

d. Pharmaceutical Data: Arginine Butyrate (50 mg/ml) solution is stable for at least 21 months (the longest time tested) in glass bottles. Plasma levels of butyric acid that have been achieved are 0.02 to 1.2 mM (millimolar). Butyric acid is cleared within 15 minutes. Arginine is cleared between 2 and 18 hours. Erythropoietin has a half-life of 4-13 hours in patients with chronic renal failure and of a few hours in normal volunteers. The shelf-life of each will be followed and no expired batch product will be used.

e. Drug Supply: A GMP source of the drug substance butyric acid for this IND has been established with Aldrich Chemical Company. The drug product is manufactured under GMP conditions at the University of Iowa College of Pharmacy. Rhu-Erythropoietin will be supplied from the manufacturer.

f. Administration: Since the 5% Arginine Butyrate solution is hypertonic (50 mg/ml), it must be infused through a long or deep IV access to avoid peripheral venous irritation, such as a port-a-cath or central line. Arginine Butyrate should be infused via a portable cassette infusion pump. Usual doses will be 800 mg/kg/dose infused over 10-12 hours. The dose may be adjusted to either 500 mg/kg, given over 6 hours, or to 1200 mg/kg, which should be given over 14 hours, or slower, as tolerated. Patients will be taught how to self-administer the Butyrate.

Erythropoietin will be given subcutaneously, or intravenously on those days/month when the patient's port is accessed.

4.0 **ELIGIBILITY CRITERIA**

Patients will be eligible for inclusion in the study if they have a beta thalassemia syndrome, with evidence of two beta thalassemia mutations, have been splenectomized, and are not receiving transfusions on a regular basis. A record of steady-state total hemoglobin and

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hematocrit should ideally be available. Patients must be able to reliably return to the hospital or receive therapy at home with appropriate vascular access and after training in the clinic, with home nursing services. Informed consent must be signed and witnessed. Specific eligibility criteria are as follows:

- 4.1 Patients must have a beta thalassemia syndrome with hemoglobin levels less than or equal to 10 gram/dl
- 4.2 Patients must be splenectomized or he/she must have no palpable spleen.
- 4.3 Patients must stop transfusions for the study, except for acute problems.
- 4.4 Patients must have normal renal and hepatic function.
- 4.5 Patients must be at least 3 years of age.
- 4.6 Patients must not have severe iron overload, or ferritin > 5000 ng/ml.
- 4.7 Patients must not have evidence of active hepatitis or other viral disease determined by an ALT > 2 standard deviations above normal.
- 4.8 Pregnant women may not participate.
- 4.9 Patients must not have any difficulties that would interfere with compliance with the treatment plan.
- 4.10 All patients must be informed of the investigational nature of this study and must sign an informed consent written in accordance with institutional and federal guidelines.
- 4.11 Patients must be willing to have appropriate vascular access placed (if they do not already have a port device or indwelling catheter.)
- 4.12 Patients must have a Southwest Oncology Group performance status of 0-2, as described below.

GRADE	SCALE
0	fully active; able to carry on all predaceous activities without restriction (Karnofsky 90-100).
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg,

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light-housework or office work. (Karnofsky 70-80).

- 2 Ambulatory and capable of all self-care but unable to out any work activities. Up and about more than 50% of waking hours. (Karnofsky 50-60).
- 3 Capable of only limited self-care; confined to bed or more than 50% of waking hours. (Karnofsky 30-40).
- 4 Completely disabled. Cannot perform any self-care. Totally confined to bed or chair. (Karnofsky 10-20).
- 5 Dead.

5.0 TREATMENT PLAN

(a) Overview

Beta thalassemia patients in our previous Phase I/II studies have usually required Butyrate doses of 800 mg/kg or higher to produce an increase in γ -globin synthesis and a subsequent correction of globin chain ratios. As thalassemia patients may have 100 different mutations and have variable pathology, an optimal dose must be identified for each patient. Dose of 800 mg/kg and 1200 mg/kg will be tested in the patients unless there is a good response, of at least a 0.5 gram/dl increase in total hemoglobin or some improvement in globin chain balance, to the first course dose. Mildly affected patients with Hgb 8.0 g/dl or higher may receive a lower dose, 500 mg/kg. Assays of globin chain biosynthesis and quantitation of γ and β globin mRNA will be performed on the third day after completion of a treatment cycle, at each dose level, to assess biochemical response. The dose that is most effective in producing the optimal globin chain balance, or which induces a rise in total hemoglobin and hematocrit, in each patient will then be continued during the maintenance phase of the study. The globin synthesis ratios will be performed before and at two or three days after a treatment course. Quantitation of $\gamma/\gamma+\beta$ globin mRNA, and or γ globin relative to α globin mRNA will also be analyzed.

(b) Determination of a Patient's Baseline Hematologic Status

To obtain baseline data on each patient, a "**Baseline Evaluation Phase**" of two months will be used to obtain the following data on four separate occasions prior to beginning the drug:

1) Hematological Parameters

Complete blood counts including Hgb, Hct, MCV, MCHC, MCH
Reticulocytes
F-cells

Hb F quantitation (by electrophoresis and densitometry or by HPLC)

2) Globin chain synthesis

3) Quantitation of proportions of γ and β globin mRNA. These studies will also be performed during each dose escalation, and 48-72 hours after each treatment cycle during the Induction Phase.

The following will be collected 3-4 times during the baseline period and monthly during the treatment phase:

- 4) Chemistry panel, including liver and renal function tests, calcium, phosphorus, and electrolytes
- 5) LDH, Bilirubin (parameters of hemolysis)
- 6) Ferritin

(c) Determination of an optimal dose of Butyrate on an intra-patient basis, followed by maintenance cycles

An "optimal dose" will be determined for each patient (during the **Induction Phase**), (similar to the maximally tolerated dose (MTD) established on the national multi-center study of hydroxyurea in sickle cell disease). This Dose-Ranging/Induction Phase is proposed because individual thalassemia patients on the Phase I/II study responded to different doses, which may have reflected their different molecular genotypes and their rates of erythropoiesis. Optimal doses therefore must be established for each patient. During the **Fetal Globin Induction Phase**, Butyrate will be given at **500 mg/kg/dose for 4 days/week for mildly affected patients** and at **800 mg/kg/dose for 5 days/week for 4 weeks in most patients**, followed by 10 to 14 days without any therapy during which hemoglobin, hematocrit, globin biosynthesis and mRNA will be assayed. If there is no increase, or an increase of less than 0.5 gram/dl in total hemoglobin after 3 weeks of this dose, a second course of 800 or 1200 mg/kg, will be tested, for 5 days/week for 4 weeks, followed by a 2 week period of response analysis. If there is a response in total Hgb or globin chain balance, the drug will then be continued on alternate weeks for three months, or 6 maintenance treatment cycles, and a final two week response period, representing the **Maintenance Phase**. Patients who decline to participate or have medical contraindications to the EPO arm of the study will be monitored for 2 months following the treatment to determine the duration of Butyrate effect. Patients who do participate in the EPO/Butyrate Phase may continue after completion on **EPO alone** for 12 weeks. These patients will receive **EPO 300U/kg every other day three days per week for 12 weeks, or 500U/kg every other day three days per week for 12 weeks** depending on which dose was effective during the EPO/Butyrate Phase. Total hemoglobin, fetal hemoglobin, and globin mRNA and chain synthesis will be monitored monthly during this phase. If the patient has more severe anemia (hemoglobin < 7g/dl), the order of testing the two study drugs may be reversed. **The EPO Phase may be started first for 12 weeks prior to the Butyrate Induction Phase of the study.**

If there is no increase in fetal globin mRNA, no improvement in globin chain balance, and no increase in total hemoglobin and hematocrit after the two Butyrate induction regimens, the patient will be removed from the study and scored as a non-responder.

d) Treatment Protocol: Butyrate

1. Baseline Evaluation

Time Relative to Treatment (Approx)

2 months

Laboratory Tests

CBC, reticulocytes, Hb F, globin mRNA, globin chain synthesis LDH, bilirubin, BUN, creatinine, liver function tests, EPO level
Ferritin

6 weeks

CBC, Reticulocytes, Hb F, LDH, Bilirubin

1 month

All of the above

14 to -7 days

All of the above

2. Dose-Ranging/Fetal Globin Induction Phase

The following “priming” or intensive treatment will be given for two cycles of 4-week treatment followed by 2-week response analysis.

Induction Cycle 1

Week 1, 2, 3, 4

Days of therapy

1, 2, 3, 4, 5

Dose (mg/kg)

800 (most pts)
**500 (mild;
Hgb > 8g/dl)

Studies

- Globin synthesis, mRNA before and on the 2nd or 3rd day after therapy
- Chemistry panel, CBC, reticulocytes weekly
- U/A, ferritin monthly
- CBC 7, 10, 12, 14 EPO level day 14 days after Rx

Week 5-6

No drug

Induction Cycle 2 *

Week 7, 8, 9, 10 11-12 at physician's discretion	1, 2, 3, 4, 5	500, 800 or 1200	•Globin synthesis, mRNA 72 hrs post treatment
Week 11-12 or Week 13-14	No drug		•Chemistry panel, Hgb/HCT 10, 12, 14 days after the cycle •EPO level, Hb F day 14 after Rx •U/A, ferritin monthly

*If a patient responds satisfactorily to the first Induction course with an increase in total Hgb of 0.5 grams/dl above baseline, higher doses, and a second cycle will not be tested in the patient.

**If a patient has mild thalassemia, with a baseline Hgb of 8.0 grams/dl or more, a 500 mg/kg dose may be tested. This can be administered over 6 hours.

***The 800 mg/kg/dose must be administered over 10 hours minimum (preferably at night). The 1200 mg/kg/dose must be given over 15 hours (most patients should not require >800 mg/kg).

3. Maintenance Treatment Phase (study weeks 13-26)

Treatment and Frequency

Four nights,
Every other week for 3 months
(Treat on alternate weeks)

Dose Selection

Administer the dose identified during the
Induction Period which produced the best
globin chain ratios (or the highest increase in

4. Post-Treatment Studies

After completion of dose-testing and three months of maintenance therapy, any patients who declined to receive EPO or who has medical contraindications to Erythropoietin

will be followed with CBCs, Hb F levels, and Erythropoietin levels every 2 weeks for 2 months, or until the CBCs return to baseline. This will allow a determination of how long the effects of the drug persist.

5. BUTYRATE + ERYTHROPOIETIN (study weeks 27-40)

Patients will be offered participation in the study of the combination of Butyrate + EPO, unless he/she has a medical contraindication such as paraspinal extramedullary hematopoiesis, hypertension, or poorly controlled congestive heart failure. Results of the combined treatment will be compared to results achieved on butyrate alone (in each patient). Butyrate will be continued on alternate weeks. EPO will be given three times per week, IM or sub Q. Initial dose will be 300 U/kg, and 500 U/kg during week 34 if there is no response. Laboratory studies will be performed as shown below.

<u>Week</u>	<u>Optimal Butyrate Dose, 4 nights/week</u>	<u>EPO 300-500 u/kg* 3x/week</u>	<u>Lab Studies</u>
27	X	X	CBC, chem panel EPO level, HbF, retics
28		X	Ferritin, Globin biosynthesis
29	X	X	
30		X	CBC, retics, HbF, EPO, chem panel
31	X	X	
32		X	Globin biosynthesis, CBC,
33	X	X	
<u>Week</u>	<u>Optimal Butyrate Dose, 4 nights/week</u>	<u>EPO 300-500 u/kg* 3x/week</u>	<u>Lab Studies</u>
34		X	CBC, retics, HbF, chem panel
35	X	X	

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36		X	CBC, retics, HbF, EPO level, ferritin, U/A
37	X	X	
38		X	CBC, retics, HbF, chem panel, ferritin, U/A Revision #1, 7/1/99 Revision#4, 12/20/00
39	X	X	
40		X	CBC, retics, HbF, EPO
44	Post-treatment studies		Ferritin, CBC, HbF, Retics, U/A
48	Post-treatment studies		Ferritin, CBC, HbF, Retics Chem Panel

*Dose may be increased to 500 U/kg if no response occurred at 300 U/kg.

6. EPO Phase (Study weeks 41-52 or study weeks 1-12)

<u>Weeks of EPO Treatment</u>	<u>Dose</u>	<u>Lab Studies</u>
1-6	300U/kg	CBC, Retics, HbF, Globin Synthesis, EPO level, Ferritin, U/A monthly
If no response to 300U/kg:		
7-12	500U/kg	CBC, Retics, HbF, Globin synthesis, EPO level, Ferritin, U/A monthly

* If patient has severe anemia (total hemoglobin < 7g/dl), this phase may be conducted prior to the Butyrate Induction Phase. EPO will then be continued throughout the Butyrate Induction and the Maintenance Phase.

7. Administration Of Butyrate In The Home

Training for safe delivery of Butyrate is required before treatment is given at home. Patients or families must demonstrate proficiency in the following before therapy can be moved to the home.

- 1) Maintaining sterility of tubing, drug bottles, and port-a-cath devices
- 2) Flushing with saline and heparin
- 3) Preventing air bubbles in the system
- 4) Changing the dressing over the Huber needle
- 5) Adjustments and dealing with pump malfunction
- 6) Emergency procedures
- 7) Maintaining supplies
- 8) Training of two family members ideally (the patient ideally is one member)

- 9) Pass checklists for demonstrating proficiency in the clinic before implementation in the home
- 10) Ongoing surveillance by visiting nurses after implementation in the home

6.0 TOXICITIES TO BE MONITORED

6.1 Symptoms

Vital signs and symptoms will be checked and recorded during initial Arginine Butyrate infusions. All adults will be pre-treated with anti-emetics and acetaminophen and infusions will begin at 65 mg/kg/hour and increased as tolerated to 80 mg/kg/hour maximum. If nausea or vomiting occur, the infusion will be discontinued for that day and supportive therapy with anti-emetics and IV fluids given. On the next scheduled treatment day, pretreatment with a different anti-emetic or higher doses of the same anti-emetic such as Reglan, and Benadryl 25 mg po, will be given. If headache occurs, Tylenol 650 mg po, or Ibuprofen 400 mg po, will be given and used thereafter as pre-treatment medication. If premedication does not prevent recurrence of symptoms, the dose or infusion rate of Arginine Butyrate will be lowered to the previous dosage which was tolerated by the patient during the initial escalation.

6.2 Safety Monitoring will be performed with each treatment cycle and will include:

- 1) Chemistry panel, including electrolytes, BUN, creatinine, calcium, phosphorus, AST, ALT, alk phos, total protein, albumin, LDH
- 2) Urinalysis monthly
- 3) Ferritin monthly

Elevations in BUN have occurred with 1500-2000 mg/kg doses of Arginine Butyrate in patients treated previously, and has always resolved within 12 hours of discontinuation of the drug. This elevation in serum nitrogen is expected, as arginine is metabolized to urea, and thus the BUN elevation does not reflect a toxic effect on the kidneys. BUN elevation is not anticipated to be significant at the doses selected here. However, to be prudent, the upper limit of BUN that will be tolerated without dose adjustments is 50. If BUN rises higher than 50, the dose of Butyrate will be decreased by 100 mg/kg, after a 36 hour interval without infusion and return of BUN to baseline.

6.3 Supplemental KCL

Hypokalemia has not occurred with KCL supplementation in our previous studies, but other investigators, who did not supplement their patients with potassium and gave continuous high dose infusions of 2000 mg/kg/day Butyrate for prolonged periods, did report mild hypokalemia. Potassium and electrolytes will be monitored and supplements provided as necessary.

6.4 Iron Supplements

Although thalassemia patients are often iron-overloaded, not all the iron appears to be available for incorporation into new hemoglobin. Large amounts of iron are needed to increase hemoglobin levels by 1 gram/dl, which is equivalent to one unit of PRBCs. It has been observed in previous patients that responses have declined or not been observed in patients in whom the Ferritin level declined by 50% or in patients who could not tolerate iron. Ferrous gluconate supplements should be given TID if/when the Ferritin declines to approximately 50% of baseline and will be given at least once/day with EPO. If the Ferritin level is <800 ng/ml, one iron tablet should be administered from the outset.

6.5 Reporting of Side Effects

For any serious side effect or suspected side effect, the drug(s) will be discontinued, the PI must be notified and the FDA will be notified. All investigators and the Safety Monitoring Committee will discuss the case and will collectively decide whether precautions should be instituted for other patients. Whether lower doses of Arginine Butyrate or Erythropoietin are resumed in an affected patient will be decided by a consensus of all the investigators.

6.6 Maintenance and Storage of case report forms

. Case report forms will be provided in triplicate. One copy will be maintained at the treatment site, one copy will be sent to the Coordinating Center, and one copy will be stored at the Data Coordinating Center, Boston University School of Public Health. Statistical analysis will be performed by the Boston University School of Public Health.

7.0 CRITERIA FOR EVALUATION

An increase in total Hgb of at least 2.0 gram/dl above the well-documented baseline is the major goal of this study. It is projected that, based on the data obtained in pilot studies, that statistical significance will be detected with 80-90% power if the patients who were treated previously are representative of thalassemia intermedia patients. Frequency distributions and descriptive statistics will be generated for all study variables. Because we expect similar means and standard deviations for the four baseline measurements, it is assumed that taking the average of the four baseline sets of data will be representative experience for each person. Because a sufficient follow-up period needs to occur in order to detect a rise in hemoglobin, the last month's assessment will be the most sensitive and most important (or the last month of the induction period, if no response is detected and the patient is withdrawn from the trial). The major hypothesis, differences in hemoglobin and hematocrit between the baseline and the final 6-month assessment, will be examined using a paired t-test for normally distributed data. If differences are not normally distributed, we will employ a non-parametric one sample exact test. Secondly, because multiple measurements will be obtained between baseline and the 6-month treatment value, we can also employ repeated measures analysis via mixed linear models should the data suggest a non-linear response over time. Similar analysis will be conducted for the other major and minor endpoints. Correlations between changes in γ globin mRNA or globin chain synthesis ratios and hematologic responses to pulsed Arginine Butyrate will be analyzed. The interaction between different molecular mutations and hematologic responses to pulsed Arginine Butyrate will also be explored. (We may not be able to adequately detect more than 1 or 2 responsive genotypes with the sample size planned.)

Assuming a standard deviation of 1.3, a difference in total hemoglobin of 1.5 grams/dl above baseline (or, for the Butyrate + EPO study, above the last Hgb on Butyrate alone) will be detected with 9 patients with a power of 80% using a paired t-test with a 0.05 two-sided significance level. To detect a power of 90%, a sample size of 11 patients will be necessary. This small a difference can be detected with the proposed sample sizes, but if the difference before and at the end of treatment is larger, fewer patients may be needed. (To allow for a 20% drop-out rate, 15 patients will be recruited.)