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## Evaluation of Safety and Pharmacokinetics of Sodium 2,2-Dimethylbutyrate, a Novel Short Chain Fatty Acid Derivative, in a Phase 1, Double-Blind, Placebo-Controlled, Single- and Repeat-Dose Studies in Healthy Volunteers

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### Abstract

Pharmacologic induction of fetal globin synthesis is an accepted therapeutic strategy for treatment of the beta hemoglobinopathies and thalassemias, as even small increases in hemoglobin F (HbF) levels reduce clinical severity in sickle cell disease and reduce anemia in beta thalassemia. Prior generation short chain fatty acid therapeutics, arginine butyrate and phenylbutyrate, increased fetal and total hemoglobin levels in patients, but were limited by high doses or intravenous infusion. A fetal globin-inducing therapeutic with convenient oral dosing would be an advance for these classic molecular diseases.

Healthy adult human subjects were treated with a novel SCFA derivative, sodium 2,2-dimethylbutyrate (SDMB), or placebo, with one of four single dose levels (2, 5, 10 and 20 mg/kg) or daily doses (5, 10, or 15 mg/kg) over 14 days, and monitored for adverse clinical and laboratory events, drug levels, reticulocytes, and HbF assays. SDMB was well-tolerated with no clinically significant adverse events related to study medication. The terminal half-life ranged from 9–15 hours. Increases in mean absolute reticulocytes were observed at all dose levels in the 14-day study. The favorable PK profiles and safety findings indicate that SDMB warrants further investigation for treatment of anemic subjects with beta hemoglobinopathies.

### Keywords

short chain fatty acids; anemias; pharmacokinetic profiles; erythropoiesis; fetal hemoglobin

### Introduction

Fetal hemoglobin (HbF,  $\alpha_2\gamma_2$ ) is normally developmentally suppressed to levels <2% by 6 months of age.<sup>1</sup> In the beta globin diseases, residual elevated levels are associated with reduced clinical severity in sickle cell disease (SCD) and beta thalassemia.<sup>1–15</sup> Fetal ( $\gamma$ ) globin chains interfere with the polymerization of sickle hemoglobin, thereby preventing many pathologic and clinical consequences of sickling.<sup>1–5</sup> HbF levels  $\geq$  8.6% correlate with improved survival, and higher levels (>20%) of HbF and F-cells are associated with milder,

even benign, clinical courses in sickle cell disease.<sup>1–11</sup> Three classes of therapeutic agents have been shown to induce HbF expression in affected patients: chemotherapeutic agents, which cause cytostasis or demethylation, (such as hydroxyurea, 5-azacytidine, decitabine), erythropoietin, and short chain fatty acids (SCFAs) and SCFA derivatives (SCFADs), such as arginine butyrate (AB) and sodium phenylbutyrate (SPB), when administered with iron supplements.<sup>1,15–32</sup>

Unlike chemotherapeutics, the SCFAs are not known to be mutagenic, carcinogenic or teratogenic.<sup>29</sup> Sodium phenylbutyrate and arginine butyrate enhance both fetal and total hemoglobin levels in patients with sickle cell disease and beta thalassemia.<sup>1,15–21,26–32</sup> Some previously transfusion-dependent thalassemia patients even became transfusion-independent.<sup>15–16,18–20,29</sup> However, these earlier SCFAs (including AB and SPB) are rapidly cleared from plasma, and require intravenous (IV) infusion or large oral doses. These first-generation SCFA compounds also inhibit histone deacetylases, which generally inhibit erythroid cell proliferation, and therefore require dose titration and intermittent dose schedules.<sup>26–29</sup> There remains an unmet medical need in patients with beta hemoglobinopathies for convenient oral therapeutics that induce fetal ( $\gamma$ )-globin gene expression without inhibiting erythroid cell proliferation.

Sodium 2,2-dimethylbutyrate (SDMB or HQK-1001, previously designated ST-20) is a new generation SCFA that is orally bioavailable in non-human primate models and induces fetal globin synthesis in reporter gene assays, in patients' cultured cells and in transgenic mice.<sup>26–28</sup> SDMB also stimulates erythroid cell proliferation and survival,<sup>26,28</sup> and increases total hemoglobin levels in phlebotomized anemic baboons.<sup>27</sup> The compound has a favorable pharmacokinetic profile in non-human primates and is non-mutagenic.<sup>27</sup> The studies described were designed to evaluate the clinical safety of SDMB in healthy adult human subjects, before initiating trials in patients with anemias associated with beta globin disorders.

## Subjects, Materials and Methods

The safety and pharmacokinetics of SDMB were evaluated in healthy adult human subjects in two clinical studies. The first study consisted of 4 cohorts of approximately 8 healthy adult subjects treated with a single dose, with escalation to higher single doses in additional cohorts thereafter. The second study consisted of 14 days of once-daily dosing in 5 single-dose level cohorts with safety monitoring for the ensuing 28 days, followed by the same treatment program in additional cohorts with dose escalation. In addition, some subjects at all dose levels in the repeat-dose study were treated with iron, which is required for fetal globin synthesis and erythropoietic effects with other therapeutic agents.<sup>1,13,15,22,29</sup>

### Eligibility

Criteria for eligibility in both studies included healthy male and female adults, ages 18–45 years with normal screening laboratory studies including hematology, chemistry, urinalysis, and coagulation parameters. Female subjects could not be pregnant and, if of child-bearing potential and engaging in potential reproductive activity, were required to employ an effective method of birth control during the course of the study and for one month after study medication exposure. Male subjects were also required to use effective contraception throughout the study. Subjects could not have received any prescription medication, with the exception of multivitamins or contraceptives, within 2 weeks prior to study drug administration, and must have had a negative urine screen for substances of abuse. In the repeat-dose study, serum ferritin levels at screening were required to be greater than 50 ng/mL for Cohort 1 and greater than 30 ng/mL for Cohorts 2, 3, 4, and 5.

Both studies were conducted at Charles River Clinical Services Northwest, in Tacoma, Washington. Subjects in both studies provided written informed consent prior to eligibility screening and study participation. The protocol, amendments and informed consent forms for both studies were approved by Aspire Independent Review Board, La Mesa, CA, and were conducted in accordance with the International Compilation of Human Subject Research Protections 2009 ([www.fda.gov](http://www.fda.gov)) and Guidelines for Good Clinical Practice.

## Study Design

This was a Phase 1, placebo-controlled, single dose-escalation and multiple dose clinical study to evaluate and compare the safety and pharmacokinetics (PK) of SDMB compared to placebo in healthy adult human subjects. Subjects were admitted to a Phase 1 Clinical Research Unit (CRU) for the first 24 hours of both studies and treated as out-subjects thereafter. The single-dose study involved 5 cohorts of subjects each receiving either SDMB or placebo on Day 0. The multiple-dose study also involved 5 cohorts of subjects receiving either study drug or placebo from Day 0 for 14 consecutive days. Follow-up evaluations were conducted every two weeks for 28 days after discontinuation of study medication in both trials.

In the single-dose study, there were 5 planned cohorts at dose levels of 2, 5, 10, 20, or 30 mg/kg. Each cohort was comprised of 8 subjects, with 6 receiving active drug and 2 receiving placebo. All cohorts were dosed in a fasting state. Subjects in the 20 mg/kg cohort also received a second dose of investigational product under fed conditions after a 7-day washout period. The Principal Investigator (PI) reviewed all safety data to determine if dose limiting toxicity (DLT) had occurred. Once the safety of the prior cohort had been determined, study drug was administered to subjects in the next dose level cohort. The PK findings from the 20 mg/kg cohort revealed that subject plasma dimethylbutyric acid (DMB) levels were significantly higher than those required to stimulate HbF expression in erythroid cell cultures *in vitro*. It was therefore decided not to enroll subjects in the planned 30 mg/kg cohort. There were no safety observations limiting further dose escalation.

In the repeat-dose study, each cohort consisted of 8 subjects, with 6 receiving active medication and 2 receiving placebo, except for Cohort 2, which enrolled 9 subjects, with 7 receiving active drug and 2 receiving placebo. Cohorts 1, 2, and 3 tested 5 mg/kg doses under 3 conditions- fasting, fed, or fed and with iron supplements, respectively. Iron supplements were administered at 325 mg per day of oral ferrous gluconate, to determine whether iron altered pharmacokinetic profiles. Cohorts 4 and 5 received 10 mg/kg and 15 mg/kg, respectively under fed conditions with oral iron. After safety observations and PK data from the prior cohorts were reviewed, Cohorts 3 and 4 were dosed concurrently. Cohort 5 was dosed after data from Cohort 4 showed no dose-limiting toxicity (DLT).

Safety was assessed in both studies by vital signs, physical examination, screening neurological examination, clinical laboratory studies, 12-lead electrocardiogram (ECG), and monitoring for adverse events. Special hematology studies (fetal hemoglobin-containing reticulocytes [F-reticulocytes], fetal hemoglobin-containing red blood cells [F-cells], fetal hemoglobin levels) were also performed to provide potential insights into SDMB pharmacodynamic (PD) activity. In the single dose study, examinations and laboratory monitoring were performed at two baseline points and Days 0, 1, 2, 3, 7, 14, and 28. In the multi-dose study, laboratory monitoring tests were performed at 2 baseline points, and 6 times on study, at Days 0, 6, 13, 20, 27 and 41. With the PK studies listed below, approximately 500 mls of blood was withdrawn over the 6-week period. Pharmacokinetic measures were evaluated during the course of both studies. These included maximum observed DMB plasma concentration ( $C_{max}$ ), minimum concentration ( $C_{min}$ ), average concentration ( $C_{avg}$ ), time of maximum concentration ( $T_{max}$ ), area under the plasma

concentration-time curve from time 0 to time of last measurable plasma concentration ( $AUC_{(0-last)}$ ), area under the curve from time 0 to last quantifiable concentration ( $AUC_{(0-lqc)}$ ), area under the plasma concentration-time curve from 0 time extrapolated to infinity ( $AUC_{(0-\infty)}$ ), percentage of AUC that is extrapolated beyond the last measurable concentration ( $AUC_{\%extrap}$ ), apparent plasma terminal-phase elimination rate constant ( $\lambda_z$ ), terminal-phase half life ( $t_{1/2}$ ), apparent oral volume of distribution, terminal phase ( $V_z/F$ ), total body clearance ( $CL/F$ ), renal clearance ( $CL/R$ ), and cumulative urinary excretion ( $A_e$ ).

### DMB Plasma Concentration Assay

In the single dose study, plasma concentrations of SDMB were measured using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS), as previously described.<sup>36</sup> The analyte was extracted from heparinized human plasma by protein precipitation with acetonitrile, followed by derivatization to a benzyl amide. The linear concentration range (for calibration) was validated and documented from 0.2  $\mu\text{g/mL}$  to 50  $\mu\text{g/mL}$  with  $1/x^2$  weighting. The lower limit of quantification (LLOQ) was 0.20  $\mu\text{g/mL}$ . In the repeat-dose study, plasma concentrations of SDMB were measured using a validated LC-MS/MS assay method at Frontage Laboratories (Malvern, PA). The LLOQ for plasma levels was validated at 0.100  $\mu\text{g/mL}$ . Quantitation of levels higher than 50  $\mu\text{g/mL}$  was performed using dilutions with blank plasma and validated in the SOPs developed for the assay.

### Pharmacokinetic Data Analysis

Pharmacokinetic analyses for both the single-dose and repeat-dose studies were performed using noncompartmental methods employing WinNonlin® Professional versuib 5.2 (Pharsight Corp, Mountain View, CA).

### Hematology Analyses

Absolute reticulocytes were measured by an Advia Coulter Counter at Children's Hospital Oakland, Oakland, CA. The proportions of reticulocytes and red blood cells which express some HbF, (F-reticulocytes and F-cells respectively) were analyzed by FACScan analysis as previously described<sup>35</sup> employing a PE Mouse Anti-human fetal hemoglobin antibody (Invitrogen, Carlsbad, CA), a PE Mouse isotype Control (Beckman-Coulter, Brea, CA), and Retic-COUNT reagent (BD Biosciences, San Jose CA), adapted from the manufacturer's directions.<sup>33</sup>

### Statistical Analysis

In the single-dose study, pharmacokinetic linearity and dose proportionality were assessed for  $AUC_{inf}$ ,  $C_{max}$ , and  $CL/F$  versus dose using GraphPad Prism software version 5. The relationship between the log of the pharmacokinetic parameter and the dose was assessed for linearity. Dose linearity over the dose range was shown if the slope of the linear relationship was not significantly different from one, or from zero in the case of  $CL/F$ . The effect of food on the  $C_{max}$ ,  $AUC_{last}$ , and  $AUC_{inf}$  values was assessed by use of a paired t-test, and  $T_{max}$  values were compared using a Wilcoxon signed rank test. The analyses were also performed using the GraphPad software. Summary statistics were analyzed for all safety variables.

In the repeat-dose study, data were summarized by visit, fed vs. fasting condition, administration of oral iron, dose level and measurement time. Comparisons between fed and fasting conditions, dose levels, and administration of oral iron in terms of  $AUC_{(0-lqc)}$ ,  $AUC_{(0-\infty)}$ ,  $C_{max}$ ,  $C_{min}$ ,  $C_{avg}$ ,  $T_{max}$ ,  $T_{1/2}$ ,  $CL/F$ ,  $\lambda_z$  and  $CL_r$  were made using an Analysis of Variance (ANOVA) model. Comparisons were made in early PD measures (changes from baseline in absolute reticulocytes and F-reticulocytes) between dose level cohorts and

between utilization (or not) of iron supplementation using ANOVA, and Statview software. Adverse events were characterized by dose level, fed or fasting state, presence or absence of iron, system organ class (SOC), using MedDRA (Medical Dictionary for Regulatory Activities) v. 9 preferred term coding, and AE and laboratory abnormality severity grading was performed using the NCI CTC Version 3. The PI determined AE relationship to study medication.

## Results

In the single dose study, 32 subjects (12 males and 20 females) were enrolled and randomized into 1 of 4 treatment groups as described. All subjects received study drug SDMB or placebo, and completed the study. The ages of the subjects ranged from 18 to 44 years, and the majority of the subjects (90%) were Caucasian; demographic features of the study subjects are shown in Table 1. No dose limiting toxicity (DLT) was observed at any dose level studied. The 24 subjects (16 females and 2 males) who had received active drug were included in the PK analysis population.

In the repeat-dose study, 41 subjects (22 males and 19 females), ages 18 to 41 years were enrolled and randomized to 1 of 5 treatment groups; 68% of subjects were Caucasian, as shown in Table 1. Thirty-seven subjects completed the study; three subjects discontinued participation due to scheduling conflicts, and one subject was discontinued due to non-compliance with study procedures. The PK population in this study therefore consisted of 29 subjects (13 females and 16 males) who received active study medication.

## Pharmacokinetics

**Single-Dose Study**—The mean plasma drug concentrations increased at each sampling point in a dose proportional fashion up through 20 mg/kg. Measurable (DMB) concentrations were observed at the earliest time point (15 minutes post-dose) for all subjects at the lowest initial dose level of 2 mg/kg (n=6), although concentrations were below the LLQ for all subjects in this cohort at 48 and 72 hours. Only one subject in each of the 5 and 10 mg/kg cohorts had measurable concentrations at the 72 hour sampling point. The highest peak DMB plasma concentration detected in any subject was 105 µg/mL at the 0.75 hour sampling point, in the 20 mg/kg cohort.

The 20 mg/kg cohort was assessed under fasting and non-fasting conditions in a crossover fashion. Prior food ingestion was associated with a lower  $C_{max}$  and later  $T_{max}$ , but nearly identical terminal-phase pharmacokinetics (shown in Figure 1) and exposure (AUC) (shown in Table 2 and Supplemental Figures 1 and 2). Mean  $C_{max}$  was reduced by 32% after food (89 µg/mL fasting vs. 60 µg/mL non-fasting;  $p = 0.0037$ ), while median  $T_{max}$  increased 5-fold from 0.75 hours in the fasting state to 4.50 hours in the non-fasting state ( $p = 0.0355$ , nonparametric analysis based on medians). DMB half-life, clearance, and distribution were unaffected by the presence of food.

Noncompartmental analyses of individual subject concentration-time data demonstrated that the estimated mean maximal plasma concentration ( $C_{max}$ ) values increased with dose from 4.8 to 20.4, 38.1, and 88.9 µg/mL, respectively, for the 2, 5, 10, and 20 mg/kg doses.  $C_{max}$  was achieved rapidly from a mean of 0.6 hours following a 2 mg/kg dose to a mean of 1.3 hours following a 20 mg/kg dose. The mean half-life of SDMB at the differing dose levels ranged from 5.6 to 11.3 hours. Exposure (AUC) increased in a dose proportional fashion, ranging from 38.4 to 1190 h • µg/mL over the 2 to 20 mg/kg dose range. In general, the inter-subject variability in  $C_{max}$  was very low, with CV% values ranging from 14 to 19%. Higher variability was evident in AUC with CV% values ranging from 13 to 61 per cent.



**Repeat Dose Study**—Mean plasma DMB concentration-time data for the repeat dose study are shown by treatment group in Figure 2, and Supplemental Figure 1. Dose proportional increases in mean plasma concentrations of SDMB were observed over the 5 to 15 mg/kg dose range at Day 0 and at steady state on Day 13.

Dose proportionality was assessed across all cohorts. Supplemental Figures 2a and 2b present plots of the  $C_{\max}$  at Day 0 and Day 13, respectively, presented along a regression line. Values for both parameters increased in a linear fashion. Inter-subject variability was low, and, in general, the concentration-time profiles appeared similar for all subjects. Administration of SDMB with food resulted in a significant delay in  $T_{\max}$  and a slight decrease in  $C_{\max}$  (Table 3), as observed with single ascending doses. There was a nearly 4-fold increase in  $T_{\max}$  after both a single dose (Day 0) and multiple doses (Day 13), as shown in Tables 2 and 3. The maximum study medication concentration ( $C_{\max}$ ) at Day 13 decreased approximately 15% when taken after food ingestion (Figure 1, Table 2). However, there were no significant changes in total medication exposure as defined by AUC, also shown in Table 3.

The SDMB pharmacokinetic profiles include rapid absorption within 15 minutes after ingestion, a half-life probably allowing once per day dosing, dose proportionality with exposure and low inter-subject variability. In addition, well-tolerated oral doses of SDMB result in plasma concentrations greater than those required in cell culture studies to induce fetal globin expression and proliferation of erythroid precursors.<sup>28,30</sup> These attributes suggest a practicable agent for clinical application as a long-term therapeutic.

Concomitant ferrous fumarate was taken by subjects in Cohort 3 (5 mg/kg under fed conditions), and results were compared to those obtained in Cohort 2, who had received the same dose of study medication with food but without concomitant iron. There were no meaningful differences in  $C_{\max}$ ,  $T_{\max}$ , or AUC on Day 0 or Day 13 in the presence or absence of iron (Table 4).

## Pharmacodynamics

This study was designed for evaluations of SDMB safety and PK, and did not provide dosing of sufficient duration to fully characterize SDMB pharmacodynamics. Nonetheless, limited pharmacodynamic data collected in the repeat-dose study revealed changes in absolute reticulocytes between mean baseline and peak values, usually occurring 2 weeks following completion of study dosing (at Day 27), consistent with the timing required for maturation of red blood cell precursors, as shown in Figure 3 and Table 6. Mean absolute reticulocytes ( $10^9$  cells/L) in the placebo group at baseline and Day 27 were  $51 \pm 12$  and  $62 \pm 20$ , respectively, which was not statistically significant ( $p=0.19$ ). Mean absolute reticulocytes increased on average from 50% to 60% above the mean baseline values in the subjects who received study medication. For example, at baseline in Cohort 1 (5 mg/kg, fasted) mean absolute reticulocytes were  $50 \pm 12$  and mean peak reticulocytes were  $81 \pm 24$ , ( $p=0.02$ ); in Cohort 4 (10 mg/kg) mean baseline reticulocytes were  $58 \pm 8$  and mean peak reticulocytes were  $84 \pm 14$ , ( $p=0.003$ ), and in Cohort 5 (15 mg/kg; fed), the mean baseline was  $46 \pm 11$  and mean peak level was  $72 \pm 11$ , a 50% increase ( $p=0.001$ ). No changes in laboratory values consistent with hemolysis, such as elevations in LDH or bilirubin, or declines in Hgb or hematocrit were observed in treated patients to suggest that the reticulocyte changes were secondary to hemolysis. No differences were detected between Caucasian and African American subjects in reticulocyte response rates in this small sample. Although there was a trend toward SDMB associated increases in F-reticulocytes, comparison with placebo responses were not statistically significantly different in these small groups.

## Safety Results

In the single-dose study, SDMB was well-tolerated at all dose levels (Table 5a). There were no serious adverse events, deaths, or discontinuations from study due to adverse events. Most AEs were grade 1 (mild) in severity and considered unrelated to study medication. Headache was the most common AE (28.0% in treated and 25% in controls), with higher frequency in fasting subjects. Three AEs in three different subjects were considered possibly related to study drug: upper abdominal pain, in a subject receiving 2 mg/kg, and dysphoria and dizziness in subjects receiving 10 mg/kg. There were no clinical or laboratory findings suggesting significant intolerance or toxicity in any of the dose level cohorts.

In the repeat-dose study, SDMB was similarly well-tolerated at all dose levels evaluated (Table 5b). No laboratory findings or clinically significant AEs, or SAEs, leading to discontinuation were reported. The most common AE was mild headache, which occurred in 23%, whereas 40% of subjects in the placebo-treated subjects reported headache. No DLT was noted in the dosing range evaluated.

Elevated creatine phosphokinase (CK) levels were observed at baseline and at later time points on study in 6 of 10 subjects who received placebo and 15 of 30 subjects who received SDMB, across all dose levels and laboratory sampling points. These minor elevations at random times were classified by the PI as not clinically significant and were not reported as AEs. No apparent dose-response relationship with CK levels was noted, and there were no findings suggestive of clinical myopathy or cardiac injury, *i.e.*, no myalgias, weakness, brown urine or chest discomfort. The proportion of subjects with CK elevations was not statistically different between placebo and treated subjects. The Principal Investigator (PI) did not consider elevated CK values related to study medication, as asymptomatic CK elevations are commonly observed in multiple studies at the clinical site and often attributed to prior exercise and commonly normalizing after inactivity.

## Discussion

Additional therapeutics are needed for treatment of the underlying pathology in beta globin diseases.<sup>1,2,15,29</sup> Although hydroxyurea is a centrally important therapeutic in reducing crisis frequency and prolonging survival in SCD patients,<sup>1, 23-25</sup> some patients are reluctant to utilize this agent because of concerns regarding potential mutagenicity, carcinogenicity and teratogenicity.

SDMB is an oral SCFA that stimulates fetal globin gene expression in many experimental models, proliferation of human erythroid progenitor cells in culture, and increases red blood cell counts in phlebotomized baboons.<sup>26-27</sup> SDMB does not induce mutagenicity in the Ames assay, the lymphocyte chromosomal aberration assay, or micrococcal nuclease testing. Twenty-six week repeat dose toxicology studies with SDMB in rats and dogs have not shown any significant adverse effects at dose levels up to 500 mg/kg/day. At the dose levels evaluated here, SDMB was well-tolerated without significant toxicity. There were no imbalances in rates of AEs or laboratory abnormalities when comparing SDMB safety to that associated with placebo.

Based on precedents with hydroxyurea, arginine butyrate, and phenylbutyrate, where months of treatment are typically required for augmentation of fetal globin levels, no pharmacodynamic effects from SDMB treatment were anticipated in healthy adult individuals in the brief time-frame of this study. However, increases in absolute reticulocytes, without hemolysis, suggested an erythropoietic effect at all dose levels in the 2-week dosing study, an early indication of erythropoietic effects. In summary, the favorable

safety profile, pharmacokinetic features, and early erythropoietic effects observed in this study support further investigation of SDMB in anemic subjects with beta globin diseases.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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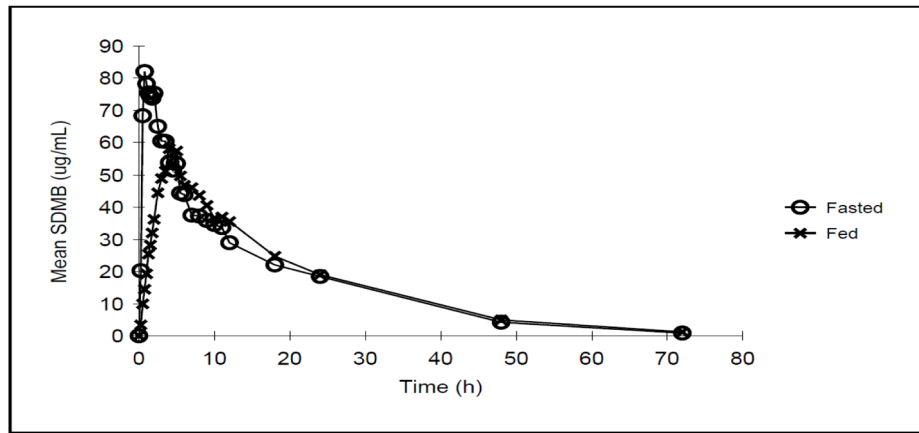
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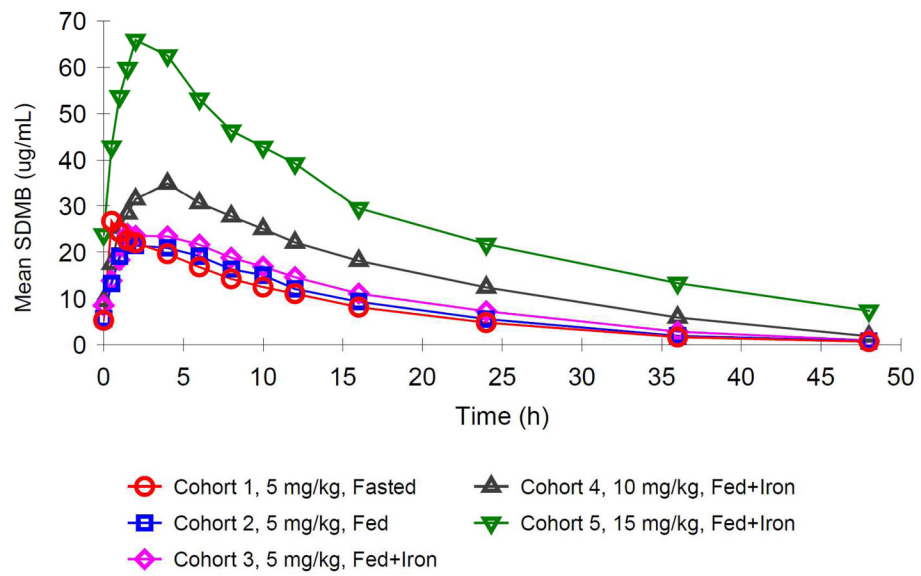
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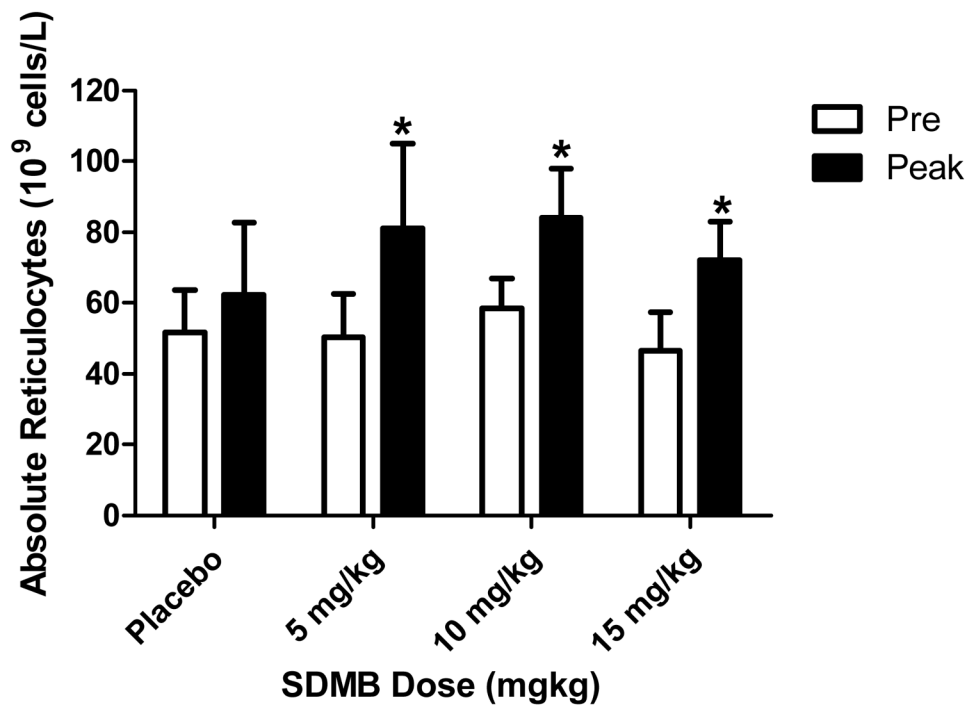
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**Figure 1.** Mean Concentration-Time Plots with administration of SDMB after fasting or fed conditions (Single Dose Study, 20 mg/kg/dose level)



**Figure 2.**  
Mean Sodium 2,2 Dimethylbutyrate Plasma Concentration vs. Time Plots by Treatment Group – Day 13 (Repeat Dose Study)



**Figure 3.** Mean absolute reticulocytes at baseline (white bars) and with treatment (dark bars). The asterisks demonstrate where significant changes were detected.

**Table 1**

## Demographics and Baseline Characteristics of Study Subjects

Demographic Parameter	Single Dose Study		Repeat Dose Study	
	Sodium 2,2 Dimethylbutyrate Total (n=32)	Placebo (n=8)	Sodium 2,2 Dimethylbutyrate Total (n=41)	Placebo (n=10)
Gender, n (%)				
Male	12 (37.5)	4 (50.0)	19 (46)	5 (50)
Female	20 (62.5)	4 (50.0)	22 (54)	5 (50)
Race, n (%)				
Caucasian	29 (90.6)	7 (87.5)	28 (68)	6 (60)
African American	3 (9.38)	1 (12.5)	12 (29)	4 (40)
Asian/Pacific		0	1 (2)	0
Islander				
Mean Age, years	29.8	28.9	28.3	31.4
Range (min, max)	18, 44	18, 43	18, 41	18, 41



**Table 2**

Comparison of Sodium 2,2-Dimethylbutyrate Administered under Fasted and Non-Fasting Conditions. Studies were performed at the 20 mg/kg dose level.

Dependent Variable	Mean		Difference	F <sub>rel</sub> %	p-value
	Fasting	Nonfasting			
C <sub>max</sub> (µg/mL)	88.92	60.40	28.52	67.9	0.0037
AUC <sub>(0-1ast)</sub> h•µg/mL	1174	1162	12.10	99.0	0.8559
AUC <sub>(0-∞)</sub> h•µg/mL	1190	1186	3.267	99.7	0.9638
T <sub>max</sub>	0.750	4.50	-3.75	NC	0.0355

Abbreviations: CI: confidence interval; F<sub>rel</sub>: relative bioavailability; NC: not calculated; SD: standard deviation

**Table 3**

Mean Plasma Non-Compartmental SDMB Pharmacokinetic Parameters – Food Effect at Day 0 and Day 13 (Cohorts 1 and 2; Repeat Dose Study)

Cohort	Dose Level <sup>a</sup> (mg/kg)	Food Status	Day 0			Day 13		
			C <sub>max</sub> (µg/mL)	*T <sub>max</sub> (h)	AUC <sub>INF</sub> (h·µg/mL)	C <sub>max</sub> (µg/mL)	*T <sub>max</sub> (h)	AUC <sub>T</sub> (h·µg/mL)
1	5	Fasted	21.2	1.11	275	26.8	0.58	293
2	5	Fed	17.7	4.40	303	22.4	2.33	312

The \* denotes a significant difference between fasted and fed states.

**Table 4**

Day 0 Mean Plasma Non-Compartmental SDMB Pharmacokinetic Parameters *versus* Dose – Cohorts 2 and 3 (without and with Iron)

Cohort	Dose Level <sup>a</sup> (mg/kg)	Food Status	Iron Status	Day 0			Day 30		
				C <sub>MAX</sub> (µg/mL)	T <sub>MAX</sub> (h)	AUC <sub>INF</sub> (h·µg/mL)	C <sub>max</sub> (µg/mL)	T <sub>max</sub> (h)	AUC <sub>T</sub> (h·µg/mL)
2	5	Fed	No	17.7	4.40	303	22.4	2.33	312
3	5	Fed	Yes	16.3	4.35	287	24.8	2.88	360

**Table 5a**

Subject Incidence of Adverse Events in the Single Dose Study

Adverse Events	Placebo N=8	SDMB N=24
Headache	2	7
Vessel puncture site hematoma	1	4
Dizziness	2	3
Nausea	1	2
URI	1	2
Diarrhea	1	2
Anxiety	1	
Dysmenorrhea	1	
Injection	1	
Menorrhagia	1	
Pain in extremity	1	
Pharyngitis	1	
Pharyngolaryngeal pain	1	
Sialoadenitis	1	

**Table 5b**

Subject Incidence of Adverse Events in the Multi-dose Study

Adverse Events	Placebo N=10	SDMB N=31
Headache	4	7
Menstrual Cramps	1	2
Diarrhea		2
Flatulence		2
Nausea		2
Pharyngitis		2
Abdominal distention	1	
Contusion of hand (s)	1	
Dyspepsia	1	
Emesis	1	
Euphoria	1	
Foot pain	1	
Intra-ocular infection	1	
Lymphadenopathy, axillary	1	



**Table 6**

Mean absolute reticulocytes ( $10^9$  cells/L) at baseline and peak values.

SDMB dose	Pre		Peak		p Value vs. placebo
	Mean	SD	Mean	SD	
Placebo	52	12	62	20	
5 mg/kg (fasted)	50	12	81	24	0.03
10 mg/kg (fed)	58	8	84	14	0.055
15 mg/kg (fed)	46	11	72	11	0.02