Comparing the Efficacy and Tolerability of a New Daily Oral Vitamin B_{12} Formulation and Intermittent Intramuscular Vitamin B_{12} in Normalizing Low Cobalamin Levels: A Randomized, Open-Label, Parallel-Group Study

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ABSTRACT

Background: Vitamin B_{12} deficiency is routinely treated with parenteral dosing and less often with high-dose oral vitamin B_{12}. Oral vitamin B_{12} formulations have low bioavailability in patients with malabsorption and are considered less reliable than parenteral treatments.

Objective: The objective of this study was to compare the efficacy and safety profile of a new proprietary oral vitamin B_{12} formulation (oral B_{12}) with intramuscular (IM) vitamin B_{12} (IM B_{12}) in restoring normal serum B_{12} concentrations in patients with low cobalamin levels (<350 pg/mL).

Methods: Patients were recruited from 5 centers and randomly assigned to receive oral B_{12} 1000 μg, taken daily for 90 days, or IM B_{12} 1000 μg, given on study days 1, 3, 7, 10, 14, 21, 30, 60, and 90. The patients were aged ≥60 years or aged ≥18 years and had gastrointestinal abnormalities or were on a restricted diet. The primary efficacy outcome compared the proportion of patients in each treatment arm in whom cobalamin levels were normalized (≥350 ng/mL) following 60 days of treatment. Secondary objectives included comparing the efficacy of the 2 formulations after 90 days of treatment, assessing time to normalization of B_{12} levels, and evaluating the changes in the levels of biomarkers methylmalonic acid (MMA) and homocysteine (HC). The effect on holotranscobalamin II (active B_{12}) levels was assessed as an exploratory end point and correlated to serum cobalamin levels in both treatment groups. Blood samples were collected at baseline (day 1) and on days 15, 31, 61, and 91.

Results: Fifty patients were recruited. Forty-eight patients (96.0%) completed the study (22 patients [91.7%] in the oral B_{12} group and 26 patients [100%] in the IM B_{12} group). All patients (100%) in both treatment groups and in both populations had a cobalamin level ≥350 pg/mL on day 61 and maintained it on day 91. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance ($P < 0.05$) for mean percent change from baseline (PCFB) in serum cobalamin levels on day 61 and day 91. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance for mean PCFB in serum MMA levels on day 61. There was a statistical difference between the IM and oral treatment groups for mean PCFB in serum MMA levels on day 91 ($P = 0.033$), with lower values in the oral B_{12} group. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance for mean PCFB in plasma HC levels on day 61 and day 91. All patients in each treatment group achieved normalization of serum cobalamin levels by day 15. All patients in both treatment groups and in both populations had plasma holotranscobalamin levels ≥40 pmol/L on day 61 and on day 91. No statistical analysis was planned or performed for safety end points, which were reported only descriptively. Most observed adverse effects were considered mild or moderate in intensity. All adverse effects that were considered severe in intensity were also considered by the investigator to be not related to the study drug.

Conclusions: In this selected study population comprising individuals with low cobalamin levels but who otherwise were in good health, patients received oral B_{12} (1000 μg/d) or IM B_{12} (1000 μg in 9 injections over 3 months) for a total of 3 months. Both the oral and IM formulations were effective in

restoring normal levels of serum cobalamin in all patients studied (100%). Both formulations used in this study were well tolerated at the dose studied. ClinicalTrials.gov identifier: NCT01312831. (*Clin Ther*. 2011;33:358–371) © 2011 Elsevier HS Journals, Inc. All rights reserved.

Key words: B_{12}, cobalamin, deficiency, Eligen, holotranscobalamin, homocysteine, intramuscular, methylmalonic acid, SNAC.

INTRODUCTION

The traditional treatment for B_{12} deficiency is intramuscular (IM) injections. In the United States, injections of 1-mg cyanocobalamin are given frequently within the first month (6–9 injections), followed by maintenance injections as prescribed by the physician. After the initial treatment period or once clinical remission occurs, treatment is continued for life on a monthly schedule. Various treatment schedules and doses are described in the literature, commencing with frequent dosing and transitioning to less frequent maintenance dosing.[1]

The use of oral crystalline cyanocobalamin to treat vitamin B_{12} deficiency was reported in 1968.[2] Patients with pernicious anemia and vitamin B_{12} malabsorption were treated with 500- or 1000-μg/d oral cyanocobalamin for up to 60 months. The study reported successful use of oral cyanocobalamin at 1000 μg/d for maintenance therapy in B_{12} deficiency, with no adverse effects. No statistical analyses were presented. Absorption of oral cobalamin was reported to occur in the absence of intrinsic factor. Oral cyanocobalamin was reported to be absorbed to the extent of ~1% through a passive diffusion process, when doses in the range of 100 μg to 5 mg were used.[2] However, individual differences in B_{12} supplementation requirements exist.[3]

The use of oral versus IM cyanocobalamin to treat B_{12} deficiency has been investigated in 2 randomized controlled studies in which B_{12} levels were measured and biochemical or hematologic parameters were monitored for 4 months in 33 patients[4] or 3 months in 60 patients.[5] In both studies it was concluded that oral B_{12} at doses of 1000 or 2000 μg/d or less frequently had at least comparable efficacy and perhaps even superior efficacy compared with parenteral treatment (1000 μg IM monthly after a loading period) for B_{12} deficiency. These studies were also the subject of a Cochrane Group analysis to assess the evidence support-

ing the study conclusions.[6] This systematic review supported the study conclusions in both cases.

During the past 10 years there have been several reports on the effect of oral cobalamin alone on B_{12} levels in the elderly. These reports were mostly small cohort studies in which enrollment varied between 10 and 30. Two studies were conducted with daily dosing for at least 3 months,[7,8] and one for a mean (SD) of 2.5 (1.2) years.[9] Oral doses were 650 to 1000 μg/d. One study included patients with pernicious anemia; 2 studies included patients with food cobalamin malabsorption. The results indicated that 80% to 90% of patients achieved normal serum B_{12} levels in the 3-month studies and that 95% achieved normal levels in the 2.5 year study. Clinical improvements were reported in 20% to 30% of patients.[10] A short-term study of 1-week duration with 1000-μg/d oral B_{12} indicated that B_{12} levels increased by a mean value of 0.23 μg/L (230 pg/mL) in 17 of 20 elderly patients.[11]

Despite evidence that oral B_{12} administered at doses $\geq$100 μg can treat B_{12} deficiency in certain patients,[2] the ability of oral treatments to match parenteral B_{12} in rapidly and reliably restoring or maintaining B_{12} stores in most or all patients is still in question. As a result, IM treatment remains in wide clinical use in some regions, including the United States. A recent systematic review of randomized controlled oral B_{12} intervention studies[12] examined biomarker responses to intervention with oral B_{12} in 8 studies. Cobalamin, methylmalonic acid (MMA), homocysteine (HC), and holotranscobalamin responses were evaluated. It was found that the B_{12} intakes produced highly variable effects on blood B_{12} concentrations and that gender or age subgroup analysis failed to account for the variability. MMA and HC responses were more closely linked to B_{12} intake, but there was insufficient suitable data to assess holotranscobalamin response. The authors noted that some papers failed to report sufficient information to impart a clear picture of the clinical response. Similarly, Carmel[3] commented in part on published B_{12} surveys as follows: " . . . reliance of these studies on mean values and group statistics can often hide subsets of persons with little or no improvement within the overall association of supplementation and better biochemical status."

The two common B_{12} treatment methods of IM injection and oral administration both suffer from certain drawbacks. IM B_{12} administration is inconvenient, relatively costly when medical personnel are

involved in dosing, and may be painful.[6] As noted earlier, current oral treatment, despite active study for several decades and acceptance in some quarters, is still viewed as less reliable than parenteral administration, and patient monitoring is advised more frequently than with parenteral administration.[13]

The purpose of this study was to provide clinical data in patients with low B_{12} concentrations but otherwise good health on the efficacy and safety profile of oral B_{12}, a new proprietary oral formulation of B_{12}* with enhanced bioavailability, as a possible treatment alternative to IM cyanocobalamin. This new formulation contains an amphiphilic carrier molecule, sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC). When the carrier is coformulated with poorly absorbed molecules, it allows them to be more easily absorbed from the gastrointestinal tract.[14] Absorption of this new proprietary oral B_{12} formulation was studied in a pharmacokinetic study in healthy male subjects (Castelli MC, Wong DF, Friedman K, Riley MG. An open-label, randomized, single-dose, parallel-group pharmacokinetic study in healthy male subjects of oral cyanocobalamin formulated with sodium N-[8-(2-hydroxybenzoyl)amino]caprylate [SNAC]. Submitted for publication). Mean peak B_{12} concentrations with this oral B_{12} formulation were 10 times higher than with the same 5-mg dose of B_{12} in a conventional oral formulation (mean [SD] C_{max}, 12,847 [6613] pg/mL vs 1239 [450] pg/mL; $P = 0.002$). Peak concentrations of B_{12} with the test formulation were observed at 30 to 45 minutes compared with 6 hours for the conventional formulation. Absolute bioavailability of the test formulation based on AUC_{last} data was estimated at 5.09% versus 2.16% for the conventional formulation. In the present study, the safety profile and therapeutic efficacy of the new proprietary oral tablet formulation of B_{12} (1000 μg) was compared with that of IM B_{12} (1000 μg) in patients with mild B_{12} deficiency.

PATIENTS AND METHODS
Study Design
This was an open-label, parallel-group, randomized 60-day study, with a 30-day extension, conducted in B_{12}-deficient patients at 5 medical centers. Patients were assigned to treatment according to a randomization schedule prepared by a statistician at the start of

the study. The randomization schedule was computer generated, using random permuted blocks of 2. Allocation numbers were assigned sequentially in an ascending order. The randomization scheme was stratified such that a balanced number of men and women were assigned to both treatment groups. Patients were randomly assigned to receive either oral B_{12} 1000-μg tablet, administered once daily for 90 days, or IM cyanocobalamin 1000 μg, administered on days 1, 3, 7, 10, 14, 21, 30, 60, and 90. The IM dosing schedule adopted in this study was the same as that recommended by Schilling[15] as a clinical standard and subsequently used in a widely cited comparison of oral and IM dosing reported in 1998.[4] In addition, daily oral dosing was used in the latter study.

Oral B_{12} was self-administered in the fasted state at least 1 hour before the morning meal as a single tablet with 50 mL of water. IM study drug administration was performed by study personnel in the research clinic in the morning, in the fasted state, and at least 1 hour before the morning meal on study days 1, 3, 7, 10, 14, 21, 30, 60, and 90. For patients assigned to receive oral B_{12}, treatment compliance was based on patient diary entries. Blood samples for pharmacodynamic assessments (efficacy variables) were collected from all patients at baseline prior to dosing (day 1) and on days 15, 31, 61, and 91. Sample collection was carried out approximately 24 hours after the last dose administration, before that day's dose administration, and in the fasting state.

The study was conducted from February 21, 2009 to May 20, 2010. The study protocol, all study protocol amendments, written study patient information, informed consent forms, and any other appropriate study-related information were reviewed and approved by an independent Institutional Review Board, IntegReview Ethical Review Board (Austin, Texas).

Patients
During enrollment, patients were informed that they could receive either a tablet of vitamin B_{12} taken by mouth once per day for 90 consecutive days or an injection of vitamin B_{12} 9 times during a 90-day period. Patients were also informed that the formulation they would receive was going to be assigned by chance, like the flip of a coin, and disclosed to them on the first day of the study. Fifty adult male and female patients were randomly assigned to receive a study drug. Twenty-two of the 24 patients (91.7%) who were randomized

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to receive the proprietary oral B_{12} completed the study as planned. Two patients (8.3%) discontinued the study drug prematurely. One patient voluntarily withdrew consent after 1 dose of oral B_{12} and 1 patient voluntarily withdrew consent after 67 doses of oral B_{12}. Physical examination and review of laboratory tests and medical history for reported adverse events (AEs) revealed no relationship to oral B_{12}. Twenty-six patients were randomized to receive IM B_{12} and all 26 patients (100%) completed the study as planned.

Eligible patients were men or women whose clinical laboratory tests showed vitamin B_{12} deficiency, defined as serum cobalamin <350 pg/mL. Patients were required to be aged ≥60 years or ≥18 years with gastrointestinal abnormalities including, but not limited to, gastrointestinal surgery (eg, gastrectomy, gastric bypass), ileal resection, gastric atrophy, celiac disease, and Crohn's disease, history of prolonged use (>3 months) of proton pump inhibitor drugs, or a restricted diet (such as vegetarian or vegan). Additional inclusion criteria were general good health and normal kidney function. Good health was determined by lack of significant findings in medical history, on physical examination, and in clinical laboratory tests (chemistry, hematology, and urinalysis), vital sign measurements, and electrocardiogram (ECG) results. Normal kidney function was determined by estimated creatinine clearance computed with the Cockcroft-Gault formula, because impaired renal function may elevate HC, MMA, and holotranscobalamin values.[16] Vital sign measurements included body temperature (°C), heart rate (beats/min), and blood pressure (systolic and diastolic mm Hg, measured in a supine position after 3 minutes of rest). Body temperature was measured with a digital oral thermometer (Welch Allyn Inc, Skaneateles Falls, New York) or an ear thermometer (ThermoScan Exact Temperature; Braun/Kaz USA, Southborough, Massachusetts) while the patient was seated. Heart rate was measured manually for at least 30 seconds by qualified personnel. Blood pressure was taken at rest while the patient was seated, usually from the left arm. A wall-mounted sphygmomanometer and a stethoscope were used to obtain systolic and diastolic blood pressure readings (Welch Allyn). Physical examinations and collection of vital sign measurements were conducted by a professionally trained physician or health professional licensed to perform physical examinations.

Exclusion criteria included current treatment from a health care provider to treat vitamin B_{12} deficiency or symptoms; daily use of neutralizing antacids; inability to ingest oral medication; any clinically significant laboratory value at screening; hypersensitivity or allergic reaction to vitamin B_{12}; participation in a clinical research study involving a new chemical entity within 30 days of the first study dose; and folate levels below the reference range provided by the clinical laboratory.

Confounding factors in the diagnosis and treatment of vitamin B_{12} deficiency include folate deficiency, renal insufficiency, and vitamin B_6 deficiency. Folate deficiency can cause many of the same symptoms as B_{12} deficiency, such as elevated total HC. Renal insufficiency may elevate MMA levels in the blood, and vitamin B_6 deficiency or hypothyroidism may lead to elevated total HC. These clinical parameters of B_{12} deficiency, when attributed to other causes, would not respond to B_{12} treatment. Patients with the aforementioned deficiencies were excluded from the study.

Patients recorded concomitant medications (including vitamins, herbal supplements, and antacids) and AEs in diaries distributed on study day 1. Patients randomized to receive the proprietary oral B_{12} formulation also recorded dosing information (date and time) and time of meal consumption before and after each dose.

Test and Reference Formulations

The branded B_{12} 1000-μg oral tablets were manufactured and supplied by Emisphere Technologies, Inc (Cedar Knolls, New Jersey) as open-label containers (lot number 112-08-04; batch number EM0411; expiry date July 2010). The investigational pharmacist or designee at the central site (Advanced Biomedical Research Inc, Hackensack, New Jersey) packaged and labeled the study product for administration according to a randomization schedule prepared before the start of the study. Oral B_{12} was stored at controlled room temperature (15°C–30°C) and protected from light according to the instructions on the package label.

Sterile cyanocobalamin for injection USP 1000 μg/mL was purchased from a pharmacy by the investigational pharmacist at Advanced Biomedical Research as individual 1-mL vials (NDC number 0517-0031-25; lot numbers 8786 and 8798, expiry date November 2010; and lot number 8829, expiry date December 2010). IM B_{12} was stored at controlled

room temperature (15°–30°C) and protected from light according to the instructions on the package label.

Treatments

The 1000-μg oral tablet was taken in the fasted state as a single tablet with 50 mL water. Each dose was self-administered daily for 90 days, after an overnight fast, and 1 hour before the morning meal. No liquid was consumed for at least 1 hour before and 1 hour after dosing. The commercially available 1000-μg cyanocobalamin was administered IM as 1 mL from a vial containing 1000 μg/mL drug. Study drug administration was performed by study personnel in the research clinic, in the morning, and in the fasted state at least 1 hour before the morning meal on study days 1, 3, 7, 10, 14, 21, 30, 60, and 90.

Blood Sample Processing
Standard Clinical Laboratory Tests

Standard clinical laboratory tests, which included screening for serum cobalamin, were centralized and performed by BioReference Laboratories (Elmwood Park, New Jersey), a CLIA- and CAP-certified laboratory. The tests performed were chemistry, hematology, urinalysis (chemistry and microscopic), marker for hepatitis B (HBsAg test, chemiluminescence method; Ortho Clinical Diagnostics, Raritan, New Jersey), HIV (EIA/ELISA test, chemiluminescence method; Ortho), hepatitis C (hepatitis C virus antibody test, chemiluminescence method; Ortho), vitamin B_{12} (serum cobalamin, Cobas electrochemiluminescence immunoassay kit; Roche Diagnostic, Indianapolis, Indiana [package insert available upon request]), vitamin B_6 (HPLC method available upon request), and folate (Folate-E170 electrochemiluminescence immunoassay kit; Roche).

Efficacy Assessments

All bioanalytical determinations for efficacy assessments (pharmacodynamic variables) were performed at Frontage Laboratories (Malvern, Pennsylvania).

For isolation of serum (for serum cobalamin and MMA determinations), approximately 7 mL of venous blood was collected in serum-separator blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey). Tubes were immediately wrapped in aluminum foil, inverted, and placed on ice until centrifugation at 1200g for 10 minutes at 4°C. Serum was withdrawn and placed in equally divided aliquots into polypropylene tubes. Tubes were wrapped with alumi-num foil and stored at −70°C until shipment to the bioanalytical laboratory.

For isolation of plasma (for HC and holotranscobalamin determination), approximately 10 mL of venous blood was collected in blood collection tubes containing lithium-heparin. Tubes were inverted and placed on ice until centrifugation at 1200g for 10 minutes at 4°C. Plasma was withdrawn and placed in equally divided aliquots into polypropylene tubes and stored at −70°C until shipment to the bioanalytical laboratory.

Serum cobalamin (B_{12}) levels were determined by a validated microparticle enzyme immunoassay (MEIA) detection method with a calibration range of 100 to 2000 pg/mL. Sample dilution procedures were validated up to 4-fold dilution. Serum MMA levels were determined by a validated LC/MS/MS method with a calibration range of 5 to 200 ng/mL. Plasma HC levels were determined by a validated fluorescence polarization immunoassay detection method with a calibration range of 2.5 to 50 μmol/L, and plasma holotranscobalamin (active B_{12}) levels were determined using a validated MEIA method with a calibration range of 8 to 128 pmol/L. Sample dilution procedures were validated up to 32-fold dilution. All final bioanalytical method, validation, and data reports are available upon request.

Safety Profile Assessments

Safety profile assessments consisted of monitoring AEs, laboratory test results, concomitant medications, vital sign assessments, ECG, and physical examination findings. Laboratory tests included hematology, chemistry, and urinalysis. Information regarding all AEs, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test, or other means, was collected and recorded on the Adverse Event Case Report Form (AE CRF) and followed as appropriate. No script was used for solicitation of patient complaints. AEs were assessed by asking "How are you feeling today?" or by patient self-report. Once a patient had offered a complaint, the nurse would inquire further regarding onset, associated symptoms, precipitating/aggravating/relieving factors, and resolution time. Data for AEs were analyzed using the treatment-emergent adverse event (TEAE) philosophy. TEAEs were defined as AEs that emerged during treatment, having been absent at pretreatment, or that worsened in severity or frequency

relative to the pretreatment state. All reported AEs occurring during the study were listed by patient. Data were also reported as a summary with descriptive statistics (mean [SD]). An overall summary of TEAEs by treatment and a summary of TEAEs by MedDRA System Organ Class, MedDRA preferred term, and treatment group were provided. The principal investigator at each site reviewed individual AEs and serious AEs (SAEs) data. The investigators who assessed AEs are as follows: BGR, NEA, MMR, MKS, and PV. SAEs were also to be reported to the international review board within 48 hours.

Subjects with AEs ongoing at the last study visit were to be followed until resolution or for 30 days after the patient's last study visit, whichever came first. AEs that were reported within 7 days following the patient's last study visit were to be recorded on the AE CRF and followed until resolution or for up to 30 days after the patient's last study visit. AE information to be collected and reviewed by the investigator included start and stop date, severity (mild, moderate, severe), action taken, relationship to study product (possibly, probably, not related), outcome, and seriousness. Samples for clinical laboratory safety profiling tests (chemistry, hematology, and urinalysis) were obtained at screening, at baseline, and at the end-of-study visit before discharge from the study. Any clinical laboratory finding considered clinically significant was recorded as an AE. Systolic and diastolic blood pressure (mm Hg), heart rate (beats/min), and oral body temperature (°C) were assessed in the supine position after 3 minutes at rest at screening, baseline, and each clinic visit required for blood sample collection and IM dose administration and at the end-of-study visit before discharge from the study.

A resting 12-lead ECG was recorded at screening and at the end-of-study visit before discharge from the study. The ECG parameters recorded included ventricular rate (beats/min), PR interval (msec), QRS duration (msec), QT interval (msec), and QTc interval (msec; Bazett's correction). A physical examination was performed at screening and at the end-of-study visit before discharge from the study.

Efficacy Assessments

The primary efficacy variable was serum cobalamin levels on day 61. The primary efficacy outcome compared the proportion of patients in both treatment groups whose cobalamin levels were normalized ($\geq$350 pg/mL) on day 61. The secondary efficacy variables were serum cobalamin levels on day 91, serum MMA levels on days 61 and 91, and plasma total HC levels on days 61 and 91.

The secondary efficacy outcomes were comparison of the proportion of patients in both treatment groups whose cobalamin levels were normalized ($\geq$350 pg/mL) on day 91; comparison of the mean percent change from baseline (PCFB) in serum cobalamin levels among patients in both treatment groups on days 61 and 91; comparison of the mean PCFB in serum MMA levels among patients in both treatment groups on days 61 and 91; comparison of the mean PCFB in total plasma HC among patients in both treatment groups on days 61 and 91; and comparison of the mean first time to normalization of serum cobalamin ($\geq$350 pg/mL) among patients in both treatment groups on days 61 and 91.

Exploratory efficacy variables and outcomes were plasma holotranscobalamin levels on days 61 and 91 (measured by comparison of the proportion of patients in both treatment groups whose holotranscobalamin levels were normalized [$\geq$40 pmol/L] on days 61 and 91) and the relationship between cobalamin and holotranscobalamin levels on days 61 and 91.

Statistical Analysis

Programming and statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, North Carolina). Three analysis populations were used. The intent-to-treat (ITT) population included all patients randomly assigned into the trial, regardless of whether they received study product. Analyses using the ITT population assigned patients to the group to which they were randomly assigned. The per-protocol population included all randomly assigned patients who received at least 90% of their assigned study treatment, who had nonmissing baseline and day 61 serum cobalamin assessments, and who met all inclusion criteria and no exclusion criteria. For patients receiving the new branded oral B_{12}, compliance was based on diary entries. The principal analyses were conducted on the ITT population.

The safety population included all randomly assigned patients who received $\geq$1 administration of study product and who had $\geq$1 subsequent safety profiling assessment. Patients were included in this group based on the actual treatment received. All

safety profiling analyses were performed on the safety population.

The analysis of the primary efficacy outcome compared the proportion of patients in both treatment groups whose cobalamin levels were normalized (cobalamin ≥350 pg/mL) on day 61. The number and frequency of patients whose levels were normalized were calculated. The 90% confidence interval (CI) of the difference in the proportions (oral B_{12} – IM B_{12}) was calculated and presented using exact procedures.[17]

The analyses for all secondary outcomes used only available data (ie, no imputation for missing data occurred after first dose of study medication). For all tests of secondary hypotheses, $P < 0.05$ indicated a statistically significant result. No adjustments were made for multiplicity. For patients with missing baseline data, the latest screening value was used as baseline for the calculation of change from baseline and PCFB. Change from baseline was defined as (X–B). PCFB was defined as PCFB = 100(X–B)/B, where B is the baseline (predose day 1) measurement and X is the measurement at day 61 or day 91, as required.

The proportion of patients who achieved normalization of cobalamin levels (cobalamin ≥350 pg/mL) at day 91 was analyzed using the same methodology as given earlier for the primary analysis (ie, day 61). The PCFB for cobalamin levels at days 61 and 91 was analyzed using ANCOVA with treatment group and baseline cobalamin measurement as fixed effects. For each outcome and each time point the mean difference between the groups was tested. The PCFB for MMA and HC levels at days 61 and 91 was analyzed using ANCOVA with treatment group and baseline MMA or HC measurement as fixed effects. For each outcome and each time point the mean difference between the groups was tested.

Time to first normalization of cobalamin (≥350 pg/mL) from day 1 to day 61 and from day 1 to day 91 was analyzed using a log-rank test. The Kaplan-Meier curves, hazard ratio, and 95% CI were calculated. Time zero was taken as study day 1. Patients whose levels were not normalized by day 61 were censored at day 61. Those who discontinued before day 61 without achieving normalization were censored at the time of discontinuation.

The proportion of patients who achieved normalization of holotranscobalamin levels (≥40 pmol/L) at day 61 and day 91 were analyzed using the same methodology as given for the primary analysis. The relation-

ship between cobalamin and holotranscobalamin levels at day 61 and day 91 and the PCFB to day 61 and day 91 were explored. The relationship was analyzed graphically.

RESULTS

Patient Demographic and Baseline Characteristics

A total of 50 healthy patients (11 men [22.0%], 39 women [78.0%]) were randomized to receive a study drug. Mean (SD) age was 53.2 (15.33) years and race was indicated as Caucasian (40 [80.0%]) or black (10 [20.0%]). Mean (SD) serum cobalamin levels at screening were similar among patients randomized to receive oral B_{12} (285.5 [54.27] pg/mL) compared with patients randomized to receive IM B_{12} (262.0 [54.61] pg/mL). Demographic and other baseline characteristics of this population are summarized in Table I.

Efficacy

All patients in both treatment groups and in both populations had cobalamin levels ≥350 pg/mL on day 61 and day 91; therefore, P could not be calculated for this comparison. The difference between the IM and oral treatment groups did not reach the planned level of statistical significance for mean PCFB in serum cobalamin levels on day 61 or day 91. On day 61, the mean (SD) PCFB in cobalamin levels was 452.5 (353.19) and 714.6 (732.11) for patients in the oral B_{12} and IM B_{12} treatment groups, respectively. On day 91, the mean (SD) PCFB in cobalamin levels was 526.9 (350.62) and 608.9 (446.68) for patients in the oral B_{12} and IM B_{12} treatment groups, respectively. The results were identical for the ITT population and for the per-protocol population. Figure 1 displays the mean PCFB in cobalamin levels by study day for the ITT population.

The difference between the oral and IM treatment groups did not reach the planned level of statistical significance for mean PCFB in serum MMA levels on day 61. On day 61, the mean (SD) PCFB in MMA levels was –30.55 (26.66) and –28.17 (24.75) for patients in the oral B_{12} and IM B_{12} treatment groups, respectively. There was a statistical difference between treatment groups for mean PCFB in serum MMA levels on day 91 ($P = 0.033$). On day 91, the mean (SD) PCFB in MMA levels was –39.29 (24.94) and –26.58 (25.64) for patients in the oral B_{12} and IM B_{12} treat-

Table I. Demographic and baseline characteristics of the intent-to-treat population.

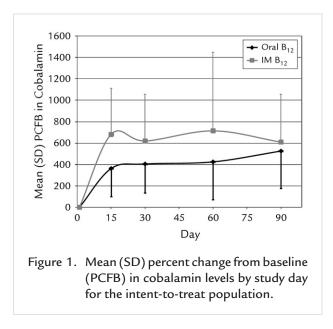| Characteristic	Oral B_{12}* (n = 24)	IM B_{12} (n = 26)	Overall (N = 50)
Age, y			
Mean (SD)	52.6 (15.27)	53.8 (15.68)	53.2 (15.33)
Quartiles	45.5, 58.0, 63.0	47.0, 60.5, 65.0	47.0, 60.0, 63.0
Min, Max	19, 76	22, 73	19, 76
Gender			
Male, n (%)	5 (20.8)	6 (23.1)	11 (22.0)
Female, n (%)	19 (79.2)	20 (76.9)	39 (78.0)
Race			
Caucasian, n (%)	18 (75.0)	22 (84.6)	40 (80.0)
Black, n (%)	6 (25.0)	4 (15.4)	10 (20.0)
Ethnicity			
Hispanic or Latino, n (%)	2 (8.3)	7 (26.9)	9 (18.0)
Non-Hispanic or Non-Latino, n (%)	22 (91.7)	19 (73.1)	41 (82.0)
Weight, kg			
Mean (SD)	85.1 (19.86)	84.9 (19.76)	85.0 (19.61)
Quartiles	69.9, 82.0, 99.3	71.7, 85.6, 96.6	70.0, 84.4, 96.6
Min, Max	57.2, 129.0	53.6, 127.0	53.6, 127.0
Height, cm			
Mean (SD)	166.0 (6.38)	163.8 (9.72)	164.8 (8.28)
Quartiles	160.5, 166.4, 171.1	157.5, 162.6, 167.6	160.0, 163.0, 170.2
Min, Max	154.5, 176.5	148.0, 188.0	148.0, 188.0
BMI, kg/m^2			
Mean (SD)	30.9 (7.09)	31.8 (7.67)	31.4 (7.33)
Quartiles	25.2, 30.3, 36.0	24.9, 30.1, 37.8	24.9, 30.1, 37.3
Min, Max	20.4, 50.4	22.0, 49.6	20.4, 50.4
Cobalamin, pg/mL			
Mean (SD)	285.5 (54.27)	262.0 (54.61)	273.3 (55.17)
Quartiles	265.5, 293.5, 323.5	231.0, 269.0, 308.0	240.0, 284.5, 319.0
Min, Max	115.0, 347.0	111.0, 335.0	111.0, 347.0

BMI = body mass index; IM = intramuscular; Max = maximum; Min = minimum.
*Trademark: Eligen® B12 (Emisphere Technologies, Inc, Cedar Knolls, New Jersey).

ment groups, respectively. Figure 2 displays the mean PCFB in MMA levels by study day for the ITT population.

The difference between the oral and IM treatment group did not reach the planned level of statistical significance for mean PCFB in plasma HC levels on day 61 and day 91. On day 61, the mean (SD) PCFB in HC levels was –21.94 (19.03) and –17.49 (24.10) for pa-tients in the oral B_{12} and IM B_{12} treatment groups, respectively. On day 91, the mean (SD) PCFB in HC levels was –14.36 (28.38) and –8.81 (28.54) for pa-tients in the oral B_{12} and IM B_{12} treatment groups, respectively. Figure 3 displays the mean PCFB in HC levels by study day for the ITT population.

Time (days) to first normalization of serum cobala-min levels (≥350 pg/mL) by day 61 and by day 91

Figure 1. Mean (SD) percent change from baseline (PCFB) in cobalamin levels by study day for the intent-to-treat population.

Figure 3. Mean (SD) percent change from baseline (PCFB) in homocysteine levels by study day for the intent-to-treat population.

(secondary efficacy outcome) was compared among patients in both treatment groups. All patients in both treatment groups achieved normalization of serum cobalamin levels by day 15; therefore, P could not be calculated for this comparison. All patients in both treatment groups and in both populations had plasma holotranscobalamin levels ≥ 40 pmol/L on day 61 and day 91; therefore, P could not be calculated for this comparison.
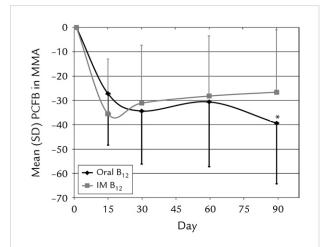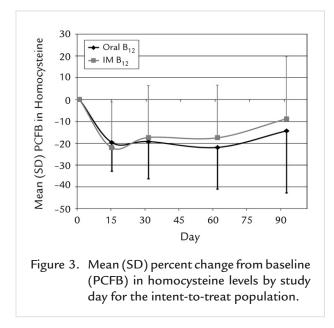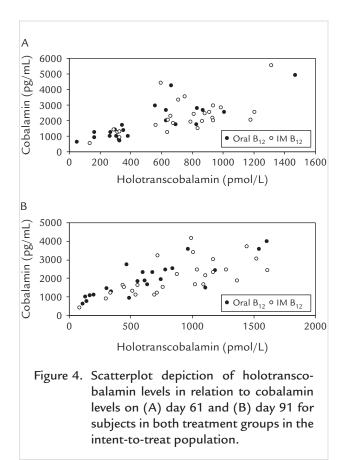

Figure 2. Mean (SD) percent change from baseline (PCFB) in methylmalonic acid (MMA) levels by study day for the intent-to-treat population.

Holotranscobalamin levels in relation to cobalamin levels on days 61 and 91 for patients in both treatment groups in the ITT population are displayed as scatterplots in Figure 4. Because a single dose (1 mg) was used for all patients enrolled whose body weight (BW) ranged from 53.6 to 127 kg, a correlation and a regression analysis (using Excel 2007, SP2 version 12) between response and BW was run for the IM and oral treatment groups. There was no relationship between BW and each of the analytes (serum cobalamin, $r^2 = 0.0191$; serum MMA, $r^2 = 0.0127$; plasma homocysteine, $r^2 = 0.00160$; and plasma holotranscobalamin, $r^2 = 0.000005$). Individual serum cobalamin, plasma holotranscobalamin, serum MMA, and plasma HC data following IM and oral treatment are available as supplemental tables (supplementary material associated with this article can be found in the online version at doi:10.1016/j.clinther.2011.03.003).

Figure 5 is a bar graph of mean (SD) serum cobalamin concentrations at baseline (day 1) and days 15, 31, 61, and 91 following both treatments. All enrolled patients screened by the clinical laboratory had cobalamin values <350 pg/mL. When the validated cobalamin method was used, 13 patients in the oral B_{12} group and 14 patients in the IM B_{12} group had values ≥ 350 pg/mL. All patients showed cobalamin and holotranscobalamin normalization at day 61 and normalization was maintained at day 91. The biomarker MMA decreased in patients who had elevated levels at screening (patients 102, 103, 202, 203, and 239 for the IM group

Figure 4. Scatterplot depiction of holotranscobalamin levels in relation to cobalamin levels on (A) day 61 and (B) day 91 for subjects in both treatment groups in the intent-to-treat population.

and 101, 228, 232, and 233 for the oral group). A total of 79% and 83% of people who were in the normal range at baseline had their level decreased at day 61 for the IM and oral treatment groups, respectively. The biomarker HC decreased in patients who had elevated levels at screening (patients 103, 105, 109, 202, 203, 214, 215, 220, 221, and 236 for the IM group and 101, 104, 107, 110, 213, 224, 228, 230, and 233 for the oral group). A total of 73% and 66% of people who were in the normal range at baseline had their level decreased at day 61 for the IM and treatment oral groups, respectively.

Safety Profile

A total of 50 healthy adult male and female patients were exposed at least once to a study drug during this study. The safety profile is presented descriptively as no statistical analysis was planned or performed for tolerability end points. Overall, 28 patients (56.0%) reported ≥1 AE during the study. All AEs reported were considered to be TEAEs. Following administration of oral B_{12} and IM B_{12}, 13 patients (54.2%) and 15 pa-

tients (57.7%) reported ≥1 AE, respectively. Three patients (11.5%), all receiving IM B_{12}, reported SAEs. No patients discontinued study treatment because of an AE. An overview of reported AEs during this study is presented in Table II.

Analysis of Adverse Events

The most common TEAEs recorded, occurring in >5% of patients in both treatment groups were upper abdominal pain, constipation, diarrhea, nausea, fatigue, bronchitis, upper respiratory tract infection, procedural pain, arthralgia, back pain, headache, and oropharyngeal pain. Most AEs were considered mild or moderate in intensity. All AEs that were considered severe in intensity were also considered by the investigator to be not related to study drug. Severe AEs reported by patients in the IM B_{12} treatment group include hunger, pain, and sinus headache; diarrhea, groin pain, abscess, procedural pain (patient 007-236); Lyme disease, procedural pain, headache (patient 007-239). One patient in the oral B_{12} treatment group (patient 007-230) reported 3 severe AEs: back pain, constipation, and increased heart rate.

No patients discontinued treatment due to an AE. Three patients, all randomized to receive IM B_{12}, experienced an SAE during this study. Patient 001-215, a 61-year-old black female, received her first dose of study drug October 7, 2009. On day 47 of the study, the patient was diagnosed with the SAE of diverticulitis that was assessed as moderate in intensity, not related to study drug, and resolved November 27, 2009. Patient 007-236, a 54-year-old Caucasian female, received her first dose of study drug on December 22,
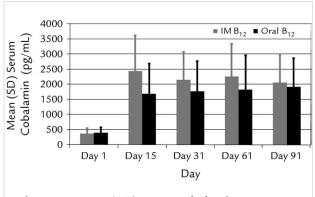

Figure 5. Mean (SD) serum cobalamin concentrations at baseline (day 1) and days 15, 31, 61, and 91 following both treatments.

Table II. Overview* of adverse events (AEs) (safety population).

| | Treatment | | |
Category[†]	Oral B$_{12}$[‡] (n = 24)	IM B$_{12}$ (n = 26) No. (%)	Overall (N = 50) No. (%)
Patients with AEs	13 (54.2)	15 (57.7)	28 (56.0)
Patients with TEAEs	13 (54.2)	15 (57.7)	28 (56.0)
Patients with SAEs	0 (0)	3 (11.5)	3 (6.0)
Patients who discontinued because of an AE	0 (0)	0 (0)	0 (0)

IM = intramuscular; SAE = serious AE; TEAE = treatment-emergent AE.
*No statistical analysis was planned or performed for safety end points, which are reported in this table only descriptively.
[†]Patients may be counted in more than 1 category.
[‡]Trademark: Eligen® B12 (Emisphere Technologies, Inc, Cedar Knolls, New Jersey).

2009. On day 30 of the study, the patient reported an SAE of abscess (left perianal and left vulvar-inguinal). The SAE of abscess was assessed as moderate in intensity, not related to study drug, and ongoing as of database lock (August 26, 2010).

Patient 007-239, a 47-year-old Caucasian female, received her first dose of study drug on January 12, 2010. On day 53 of the study, the patient reported an SAE of back pain, which was assessed as moderate in intensity, not related to study drug, and resolved March 13, 2010.

DISCUSSION

An oral B$_{12}$ formulation such as the branded test formulation with increased bioavailability and more rapid systemic uptake than conventional oral B$_{12}$ formulations may be beneficial to B$_{12}$-deficient patients who are currently treated with IM B$_{12}$ injections.

This study reported that both the test oral B$_{12}$formulation and reference IM B$_{12}$ treatment produced cobalamin normalization (>350 pg/mL) in all members of this population of patients with low levels of B$_{12}$ in as few as 15 days. This effect was maintained for the 3-month duration of the study. Normalization of holotranscobalamin levels was also observed. The study population included patients aged ≥60 years, some of whom could be expected to have age-related B$_{12}$ deficiency and some with underlying gastrointestinal conditions known to interfere with B$_{12}$ absorption. Despite normalization of cobalamin in all patients, differences were apparent between the treatment regimens. The standard IM dosing regimen used in this study involved multiple injections in the first 2 weeks of the study and produced higher values for cobalamin than did the oral treatment. On study day 15, mean serum cobalamin levels were 2434 pg/mL for the IM group and 1687 pg/mL for the oral group ($P = 0.022$). On study day 61, mean serum cobalamin levels were 2258 pg/mL for the IM group and 1828 pg/mL for the oral group ($P = $ NS). With IM dosing frequency changed to monthly, mean cobalamin values trended lower between months 2 and 3. Thus, by day 91 the difference between the mean values for the 2 treatment groups was reduced (2057 pg/mL vs 1957 pg/mL for IM and oral, respectively; $P = $ NS).

Total cobalamin and holotranscobalamin values for IM B$_{12}$ measured 24 hours after dosing would be expected to decrease somewhat over the course of a month. For example, Herbert[18] reported that cobalamin levels in vegetarians decreased from 450 pg/mL to 350 pg/mL (22%) in 1 week when vitamin B$_{12}$ intake was halted. Eussen et al[19] reported a decline in B$_{12}$ levels in elderly B$_{12}$- deficient patients when conventional oral replacement treatment (1000 µg/d) was halted. The mean decrease in 16 patients was 43% (20%) 3 months following a 6-month period of oral treatment.

The assay for total cobalamin used in this study measures cobalamin bound to transcobalamin and haptocorrin.[20] Residual unbound cobalamin may have contributed slightly to the values obtained for total cobalamin 24 hours after dosing. However, with rare

exceptions, most unbound cobalamin from either oral or IM dosing is eliminated in urine by this time even when doses in the milligram range are administered parenterally.[21–24]

In general, no differences were observed between patients treated with oral B_{12} versus IM B_{12} in change from baseline levels of cobalamin, MMA, or HC. However, there was a statistical difference between the IM and oral treatment groups for mean PCFB in serum MMA levels on day 91. The reason for this difference is not clear. One possibility is that the regimen of the daily oral B_{12} consistently maintained adequate cobalamin status whereas the periodic monthly B_{12} injection regimen resulted in peaks and troughs in cobalamin status, such that 1 month after the previous B_{12} injection on day 60 stores had become relatively depleted. The injection on day 90 would not immediately remedy the metabolic deficit in cobalamin as it has been reported that it may take up to 1 week for elevated MMA levels in B_{12} deficiency to return to normal following B_{12} administration.[25,26] There appeared to be a gradual trend of the PCFB in MMA to diverge with time, further supporting this explanation.

In Emisphere Technologies' proprietary oral drug delivery system, carriers induce a temporary increase in mucosal membrane permeability when their concentration at the membrane surface exceeds approximately 60 mM (~20 mg/mL). Under these conditions transcellular uptake of certain coformulated drugs increases.[27,28] These conditions can only be met soon after dosing with a solid oral dosage form containing SNAC carrier, as in the current B_{12} formulation. However, when the components move along the gastrointestinal tract, where they are dispersed and diluted by physiologic mixing and dilution of ingesta,[29] enhancement of drug uptake is reduced. A similar outcome may result when excess water (>50 mL) is delivered with the dosage form or dosing is not coordinated so as to limit a food effect.[30] The results of an earlier pharmacokinetic study conducted with the current oral B_{12} formulation in normal males are consistent with this mode of action (Castelli MC, et al. Submitted for publication.). Group mean bioavailability of the current oral B_{12} formulation was increased compared with a commercial oral formulation. In addition, T_{max} was greatly reduced and peak concentrations were approximately ten times higher than the commercial formulation (Vitalabs Inc, Jonesboro, Geor-

gia). Overall, the pharmacokinetic profile of the current oral B_{12} formulation resembled that of an IM injection. T_{max} and extent of uptake of the commercial oral formulation in this study was similar to that described elsewhere.[31]

The population examined in the current study had low cobalamin levels but did not exhibit clinical signs of B_{12} deficiency such as hematologic or neurologic deficits. Accordingly, the study focused on the changes in biomarker values in a multiple dose setting. Further investigations are warranted in more severely deficient patients to evaluate the efficacy of the current oral B_{12} formulation in meeting defined clinical response end points. The current study was conducted in a selected population of predominantly white patients living in the northeastern United States. In view of general concerns relating to extrapolation of clinical data to other regions and ethnic groups,[32] as well as such factors as gender, diet, and age that directly affect B_{12} status and response to supplementation,[33] the current findings require confirmation in relevant clinical settings. Although the study population was relatively small and heterogeneous in terms of etiology and predisposing factors for B_{12} deficiency, the magnitude of the effect obtained in both arms of this study was large. All patients (100%) met the primary end points with no variance.

Future studies of this proprietary formulation of oral B_{12} should focus on gaining a better understanding of the ADME processes (ie, absorption, distribution, metabolism, excretion) as they relate to the diverse causes of B_{12} malabsorption and deficiency. Based on the time course and physical requirements of this oral B_{12} drug uptake, absorption is thought to occur mainly in the stomach and upper small intestine, but experimental data on this point is lacking at present.

CONCLUSIONS

In this selected study population comprising individuals with low cobalamin levels but otherwise good health, patients received a new proprietary oral B_{12} formulation (1000 μg /d) or IM B_{12} (1000 μg in 9 injections over 3 months) for a total of 3 months. Both the oral and the IM formulations were effective in restoring normal levels of serum cobalamin in all patients studied (100%). Both formulations were well tolerated at the doses studied.

SUPPLEMENTARY MATERIAL

Supplementary tables accompanying this article can be found in the online version at doi:10.1016/j.clinther.2011.03.003.

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Supplementary Table I. Total and active B_{12} and biomarkers before and after 2 and 3 months of IM therapy.

Patient Number	Total B_{12} (pg/mL)				HTC, pmol/L			MMA, nmol/L			Homocysteine, μmol/L		
	Screen	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91
102	274	186.1	2030.0	1105.7	27.13	641.20	692.88	331.010	156.660	194.199	12.44	11.15	12.28
103	335	278.1	1487.5	1657.5	25.40	837.72	1091.96	362.512	217.749	221.213	15.20	10.30	11.94
105	240	261.5	2965.1	2467.1	35.33	935.40	1039.48	101.668	83.987	100.068	19.21	11.49	15.31
108	111	395.9	2454.8	3219.6	36.79	881.08	716.84	147.159	115.683	177.153	11.97	18.60	14.67
109	324	589.4	1901.6	2157.5	56.49	787.56	1110.04	70.997	57.659	57.693	14.58	11.97	11.30
112	284	326.0	1258.2	1313.8	35.36	314.28	515.40	272.817	274.189	258.455	12.61	8.48	10.07
202	225	242.6	2169.0	2463.5	28.23	930.60	1274.60	351.698	113.227	133.280	14.10	9.03	10.88
203	167	111.8	4408.5	1683.6	31.91	595.00	1024.92	572.004	173.486	194.191	14.90	10.25	14.12
205	289	NS	557.0	432.9	NS	134.92	82.44	NS	181.751	162.969	NS	8.54	8.20
208	324	405.9	1724.9	1634.3	35.52	563.80	434.20	141.189	116.953	97.349	7.15	6.14	6.05
209	292	278.6	1272.6	1622.6	34.21	326.04	554.68	105.377	122.000	107.731	8.83	9.32	8.76
212	330	437.1	3332.7	4171.3	33.03	709.48	992.36	425.989	172.631	164.536	12.26	14.60	21.43
214	179	117.6	1435.8	1511.9	20.10	289.56	447.48	201.846	102.007	79.677	21.36	11.20	10.69
215	231	308.4	2555.0	3022.1	43.21	910.44	1168.60	122.339	91.854	80.972	14.08	13.70	13.31
220	246	548.1	2284.4	2331.3	26.27	659.80	1169.96	249.047	100.830	127.996	15.17	11.22	8.45
221	225	174.1	2043.8	3401.1	32.13	1179.32	1008.60	149.140	116.259	120.307	14.64	12.74	19.44
223	252	500.6	5555.8	3708.5	22.59	1314.92	1442.44	187.823	163.401	198.162	12.81	10.82	12.72
225	321	364.8	3561.0	1216.8	30.19	753.24	338.56	257.719	114.616	219.951	11.55	10.59	8.96
227	312	334.2	897.6	895.4	29.14	326.52	299.96	110.035	97.587	96.706	10.15	7.82	6.40
229	260	705.0	1824.4	1539.0	34.98	675.20	759.92	161.114	104.166	138.699	11.87	7.18	7.52
231	272	844.2	2556.2	2416.8	51.46	1204.92	1613.08	128.563	104.818	134.016	11.13	7.41	9.10
234	308	317.9	1951.8	1890.6	37.19	864.84	1366.04	136.286	125.819	125.591	11.01	7.52	9.50
236	285	402.0	1983.6	1121.0	39.85	937.24	540.12	154.679	190.482	138.953	18.00	9.66	12.30
237	266	225.5	2843.4	3057.6	34.37	985.80	1524.76	209.916	157.524	140.511	13.89	13.58	12.77
239	285	585.8	2397.6	2215.2	37.61	811.08	878.12	614.658	435.956	331.349	13.69	8.87	13.46
241	261	273.4	1261.8	1219.6	39.51	637.84	716.36	242.739	BLQ	72.242	10.39	12.66	15.53
Mean	265	369	2258	2057	34.32	739	877	232	148	149	13	11	12
SD	54	180	1082	935	8.11	295	405	142	77	63	3	3	4
%CV	20	49	48	46	24	40	46	61	52	42	24	26	31

HTC = holotranscobalamin; IM = intramuscular; MMA = methylmelonic acid; BLQ = below limit of quantification; NS = no sample.

Supplementary Table II. Total and active B$_{12}$ and biomarkers before and after 2 and 3 months of oral* therapy.

Patient Number	Total B$_{12}$, pg/mL				HTC, pmol/L			MMA, nmol/L			Homocysteine, μmol/L		
	Screen	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91	Day 1	Day 61	Day 91
101	210	125.6	1019.4	1672.0	26.92	382.84	636.08	566.763	151.368	123.499	17.01	11.18	9.13
104	311	273.5	1262.4	2328.5	29.14	165.16	674.92	199.052	114.853	94.674	17.16	12.61	14.79
106	314	347.7	2001.0	1288.2	35.47	631.08	343.48	249.428	117.046	163.435	14.10	14.29	16.94
107	337	236.3	2938.4	2739.8	33.13	557.96	466.68	234.211	97.900	91.735	15.94	11.45	10.61
110	347	461.4	2791.5	2343.0	59.68	830.20	597.68	160.632	115.607	220.738	15.80	10.27	8.54
204	296	NS	906.2	1073.7	NS	162.68	168.60	NS	77.399	91.202	NS	8.13	13.02
206	263	440.2	4932.3	4002.5	42.25	1470.28	1603.96	137.903	92.201	89.432	8.72	7.29	7.15
207	287	354.4	1698.6	1864.1	29.91	343.40	616.52	142.425	93.285	90.651	13.01	9.26	11.82
210	207	225.0	989.1	748.2	32.13	263.48	143.40	141.655	97.985	96.172	13.12	15.44	13.49
211	323	349.2	2654.9	3572.6	34.42	631.24	967.64	98.704	73.275	105.868	9.30	10.21	13.85
213	319	286.9	4239.3	3565.5	50.29	662.44	1545.64	133.957	89.118	79.431	20.19	14.29	17.15
216	342	277.7	1420.1	1852.5	34.90	299.00	554.20	166.559	133.424	117.978	12.82	11.22	8.67
217	324	322.8	1385.0	1473.0	31.74	351.88	305.40	145.711	73.579	90.677	9.59	10.29	10.06
219	289	587.1	798.5	2449.8	50.62	323.00	780.84	63.773	75.290	54.450	11.76	8.54	7.39
222	268	258.9	981.6	1094.9	45.63	306.76	200.04	262.071	103.751	119.765	9.04	6.38	7.10
224	339	548.0	623.7	619.4	31.99	48.92	112.20	237.709	297.460	242.798	14.02	11.89	12.93
226	343	339.8	786.4	1478.0	23.72	NS	1107.80	162.334	113.117	127.928	10.41	NS	15.12
228	240	794.3	1737.4	1957.2	25.36	829.12	746.88	448.776	365.035	226.412	14.74	9.70	8.37
230	298	777.6	2524.6	NI	40.27	1008.92	NI	298.806	134.939	NI	19.91	9.49	NI
232	291	682.7	1237.0	1020.8	26.70	270.48	132.72	332.492	361.648	137.226	12.60	8.26	8.32
233	115	149.3	725.6	927.8	64.52	328.36	487.64	369.574	126.886	166.729	26.75	12.95	11.86
235	273	457.1	2647.4	2527.6	35.09	867.24	838.04	213.972	214.751	220.772	10.20	6.60	8.58
240	279	295.1	1740.2	2444.4	95.17	691.64	1184.44	81.633	71.488	BLQ	6.20	5.82	6.55
Mean	288	390	1828	1957	40	519	646	220	139	124	14	10	11
SD	55	186	1134	946	16	337	431	123	87	47	5	3	3
%CV	19	48	62	48	41	65	67	56	63	38	34	26	29

HTC = holotranscobalamin; MMA = methylmalonic acid; NS = no sample; BLQ = below limit of quantification; NI = not included. This subject withdrew consent after 67 doses of oral B12.
*Trademark: Eligen® B12 (Emisphere Technologies, Inc, Cedar Knolls, New Jersey).