SYNOPSIS

Name of Sponsor:
Abbott Laboratories GmbH

Name of Finished Product:
Ademetionine

Name of Active Ingredient:
Ademetionine 1,4-butanedisulfonate

Individual Study Table:

(For National Authority Use only)

Study Title:
Open-label, Randomized, Parallel-Group, Exploratory Study to Investigate the Effects of Different Doses of S-adenosyl-L-methionine (SAMe) in Subjects with Nonalcoholic Steatohepatitis (NASH) and Non-treated Matched Healthy Volunteers as Control Group

Investigator(s):
Professor Vlad Ratziu (Coordinating Investigator)

Study Center(s):
25 study centers in Germany (10 centers), Poland (5 centers), Russia (5 centers), and France (5 centers)

Publication (Reference):
Not applicable

Study Period:
27 DEC 2012 (first subject first visit, prior to study hold) and 30 SEP 2013 (after restart) to 25 SEP 2014 (last subject last visit)

Phase of Development:
III

Objectives:

Primary Objective(s)

NASH subjects

The primary objective of this study was to explore the effects of different doses of SAMe on the liver using the methionine tolerance test. The primary efficacy parameter was the methionine elimination half-life measured in blood.

Healthy volunteers

The healthy volunteer group served as a control group to establish the reference values for the methionine tolerance test.

Secondary Objective(s)

Secondary objectives of this study were the following:

NASH subjects

- To explore the effect of different doses of SAMe on the liver using the methionine tolerance test. The secondary efficacy parameter was the fasting methionine concentration and area

under the curve AUC of average methionine concentration versus time curve, the metabolic clearance rate and volume of distribution measured in blood.

- To explore the effect of different doses of SAMe on the liver using the methionine breath test. The methionine breath test was used to determine the effect of different doses of SAMe on the liver. The parameters cumulative percentage dose recovered at 30 minutes (cPDR30), 60 minutes (cPDR60), 90 minutes (cPDR90), peak, and time to peak were evaluated. The breath test was performed 1 day following the oral loading test at the start and end of the trial.
- To explore different doses of SAMe on the following laboratory parameters:
  - Hepatic panel: serum total bilirubin, serum conjugated bilirubin, alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), gamma glutamyl transpeptidase gamma-glutamyl transpeptidase (GGT), ALT/AST ratio
  - Metabolic panel: fasting lipid profile (cholesterol, high density lipoprotein, low density lipoprotein), amino acid profile, homeostasis model assessment, fasting plasma insulin, fasting glucose, glycosylated hemoglobin, as well as adiponectin
  - Immunological/antioxidant panel: C-reactive protein, cytokine profile (IL-6, IL-8, IL-10, TNF-α, MCP-1, and G-CSF), glutathione in erythrocytes, oxidative stress marker (isoprostane level)
  - Fibrosis /apoptosis panel: caspase-cleaved cytokeratin 18 (M30), hyaluronic acid, ActiTest/Fibrotest: score calculated from the results of a 6-parameter blood test, combining 6 serum markers with the age and gender of the subject: alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, serum total bilirubin, and ALT.
- To explore the efficacy of different doses of SAMe on clinical global impression (severity of illness and treatment effect)
- To explore the efficacy of different doses of SAMe on EQ/5D

Healthy volunteers

The healthy volunteer group served as a control group, to establish the reference values for the methionine tolerance test and the ^{13}C-methionine breath test.

Methodology:

This was an open-label, parallel-group, randomized, multicenter, exploratory study. The NASH subjects were screened and once the inclusion and exclusion criteria were fulfilled, the subjects were randomly assigned on Day 1 (baseline), followed by a methionine tolerance test, a methionine ^{13}C-breath test and 6-week open-label period with either 1000 mg, 1500 mg, 2000 mg SAMe per day, or no study drug treatment. The nontreated subjects stayed on their standard therapy. During the last 2 days of the open-label period, a second methionine tolerance test and methionine ^{13}C-breath test were performed. Both tests were performed after 6 weeks (plus up to 3 days for flexibility) of the open-label period.

In addition, after completion of the subject part, a group of healthy volunteers were screened and underwent baseline assessment followed by a methionine tolerance test and a ^{13}C-methionine breath test to control for these tests in NASH subjects. Healthy volunteers were

released after the completion of the methionine tolerance test and the ^{13}C-methionine breath test. They were not treated with SAMe nor did they have a second methionine tolerance or breath test.

In this study, a selected patient population with NASH was included, which has not yet been investigated in a clinical study with long-term treatment of SAMe.

Number of Subjects (Planned, Consented, Randomized and Analyzed):
(Planned: 120 in each treatment group and 15 healthy volunteers, consented: 179, randomly assigned: 108 [17 healthy volunteers], and analyzed: 104)

Diagnosis and Main Criteria for Inclusion:
Each NASH subject had to meet the following main inclusion criteria:

- Signed Informed Consent
- Age ≥ 18 years

Subjects diagnosed with nonalcoholic fatty liver disease based on one of the following approaches:

1. Subjects with NASH based on histology in medical history within the last 3 years and the subjects had to be in a stable metabolic condition since histology for NASH:
   - No major treatment changes indicative for improvement of NASH, e.g., stop of antidiabetes drug(s)
   - No significant change in body weight (> 10 % weight reduction)
2. Subjects with nonalcoholic steatosis or steatohepatitis based on histology (> 3 years) in medical history and
   - Ultrasound (< 3 months prior to study start) displaying a bright echo pattern compatible with steatosis and
   - BMI > 25 kg/m^2
3. Subjects diagnosed with nonalcoholic fatty liver disease without histology, based on:
   - Ultrasound (< 3 months prior to study start) displaying a bright echo pattern compatible with steatosis and
   - BMI > 25 kg/m^2 and
   - At least one of the following metabolic risk factors:
     - Impaired fasting glucose (serum glucose > 6.1 mmol/L) or type 2 diabetes or hypertension or dyslipidemia

Each healthy volunteer had to meet all the following criteria to be eligible for the study:

- Signed Informed Consent
- Subjects ≥ 18 years
- Subjects must have been in good health as determined by vital signs, medical history, physical examination, serum/urine biochemistry and hematology
- No findings in ultrasound of the liver indicative for liver disease

Test Product, Dose and Mode of Administration, Batch Number:

Ademetionine 1,4-butanedisulfonate, 1000-mg, 1500-mg, or 2000-mg as 500-mg tablets in the morning and before dinner, batch number 28444TF01

Duration of Treatment:

6 weeks

Reference Therapy, Dose and Mode of Administration, Batch Number:

Not applicable

Criteria for Evaluation

Efficacy:

Methionine Tolerance Test

NASH subjects

The first methionine tolerance test was performed within 1 week after randomization. Study drug intake started after completion of the test. A second methionine tolerance test was performed after 6 weeks of treatment.

Healthy volunteers

The healthy control group performed only the initial methionine tolerance test and the initial methionine breath test.

^{13}C-Methionine Breath Test

One day after the methionine loading test, at the start (healthy volunteers) and at the end of the trial (NASH subjects), subjects performed the methionine breath test following 8 hours of fasting. To measure the proportion of the metabolized substrate, the results were expressed as percentage dose of ^{13}C recovered (PDR) over time for each time interval and cumulative PDR (cPDR1.5h) after 30-, 60- and 90- minute test time. Study drug intake started the same day after finishing the initial breath test (NASH subjects) with the evening dose.

Hepatic Panel

Serum total bilirubin, serum conjugated bilirubin, liver- alkaline phosphatase, ALT, AST, GGT, ALT/AST ratio

Metabolic Panel

Fasting lipid profile (cholesterol, high density lipoprotein, low density lipoprotein), amino acid profile, homeostasis model assessment, fasting plasma insulin, fasting glucose and glycosylated hemoglobin as well as adiponectin

Immunological/Antioxidant Panel

C-reactive protein, cytokine profile (IL-6, IL-8, IL-10, TNF-α, MCP-1, and G-CSF), glutathione in erythrocytes, oxidative stress marker (isoprostane level)

Fibrosis and Apoptosis Markers

- Change in caspase-cleaved cytokeratin 18 (M30) levels as compared to baseline
- Change in hyaluronic acid levels as compared to baseline
- Change in ActiTest/Fibrotest (score calculated, from the results of a 6-parameter blood test, combining 6 serum markers with the age and gender of the subject: α-2-macroglobulin,

haptoglobin, apolipoprotein A1, gamma-glutamyl transpeptidase, total bilirubin, and ALT) as compared to baseline

Clinical Global Impression

- Change in clinical global impression scores as compared to baseline, subjects answered 2 questions related to severity of illness and treatment effect

Quality of Life/Health Economics

- Change in health-related quality of life (EQ/5D) measured at the end of treatment and compared to baseline

Safety:

Adverse Events

Each subject was evaluated from screening through the safety follow-up telephone call and in the event of premature study termination.

Laboratory

Hematology and biochemistry data were collected for the determination of hematology, biochemistry, and urinalysis parameters.

Vital Signs

Height and weight were recorded. Body mass index was calculated and waist/hip circumference as well as waist-to-hip ratio were measured.

Systolic blood pressure, diastolic blood pressure and pulse rate were to be measured while the subject was in sitting position after 5 minutes of rest.

Physical Examination

A physical examination was performed.

Statistical Methods:

There were 6 subject samples (all subjects consented, all subjects allocated to treatment, safety, full analysis [FA], per protocol, and healthy volunteer subject sample). The subjects receiving no treatment were handled as a placebo control arm and were included in the safety subject sample, despite not receiving study drug.

Sample Size

A sample size of 23 in each group had a 90% power to detect a treatment effect of 20% from 4.2 hours of methionine half-life and assuming a standard deviation of 0.8 hours using a two group t-test with a 0.05 two-sided alpha level. With a 20% drop-out rate the sample size per treatment group was approximately 30 subjects. No adjustment for multiplicity was performed.

Primary Efficacy Analysis

The primary efficacy variable was methionine elimination half-life at Week 7. This was assessed by analysis of covariance (ANCOVA) on the FA subject sample. The independent variable was the response at Week 7 and the model included the main fixed effects, intercept, treatment and country, with baseline value as a covariate. Comparisons between SAMe treatment groups and the no treatment group were performed within the ANCOVA model as contrasts. As a sensitivity analysis, methionine half-life was also assessed using the PP subject

sample.

Secondary Efficacy Analysis

Methionine tolerance test

The secondary efficacy parameters of this test were the fasting methionine concentration, AUC of methionine concentration versus time curve, the metabolic clearance rate and volume of distribution measured in the blood. The responses for these parameters at Week 7 were analyzed via ANCOVA based on the FA subject sample. Fasting concentration and AUC were log(e) transformed for this analysis.

Summary statistics were produced for the FA subject sample at baseline and Week 7. In addition, summary statistics at baseline were presented for the HV population.

^{13}C-Methionine Breath Test

The parameters, cumulative percentage dose recovered at 30, 60, and 90 minutes (cPDR30, cPDR60, cPDR90) were evaluated. In addition, peak and time to peak were determined.

For the FA subject sample cPDR30, cPDR60, and cPDR90 for ^{13}C-methionine breath test at Week 7 were assessed by ANCOVA based on the raw values. Values for peak were log(e) transformed. Time to peak was evaluated via Wilcoxon tests and summarized using median, minimum, maximum, and interquartile range.

Clinical Laboratory Evaluation

Hepatic laboratory measurements, metabolic parameters, immunological/antioxidant panel parameters, fibrosis and apoptosis markers

For the hepatic, metabolic, immunological/antioxidant panels and the fibrosis and apoptosis markers summary statistics for Week 7, baseline and change from baseline were presented.

Clinical Global Impression

For each category within each of the CGI scores, the number and percentage were produced. In addition, the number of missing values was presented. For severity of illness, a shift table was produced with baseline response represented by the rows and Week 7 response via the columns.

Quality of Life

Quality of life was assessed via the EQ/5D questionnaire. The questionnaire comprised 5 questions rating the level of debility in the following areas: Mobility, Self Care, Usual Activities, Pain/ Discomfort and Anxiety/ Depression. For the total health score summary statistics including 95% confidence intervals were produced for Week 7, baseline and change from baseline. The Week 7 total health score was analyzed using ANCOVA in the same fashion as for the primary efficacy analysis.

Other Efficacy Analysis

In addition to the primary efficacy analysis considering only main effects, 2 additional ANCOVAs were performed to assess the homogeneity of treatment response across baseline methionine half-life and country.

Summary - Conclusions

Efficacy Results:

The following conclusions are based on the results of efficacy analyses:

- For the primary efficacy endpoint of methionine elimination half-life at Week 7, there was no significant difference between the 1000-mg, 1500-mg, and 2000-mg treatment groups when compared to the no treatment group (the 95% CI for the difference in the LS means between the no treatment group and each of the treatment groups contained 0). The results from the sensitivity analysis supported the primary efficacy analysis.
- For the secondary efficacy endpoint of fasting methionine concentration and AUC of average methionine concentration versus time curve, the metabolic clearance rate and volume of distribution measured in blood between the 1000-mg, 1500-mg, and 2000-mg treatment groups there was no statistically significant difference when compared to the no treatment group. However, there was a trend towards decreased mean AUC values from baseline to Week 7 in the SAMe 1000-mg and 1500-mg groups.
- The healthy volunteer results for the methionine tolerance test and ^{13}C-methionine breath test were different from the NASH subject results and therefore served as a control group.
- For the secondary endpoint of cPDR30, cPDR60, and cPDR90, peak, and time to peak, there were no significant differences across the SAMe treatments when compared to the no treatment group. However, there was a trend toward increased mean cPDR90 values from baseline to Week 7 in the SAMe 1000-mg and 1500-mg groups.
- For the secondary endpoint of the effect of the different doses of SAMe on the hepatic, metabolic, immunologic/antioxidant, and fibrosis/apoptosis panel, the laboratory measurements at baseline and Week 7 were similar among the subject samples, with low changes from baseline to Week 7 values.
- For the secondary endpoints of the efficacy of different doses of SAMe CGI and EQ/5D, no notable findings were observed.

Safety Results:

The following conclusions are based on the results of safety analyses:

- The mean (SD) treatment duration was 43.3 (2.8), 42.0 (8.6), and 42.7 (4.0) days in the SAMe 1000-group, SAMe 1500-mg group, and SAMe 2000-mg group, respectively.
- No subjects died during the study.
- There were no TESAEs reported during the study. One subject in the no treatment group reported a total of 2 SAEs (head injury and neck injury) during the study, which began before the first treatment visit.
- There were no TEAEs leading to study termination reported during the study.
- Overall, between 33.3% and 41.7% of subjects per treatment group and 14.3% in the no treatment group reported at least 1 TEAE. No subjects had TEAEs considered severe in intensity by the investigator and the majority of TEAEs were considered mild in severity.

- The proportion of subjects with treatment-related TEAEs was higher in the SAMe 2000-mg (33.3%) compared with the other treatment groups (11.1% in the SAMe 1000-mg group, 14.8% in the SAMe 1500-mg group, and 3.6% in the no treatment group).
- The TEAEs of diarrhea, abdominal pain, headache, and fatigue were experienced by at least 5% of subjects in any treatment group. The TEAEs of diarrhea, abdominal pain, and headache were expected based on previous experience with the study drug in different patient populations.
- No notable trends were observed over time or between treatment group for laboratory values or vital sign measurements.

Conclusion:

For the primary efficacy endpoint of methionine elimination half-life at Week 7, there was no significant difference between the 1000-mg, 1500-mg, and 2000-mg treatment groups when compared to the no treatment group. The 1000-mg, 1500-mg, and 2000-mg treatments administered over 7 weeks were safe and well tolerated in subjects with NASH, and no notable differences were observed in the safety variables between the treatment groups and the no treatment group.