Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Phase 2, Randomized Controlled Trial of Berberine Ursodeoxycholate (BUDCA) in Patients with Presumed Non-Alcoholic Steatohepatitis (NASH) and Type 2 Diabetes

Adrian Di Bisceglie (adibisceglie@hightidetx.com)
HighTide Therapeutics https://orcid.org/0000-0002-6075-9305
Stephen Harrison
Pinnacle Clinical Research
Nadege Gunn
Pinnacle Clinical Research
Anita Kohli
Arizona Liver Health
Liping Liu
HighTide Therapeutics
Abbey Flyer
Pacific Northwest Statistical Consulting
Lawrence Goldkind
Uniformed Services University of Health Sciences

Article

Keywords: Progressive Liver Disease, Fatty Liver Disease, MRI Proton Density Fat Fraction, Glycemic Control, Serum Lipid Levels

Posted Date: January 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-115290/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Nature Communications on September 17th, 2021. See the published version at https://doi.org/10.1038/s41467-021-25701-5.

Abstract

Background & Aims: Non-alcoholic steatohepatitis (NASH) is frequently associated with obesity and diabetes and may lead to progressive liver disease although current treatment options are limited. Berberine ursodeoxycholate is an ionic salt of berberine and ursodeoxycholic acid, representing a new molecular entity that offers the possibility of combination therapy for NASH in a single treatment.

Methods: A prospective, randomized, double-blind, placebo-controlled trial of two doses of berberine ursodeoxycholate administered orally was conducted in a cohort of 100 subjects with fatty liver disease and diabetes. Treatment was for 18 weeks and endpoints measured included reduction in liver fat content measured by MRI proton density fat fraction, improvement in glycemic control, changes in liver-associated enzymes, safety and tolerability.

Results: Subjects that received 1000 mg twice a day of berberine ursodeoxycholate had significantly greater reduction in liver fat content compared to placebo (mean absolute decrease -4.8% vs. -2.0% [p=0.011], mean relative decrease -24.1 vs -8.3% [p=0.016]). Also, compared to placebo, subjects receiving this dose also experienced significant improvement in glycemic control as well as reductions in serum alanine aminotransferase and gamma glutamyl transferase activities. Serum lipid levels decreased modestly during therapy. The higher dose of berberine ursodeoxycholate was associated with an average weight loss (LS Mean) of -3.5kg compared to only -1.1kg with placebo (p=0.012). Diarrhea and abdominal discomfort were the most frequently reported adverse events.

Conclusions: Berberine ursodeoxycholate is single molecule with a broad spectrum of metabolic activity in patients with presumed NASH and diabetes. It is relatively well tolerated and data from this phase 2 randomized controlled trial supportr its further development as a treatment for NASH with diabetes.

Lay Summary

This phase 2 clinical trial tested the effect of berberine ursodeoxycholate in patients with fatty liver disease and diabetes. The group taking the higher dose of this new drug had significant reductions in the amount of fat in their liver as well as improvement in their diabetes. Berberine ursodeoxycholate deserves further exploration as a treatment of non-alcoholic steatohepatitis (NASH).

Introduction

Non-alcoholic steatohepatitis (NASH) is a necroinflammatory disease of the liver which may lead to fibrosis and possible progression to cirrhosis. NASH is often associated with obesity, diabetes, hypertension and hyperlipidemia (1). There are currently no approved therapies for this condition, although weight loss may result in improvements in the degree of liver injury, including hepatic fibrosis (2). Unfortunately, weight loss is very difficult for many individuals to attain and maintain, so there remains a need for a drug therapy for NASH. Ideally, an adjuvant therapy to lifestyle change would not

only improve NASH histopathology, but would also have a meaningful impact on the co-morbidities associated with NASH (1).

Berberine ursodeoxycholate (BUDCA) is an ionic salt of berberine and ursodeoxycholic acid, representing a new molecular entity that offers the possibility of combination therapy for NASH and some of its comorbidities in a single treatment (3). It can be administered orally and has been shown in animal models to improve fatty liver disease (4). The aim of this study was to determine the effect of two different doses of BUDCA on liver fat content (LFC) in patients with diabetes and presumed NASH when administered for 18 weeks.

Methods

Patients with diabetes and presumed NASH were randomized, using permuted blocks without additional stratification, into one of three treatment groups, in a 1:1:1 ratio as follows: 1) BUDCA 500mg BID 2) 1000mg BID and 3) matching placebo. Through the use of a blinded randomization number, subjects and investigators were isolated from the knowledge of treatment assignment. The randomization allocation sequence was created by PharPoint Research (Wilmington, Nc) within SAS (v9.4) using proc plan. The design was a simple randomization with a block size of 3, with 50 blocks generated for a total of 150 numbers. The numbering sequence, and similarly generated scrambled kit list, were then implemented by a central interactive web response system (IWRS) managed by Medidata Solutions (new York, NY). All subjects and study personnel, except for the unblinded statistician, the IWRS vendor and the study drug packaging supplier, remained blinded to the treatment administered throughout the study. All study drugs were provided in matching white tablets, 4 tablets to a pouch, 2 pouches to be administered each day.

Presumed NASH was defined based largely on magnetic resonance imaging derived proton density fat fraction (MRI-PDFF) of 10% or more (5). Additional inclusion criteria further enriched the study population for having NASH by virtue of requiring corrected T1 (cT1) of more than 830 milliseconds and elevated serum aspartate aminotransferase (AST) $\geq$ 20 units per liter (6). Subjects had to be overweight or obese with body mass index of 25 kg/m^2 or more.

Study subjects were recruited at specialized liver centers around the United States between January and October 2019. They underwent an initial screening evaluation, including measurement of liver fat content (LFC) by MRI-PDFF. Subjects were excluded if they had known liver disease other than fatty liver disease or a history of excessive alcohol consumption. Although liver biopsies were not done as a part of this study, subjects with clear evidence of cirrhosis were excluded, based upon a platelet count of less than 150,000/mm^3, serum albumin levels less than 3.2 mg/dL or a current or previous history of clinical hepatic decompensation.

Subjects were permitted to continue their current treatment regimen for diabetes provided they had been on a stable dose regimen for at least 90 days. The treatment duration was for 18 weeks and study

subjects were seen at intervals of 2 to 4 weeks throughout. LFC was measured at baseline and after 18 weeks of therapy. In addition, changes in liver chemistry tests, measures of glycemic control and serum lipid levels were also assessed during and at the end of treatment.

The study was registered at clinicaltrials.gov (Identifier: NCT03656744). The protocol for this study was approved centrally by the WCG Institutional Review Board (WCG IRB, Payallup, Wa). All subjects gave written, informed consent for their participation.

A pre-planned interim analysis was carried out as outlined in the protocol after 51 subjects had completed the assessment of the primary efficacy endpoint to assess sample size assumptions and futility through conditional power. This interim analysis was conducted by an independent, unblinded statistician. The study was allowed to continue.

Statistical methods: Assuming a standard deviation of 6.3% for changes from Baseline in LFC, 35 subjects in each treatment group provided 90% power to show a difference of 5 percentage points between any 2 treatment groups at the 5% level of significance. To allow for a dropout rate of 10%, up to 39 subjects could have been randomized to each of the 3 treatment groups. The achieved sample size was 33 to 34 subjects per arm.

Descriptive Statistics were used to summarize continuous data, and frequencies and percentages were used to summarize categorical data. The Safety Set consisted of all subjects who received at least one dose of study treatment. The Modified Efficacy Set is a subset of the Safety Set consisting of subjects that had at least one post-dose MRI-PDFF assessment. The Efficacy Set is a subset of the Modified Efficacy Set consisting of subjects that completed at least 80 days of study drug dosing and had a week 18 or Early Termination (ET) visit MRI-PDFF assessment. The primary endpoint was an absolute change from Baseline to Week 18 (or ET) in Liver Fat Content. Primary Endpoint summary measures were provided in both the Efficacy and Modified Efficacy sets, with the Efficacy Set prespecified as the primary analysis set. All other endpoints were prespecified to use the Modified Efficacy Set. Comparisons between active treatment groups relative to placebo were tested using Analysis of Covariance (ANCOVA) with treatment group as a fixed effect and baseline value of the parameters of interest as covariates. ANCOVA based LS Means and raw means have been reported. For statistical analysis of laboratory parameters (i.e. p-values), multiple imputation was used to take missing data into account. A two-sided alpha level of 0.05 without adjustment for multiple tests was used to indicate statistical significance. Analyses were performed using SAS System version 9.4.

Results

There were 101 patients enrolled in this study. One did not meet entry criteria and was not dosed (see CONSORT diagram). Of the remaining 100 subjects, their mean age was 56 years (range 26 to 75) and they included 72 females. Most of the subjects (91%) were white and 38% were of Hispanic ethnicity. Although all patients had a history of diabetes, the mean HbA1c level of those who enrolled was 7.1% (range 5.1 to 9.4%). Of note, 94 of 100 subjects were taking medication(s) for the treatment of

their diabetes, often two or more agents at the same time (Supplemental Table 1). In addition, many were also taking lipid-lowering therapy as well (Supplemental table 1). Findings from multiparametric MRI showed that the mean liver fat content of the subjects was 19.4% (range 9.0 to 43.5%) and mean corrected T1 was 940 (range 802 to 1412). The demographic and baseline characteristics of the subjects was evenly distributed across the three dosing groups (Table 1).

Table 2 summarizes the changes seen with therapy, according to treatment group. In general, best treatment responses were seen with the higher dose of BUDCA (1000mg BID). On average, absolute LFC decreased by 4.8% in this high dose group, compared to only 2.0% with placebo (p=0.011). The relative decrease in LFC in this group was 24.1%, compared to 8.3% with placebo (p=0.016). While there was an apparent dose response with regard to the proportion achieving at least a 5% absolute decrease or a 30% relative decrease in LFC, these changes were not statistically significant. Other biochemical parameters associated with NASH and NAFLD also improved on therapy with this dose, as noted by significant decreases in serum alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) levels.

Notably, significant improvements were also seen in glycemic control with BUDCA therapy. Mean HbA1c levels decreased by 0.6% in the 1000mg BID group and 0.3% in the 500mg BID group compared to an increase of 0.1% in the placebo group. No significant changes were noted in levels of blood glucose, insulin or HOMA-IR.

Favorable decreases were also noted in plasma lipid and lipoprotein levels. Levels of LDL cholesterol decreased by an average of 16 mg/dL in the high dose group (p=0.072) and triglyceride levels decreased by 41 mg/dL in the 500mg BID dose group (p=0.04), although not with the higher dose. There was no significant change in levels of HDL cholesterol. Finally, subjects receiving the higher dose lost an average of 3.5 kg in weight (p=0.012). Figure 2 shows the decreases in liver fat content, HbA1c and body weight in individual subjects.

Changes in serum levels of bile acids were noted (Supplementary Table 2) and were consistent with changes expected to occur with UDCA therapy. Table 1 shows these changes from baseline in the highest dose group (1000mg BID). While there was an increase in the total bile acids, levels of primary and secondary bile acids and their metabolites decreased, but a dramatic increase was noted in the levels of urso- bile acid and their metabolites. There was no significant change in levels of fibroblast growth factor-19 (FGF19) or serum fibrosis markers, including ELF and Pro-C3.

BUDCA therapy was generally well tolerated. Table 3 shows that the most frequent adverse event occurring during therapy was diarrhea while some subjects reported symptoms of gastrointestinal reflux or nausea. A similar number of subjects receiving either placebo or BUDCA reported having headaches. These adverse events were generally mild (grade 1 or 2, using CTCAE criteria). Only nine subjects had to discontinue BUDCA related to adverse events compared to only one on placebo. Three serious adverse events occurred during the conduct of this study, one in a placebo recipient (bladder cancer) and two on BUDCA, neither of which were attributed to study drug (one patient in the 500mg BID group experienced a

complication of low blood oxygen following elective shoulder surgery with an intercostal nerve block and the other, taking 1000mg BID, had an acute myocardial infarction).

Discussion

This phase 2, randomized, placebo-controlled trial has shown that BUDCA is very effective in decreasing LFC in patients with presumed NASH and diabetes, as assessed by MRI-PDFF. Furthermore, BUDCA is also effective in improving glycemic control, measured by reductions in blood levels of HbA1c. In addition to these key benefits, improvements were seen in levels of liver-associated enzymes (ALT and GGT) as well as lipid levels (LDL-c and triglycerides).

The use of non-invasive tests such as MRI is now well established in early trials of novel agents to treat NASH (5,6). Although liver biopsy was not a part of this study, the inclusion criteria allowed for an enrichment of the study population for NASH. All subjects had to have at least 10% LFC by MRI-PDFF and in addition they had to have corrected T1 values on MRI at baseline of at least 830 msec. Furthermore, subjects were all diabetic and were required to have serum AST values of at least 20 U/L – both of these are risk factors for NASH among individuals known to have NAFLD.

Each of the two parent compounds of BUDCA (berberine and ursodeoxycholic acid) are likely to have contributed to the beneficial effects seen in this patient population. Berberine is known to improve glycemic control and is commonly used as an over-the-counter remedy for diabetes and pre-diabetes (7,8). Berberine has been shown to inhibit a-glucosidase *in vitro* and in animal models and this mechanism of action is likely to have contributed to the improvement seen in glycemic control (9,10). The structural changes induced by berberine in the gut microbiome also have an effect on insulin resistance (11). It is worthy of note that although their diabetes was relatively well controlled, subjects enrolled in this study were heavily treated with other agents both before and during the study period, most commonly metformin, insulin and GLP-1 agonists. Despite this intensive treatment, BUDCA provided significant further reductions in HbA1c levels. Berberine has previously been shown to reduce cholesterol levels (total and LDL-c) and this finding was confirmed in the present study. Berberine acts by increasing the clearance of cholesterol by inducing the LDL receptor (12). Although significant reductions were noted in triglyceride levels, the mechanism by which this occurs is not clear.

UDCA is not known to have any significant anti-diabetic activity, but does decrease serum cholesterol levels by decreasing cholesterol production (13). On the other hand, UDCA is well known to have substantial hepatoprotective effects (14). For instance, in cholestatic diseases such as PBC, UDCA acts as a choleretic agent, increasing the flow of bile and also decreases the presence of toxic bile acids that accumulate in cholestasis (15,16). Both berberine and UDCA appear to have anti-inflammatory effects, consistent with the reductions seen in serum levels of ALT and GGT (17,18) although these liver enzymes may have decreased simply because of the decrease in liver fat. Furthermore, both are able to modulate the gut microbiome in a favorable way (11,19) and the microbiome has been found to play a role in pathogenesis of NASH and obesity, related to diabetes. (20,21).

Although BUDCA is a single molecule (a new molecular entity), it is thought to dissociate into derivatives of its parent compounds in the gastrointestinal tract, each of which is differentially absorbed. BUDCA is formed as a salt from equimolar amounts of berberine and UDCA. After the administration of BUDCA, increased levels of UDCA are noted in serum within about 2 hours and persist for 7 hours on average. Levels of UDCA are about 1000 times higher in serum than berberine, on a molar basis, although animal studies have found accumulation of berberine within the liver (data on file). The complex tertiary structure of the BUDCA salt appears to increase the bioavailability of berberine, both locally in the GI tract and systemically, perhaps accounting for the potent effect on glycemic control (data on file).

This study found that the use of BUDCA is also associated with significant weight loss (an average of 3.5kg in the group dosed with 1000mg BID). The mechanism of this weight loss is not clear. It is also uncertain how closely related the improvements in features of NASH are with weight loss. Several other agents currently being tested for NASH are also associated with weight loss (e.g. liraglutide) (22).

BUDCA was relatively well tolerated. GI side effects including diarrhea and abdominal discomfort were the most frequently reported adverse event and necessitated discontinuation of study drug in about 12% of subjects. UDCA itself is associated with diarrhea and other GI complaints (23). Berberine too can cause GI symptoms, possibly via its inhibition of a-glucosidase, as occurs with some other anti-diabetes agents such as acarbose and miglitol (24). Metformin too frequently causes GI upset (25). The one serious adverse event occurring in the BUDCA high dose group was acute myocardial infarction thought not to be related to study drug. It is well known that patients with NASH have substantially increased risk of coronary artery disease and cardiovascular complications are the most frequent cause of death among patients with NASH (26).

The use of BUDCA is associated not only with improvement in features of NASH (LFC, ALT, GGT) but also in other metabolic parameters including Hb1Ac and serum lipid levels. In contrast, some other agents being tested as treatment for NASH may increase serum levels of cholesterol, often requiring concomitant therapy with an HMG CoA Reductase inhibitor (a “statin”) (e.g. obeticholic acid). Other agents (such as pioglitazone) may contribute to weight gain, whereas BUDCA is associated with weight loss (27). Finally, it should be noted that BUDCA is orally administered whereas some other new agents require regular subcutaneous injection or even intravenous infusion (22,28).

Two doses of BUDCA were tested in this study. While a dose-dependent effect was noted with some parameters, in general the 500mg BID dose was less effective than the 1000mg BID dose. The adverse event profile was minimal and dose dependent and the drug was generally well tolerated.

In summary, BUDCA is a single molecule with a broad spectrum of metabolic activity in patients with fatty liver disease and presumed NASH. It is orally administered and relatively well tolerated. We conclude that data from this phase 2 randomized controlled trial support further development of BUDCA as a treatment for NASH in patients with diabetes and other features of the metabolic syndrome and we anticipater that the reduction in LFC will correlate with histopathologic improvement in future studies.

Abbreviations

BUDCA: berberine ursodeoxycholate

UDCA: ursodeoxycholic acid

NASH: non-alcoholic steatohepatitis

Declarations

Data availability

Any requests for raw data (liver fat content, hemoglobin A1c levels, serum lipid levels and de-identified patient characteristics) or the full trial protocol will be reviewed by the study sponsor in consultation with the authors. Only data requests for non-commercial use will be considered and should be sent to the corresponding author (A.M.D.). Requests should outline how the specific use of the data would catalyze considerable advancement in the treatment and management of fatty liver disease, including the specific purpose of developing therapies and technology that can be used by and for patients to help manage their disease and improve their health outcomes. Any data that can be shared will need approval from the the study sponsor (HighTide Therapeutics) and a Material Transfer Agreement will need to be in place. All data shared will be de-identified.

Code availability

The treatment code cannot be made available due to proprietary reasons.

Acknowledgements

The authors thank the investigators listed below who recruited subjects into this study:

Arun Khazanchi, Florida Research Institute, Lakewood Ranch, FL; Ziad Younes, Gastro One, Germantown, TN; Bradley Freilich, Kansas City Research Institute, Kansas City, Mo; Seth Baum, Excel Medical Clinical Trials, Boca Raton, FL; Judith White, Bioclinica, Orlando, FL; Richard Manch, Institute for Liver Health, Tucson, AZ; Sam Moussa, Adobe Clinical Research, Tucson, AZ; Donald Lazaz, Digestive Health Research Hermitage, TN; Robert Jenders, National Research Institute, Panorama City, CA; John Poulos, Cumberland Research Associates, Fayetteville, NC; Rashmee Patil, DHR Health Transplant Institute and Liver Specialty Center, Edinburg, TX; James Trotter, Texas Digestive Disease Center Dallas, TX.

Conflict of interest statements

The authors listed below have the following outside financial interests

SAH: Reports grant/research support from Axcella, BMS, Cirius, CiVi, Conatus, Cymabay, Enyo, Galectin, Galmed, Genentech, Genfit, Gilead, Hepion, Hightide, Intercept, Inventiva, Madrigal, NGM Bio, Northsea,

Novartis, Novo Nordisk, Pfizer, Sagimet, and Viking. Consulting fees from Akero, Altimmune, Arrowhead, Axcella, Canfite, Cirius, CiVi, CLDF, Cymabay, , Echosens, Fibronostics, Foresite Labs, Galectin, Genfit, Gilead, Hepion, Hightide, HistoIndex, Intercept, Kowa, Madrigal, Metacrine, NGM Bio, Novartis, Novo Nordisk, Poxel, Ridgeline, Sagimet, Terns and Viking.

NG: Research grants: Axcella, BMC, CymaBay, Genetech, Genfit, Gilead, High Tide, Madrigal, North Sea, NGM Bio, Novo Nordisk; Consultant/Speaker: AbbVie, Dova, Gilead, Intercept, Salix.

GN: Grants: Ecosens, Intercept. Consultant/Speaker: Gilead, Intercept, Salix.

AK: Grant/research support from Axcella, BMS, Cirius, Conatus, Cymabay, Enyo, Galectin, Genentech, Genfit, Gilead, Hightide, Intercept, Madrigal, NGM Bio, Northsea, Novartis, Novo Nordisk, Pfizer, Poxel and Viking. Consulting fees from Intercept and Gilead

LL: employee of HighTide Therapeutics

AF: independent contractor to HighTide

LG: is a consultant to HighTide

AMD: is a consultant to HighTide and Chief Medical Officer

Financial support statement

The conduct of this study was fully supported by HighTide Therapuetics

Authors contributions

SAH: principal investigator for the study, assisted with study design, data analysis and interpretation, reviewed and edited first draft of manuscript

NG, GC, AK: investigator, recruited patients, reviewed manuscript

LL: assisted with study design and data interpretation, reviewed manuscript

AF: biostatistician, data analysis, reviewed manuscript

AMD: oversaw conduct of the study, data analysis and interpretation, wrote first draft of manuscript

Tables

Table 1: Baseline Characteristics

	Treatment Group		
	Placebo N=33	500mg BID N=33	1000mg BID N=34
Age (years)			
N	33	33	34
Mean (SD)	58 (10.7)	58 (10.2)	53 (12.2)
Min, Max	40, 75	26, 75	27, 72
Sex – n (%)			
Male	11 (33%)	7 (21%)	10 (29%)
Female	22 (67%)	26 (79%)	24 (71%)
Race – n (%)			
White	31 (94%)	29 (88%)	31 (91%)
Black	0	3 (9%)	2 (6%)
Other	2 (6%)	1 (3%)	1 (3%)
Ethnicity – n (%)			
Hispanic or Latino	13 (39%)	14 (42%)	11 (32%)
Not Hispanic or Latino	20 (61%)	19 (58%)	23 (68%)
Weight (kg)			
N	33	33	34
Mean (SD)	97.5 (22.57)	98.4 (23.05)	101.2 (20.26)
Min, Max	70.8, 180	64.9, 154.1	71.7, 159.2
BMI (kg/m^2)			
N	33	33	34
Mean (SD)	35.0 (6.18)	36.7 (6.88)	36.3 (6.28)

	Treatment Group		
	Placebo N=33	500mg BID N=33	1000mg BID N=34
Min, Max	25.4, 55.6	26.1, 55.9	25.4, 49.1
Liver fat content (%)*			
N	32	33	34
Mean (SD)	20.2 (6.23)	18.4 (6.24)	19.4 (6.96)
Min, Max	11.1, 41.7	11.0, 43.48	9.0, 33.7
Corrected T1*			
N	32	32	32
Mean (SD)	940.8 (99.27)	948.8 (119.53)	932.4 (80.06)
Min, Max	834.0, 1317.0	802.5, 1412.0	837.5, 1122.0
ALT (U/L)			
N	33	33	34
Mean (SD)	54 (26.7)	46 (27.6)	62 (31.8)
Median	47	39	55
Min, Max	17, 115	3, 141	24, 143
AST (U/L)			
N	33	33	34
Mean (SD)	38 (17.3)	36 (15.9)	45 (29.7)
Median	32	31	37
Min, Max	19, 88	15, 80	19, 147
GGT (U/L)			
N	33	33	34

	Treatment Group		
	Placebo N=33	500mg BID N=33	1000mg BID N=34
Mean (SD)	70 (105.1)	64 (50.7)	68 (57.2)
Median	38	39	45
Min, Max	19, 618	18, 223	19, 263
HbA1c (%)			
N	33	33	34
Mean (SD)	7.0 (1.05)	6.9 (0.85)	7.3 (1.16)
Median	6.9	6.9	7.5
Min, Max	5.3, 9.4	5.5, 9.1	5.1, 9.3
LDL cholesterol (mg/dL)**			
N	33	30	29
Mean (SD)	99 (35.8)	86 (29.4)	107 (35.3)
Median	98	81	111
Min, Max	37, 188	26, 150	31, 168
Triglycerides (mg/dL)**			
N	33	31	30
Mean (SD)	197 (83.3)	190 (204.6)	174 (77.1)
Min, Max	76, 423	54, 1217	58, 430
Fasting insulin (IU/mL)			
N	33	33	34
Mean (SD)	45.7 (34.64)	30.8 (15.74)	32.9 (16.43)
Min, Max	6.2, 155.4	9.5, 70.7	9.6, 82.4

	Treatment Group		
	Placebo N=33	500mg BID N=33	1000mg BID N=34
Fasting plasma glucose (mg/dL)			
N	33	33	34
Mean (SD)	136 (44.1)	140 (39.9)	155 (46.3)
Min, Max	94, 313	89, 288	90, 268
*Multiparametric MRI assessment **Modified Efficacy Set			

Table 2: Responses to therapy

	Treatment Group		
	Placebo	500mg BID	1000mg BID
Absolute Change in LFC (%)*			
N	32	30	27
Mean (SD)	-2.0 (4.88)	-2.9 (4.02)	-4.8 (4.35)
Min, Max	-13.9, 6.3	-11.5, 5.9	-14.2, 2.9
LS Mean (SE)	-1.8 (0.74)	-3.2 (0.77)	-4.7 (0.81)
p-value		0.199	0.011
Relative Change in LFC (%)*			
N	32	30	27
Mean (SD)	-8.3 (24.48)	-15.1 (22.78)	-24.1 (21.70)
Min, Max	-58.3, 46.6	-66.4, 35.3	-60.7, 22.1
LS Mean (SE)	-8.2 (4.09)	-15.9 (4.27)	-23.3 (4.53)
p-value		0.196	0.016
Proportion achieving ≥5% Absolute Reduction in LFC*[a]	8/33 (24%)	10/31 (32%)	12/30 (40%)
Proportion achieving ≥30% relative reduction in LFC*[a]	7/33 (21%)	6/31 (19%)	10/30 (33%)
Mean change in HbA1c (%)**			
N	32	29	26
Mean (SD)	0.1 (0.82)	-0.3 (0.68)	-0.6 (0.96)
Min, Max	-1.4, 2.7	-1.8, 1.3	-2.9, 1.6
LS Mean (SE)	0.1 (0.14)	-0.4 (0.15)	-0.5 (0.16)
p-value		0.029	0.005

	Treatment Group		
	Placebo	500mg BID	1000mg BID
Mean change in ALT (U/L)**			
N	32	29	26
Mean (SD)	-3 (19.2)	-4 (17.9)	-19 (27.2)
Min, Max	-50, 47	-33, 52	-89, 18
LS Mean (SE)	-2 (3.5)	-5 (3.7)	-16 (3.8)
p-value		0.674	0.007
Relative change in ALT (%)**			
N	32	29	26
Mean (SD)	-6 (30.5)	-6 (36.0)	-21 (35.2)
Min, Max	-52, 69	-60, 89	-83, 45
Mean change in GGT (U/L)**			
N	32	29	26
Mean (SD)	-2 (34.9)	-19 (26.4)	-30 (47.9)
Min, Max	-120, 124	-104, 21	-213, 18
LS Mean (SE)	-1 (4.6)	-20 (4.8)	-25 (5.0)
p-value		0.005	<0.001
Relative change in GGT (%)**			
N	32	29	26
Mean (SD)	5 (39.7)	-23 (25.8)	-29 (27.1)
Min, Max	-46, 175	-62, 64	-81, 24
Mean change in LDL-c (mg/dL)**			
N	29	27	25
Mean (SD)	0 (20.5)	5 (34.1)	-16 (26.5)

	Treatment Group		
	Placebo	500mg BID	1000mg BID
Min, Max	-54, 48	-33, 147	-103, 31
LS Mean (SE)	1 (5.3)	1 (5.4)	-12 (5.5)
p-value		0.955	0.072
Mean change in Triglycerides (mg/dL)**			
N	32	29	26
Mean (SD)	18 (142.9)	-41 (136.3)	-24 (70.4)
Min, Max	-242, 632	-710, 98	-161, 154
LS Mean (SE)	19 (18.5)	-36 (19.4)	-24 (20.4)
p-value		0.041	0.120
Mean change in Body weight (kg)***			
N	32	29	27
Mean (SD)	-1.1 (2.86)	-1.6 (3.02)	-3.5 (4.77)
Min, Max	-9.1, 3.8	-7.2, 5.9	-18.9, 4.5
LS Mean (SE)	-1.1 (0.64)	-1.6 (0.67)	-3.5 (0.70)
p-value		0.554	0.012

*Primary Endpoint results for Efficacy Set

[a]A Placebo subject had data available at Week 18/ET but did not have baseline data, so they were not included in the absolute and relative change from baseline analyses in the Efficacy Set.

**Modified Efficacy Set

***Safety Set

Note: P-values and LS Means are obtained from an ANCOVA model with treatment group as a fixed effect, and Baseline value of associated parameters as covariates.

-

Table 3: Adverse Events

		Treatment Group		
		Placebo N=33	500mg BID N=33	1000mg BID N=34
Subjects with TEAEs Related to Study Drug reported in two or more subjects in any treatment group	Diarrhea	0	4 (12%)	9 (26%)
	GERD	0	2 (6%)	0
	Nausea	0	1 (3%)	5 (15%)
	Headache	1 (3%)	2 (6%)	1 (3%)
Subjects with TEAEs requiring discontinuation of study drug	Diarrhea	0	0	2 (6%)
	GERD	0	1 (3%)	1 (3%)
	Abdominal distension	0	0	1 (3%)
	Melena	0	0	1 (3%)
	Acute myocardial infarction	0	0	1 (3%)
	Bladder Cancer	1 (3%)	0	0
	Headache and Facial Rash	0	0	2 (6%)

References

1. Cotter TG, Rinella M. Nonalcoholic fatty liver disease 2020: The state of the disease. Gastroenterology 2020:158:1851-1864.
2. Tetri BA. Therapeutic landscape for NAFLD in 2020. Gastroenterology 2020:158:1984-1998.
3. Di Bisceglie AM, Watts GF, Lavin P, Yu M, Bai R, Lu L. A randomized, double blind, placebo-controlled trial of HTD1801 (berberine ursodeoxycholate, BUDC) in patients with hypercholesterolemia: implications for its use in non-alcoholic steatohepatitis (NASH). J Hepatol 2020;73(Suppl 1):S440
4. Liu L, Bai R, Li Y, Yu M, Fu X. HTD1801 A Novel Treatment for NAFLD/NASH and Primary Sclerosing Cholangitis (PSC). Hepatology 2019;70:1261A-1262A.
5. Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, Quantitative Assessment of Liver Fat by MRI-PDFF as an Endpoint in NASH Trials. Hepatology 2018;68:763-772
6. Harrison SA, Dennis A, Fiore MM, Kelly MD, Kelly CJ, et al. Utility and variability of three non-invasive liver fibrosis imaging modalities to evaluate efficacy of GR-MD-02 in subjects with NASH and bridging fibrosis during a phase-2 randomized clinical trial. PLOS ONE 2018;13: e0203054.
7. YF Zhang, Li X, Liu W et al. Treatment of Type 2 Diabetes and Dyslipidemia with the Natural Plant Alkaloid Berberine. J Clin Endocrinol Metab 2008;93:2559-2565.
8. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008;57:712-717.
9. Pan GY, Wang GJ, Sun JG. Inhibitory effect of BBR on glucose absorption. Yao Xue Xue Bao 2003;38:911-914.
10. Jhong C-H, Riyaphan J, Lin S-H, Chia Y-C, Weng C-F. Screening Alpha-Glucosidase and Alpha-Amylase Inhibitors From Natural Compounds by Molecular Docking in Silico. Biofactors 2015;41:242-51.
11. Zhang X, Zhao Y, Zhang M, Pang X, Xu J, et al. Structural Changes of Gut Microbiota during Berberine-Mediated Prevention of Obesity and Insulin. Resistance in High-Fat Diet-Fed Rats. PLoS ONE 2012;7:e42529.
12. Kong W, Wei J, Abidi P et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nature Medicine 2004;10,1344–1351.
13. Poupon RE, Ouguerram K, Chrétien Y, Verneau C, Eschwège E, Magot T, Poupon R. Cholesterol-lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. Hepatology 1993;577-82.
14. Hofmann AF. Pharmacology of ursodeoxycholic acid, an enterohepatic drug. Scan J Gastroenterol 1994;29 Suppl 204:1-15.
15. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology 2019;69:394-419.
16. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology. 2002 Sep;36(3):525-31. doi: 10.1053/jhep.2002.36088. PMID: 12198643.

17. Cicero AF, Baggioni A. Berberine and Its Role in Chronic Disease. Adv Exp Med Biol. 2016;928:27-45. doi: 10.1007/978-3-319-41334-1_2. PMID: 27671811.
18. Bellentani S. Immunomodulating and anti-apoptotic action of ursodeoxycholic acid: where are we and where should we go? Eur J Gastroenterol Hepatol. 2005 Feb;17(2):137-40. doi: 10.1097/00042737-200502000-00001. PMID: 15674088.
19. Kim DJ, Yoon S, Ji SC, Yang J, Kim YK, Lee S, Yu KS, et al. Ursodeoxycholic acid improves liver function via phenylalanine/tyrosine pathway and microbiome remodelling in patients with liver dysfunction. Sci Rep 2018;8:11874.
20. Chen X, Devaraj S. Gut Microbiome in Obesity, Metabolic Syndrome, and Diabetes. Curr Diab Rep. 2018 Oct 18;18(12):129. doi: 10.1007/s11892-018-1104-3. PMID: 30338410.
21. Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. EMBO Mol Med. 2019 Feb;11(2):e9302. doi: 10.15252/emmm.201809302. PMID: 30591521; PMCID: PMC6365925.
22. Ipsen DH, Rolin B, Rakipovski G, et al. Liraglutide Decreases Hepatic Inflammation and Injury in Advanced Lean Non-Alcoholic Steatohepatitis. Basic Clin Pharmacol Toxicol. 2018;123:704-713.
23. Hempfling W, Dilger K, Beuers U. Systematic review: ursodeoxycholic acid — adverse effects and drug interactions. Aliment Pharmacol Ther 2003;18: 963–972.
24. Alpha Glucosidase Inhibitors. In: LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; May 13, 2013.
25. Bonnet F, Scheen A. Understanding and overcoming metformin gastrointestinal intolerance. Diabetes Obes Metab. 2017;19:473-481.
26. Sanyal AJ, Banas C, Sargeant C et al. Similarities and diffefrences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. Hepatology 2006;43:682-689.
27. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362:1675-85.
28. Charles ED, Neuschwander-Tetri BA, Frias JP, et al. Pegbelfermin (BMS-986036), PEGylated FGF21, in Patients with Obesity and Type 2 Diabetes: Results from a Randomized Phase 2 Study. Obesity 2019;27:41-49.

Figures

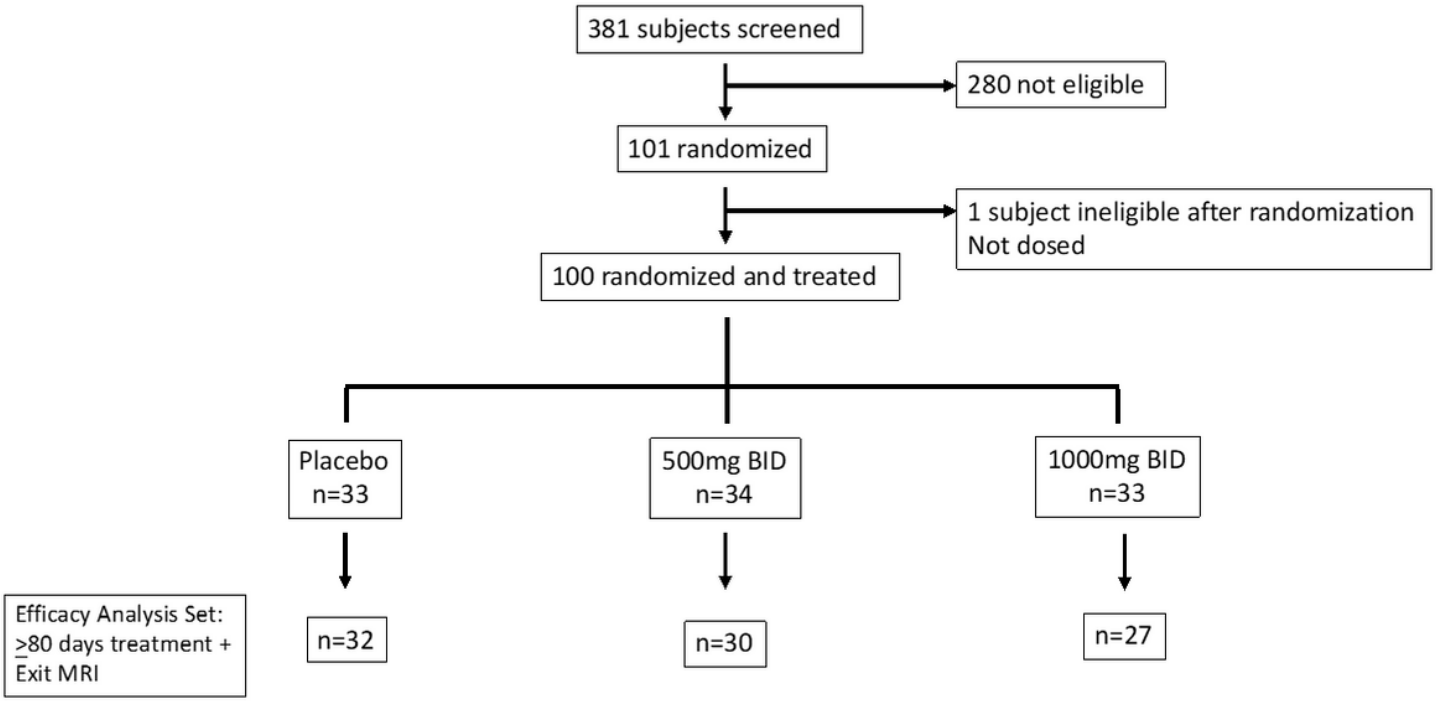

Figure 1

CONSORT Diagram

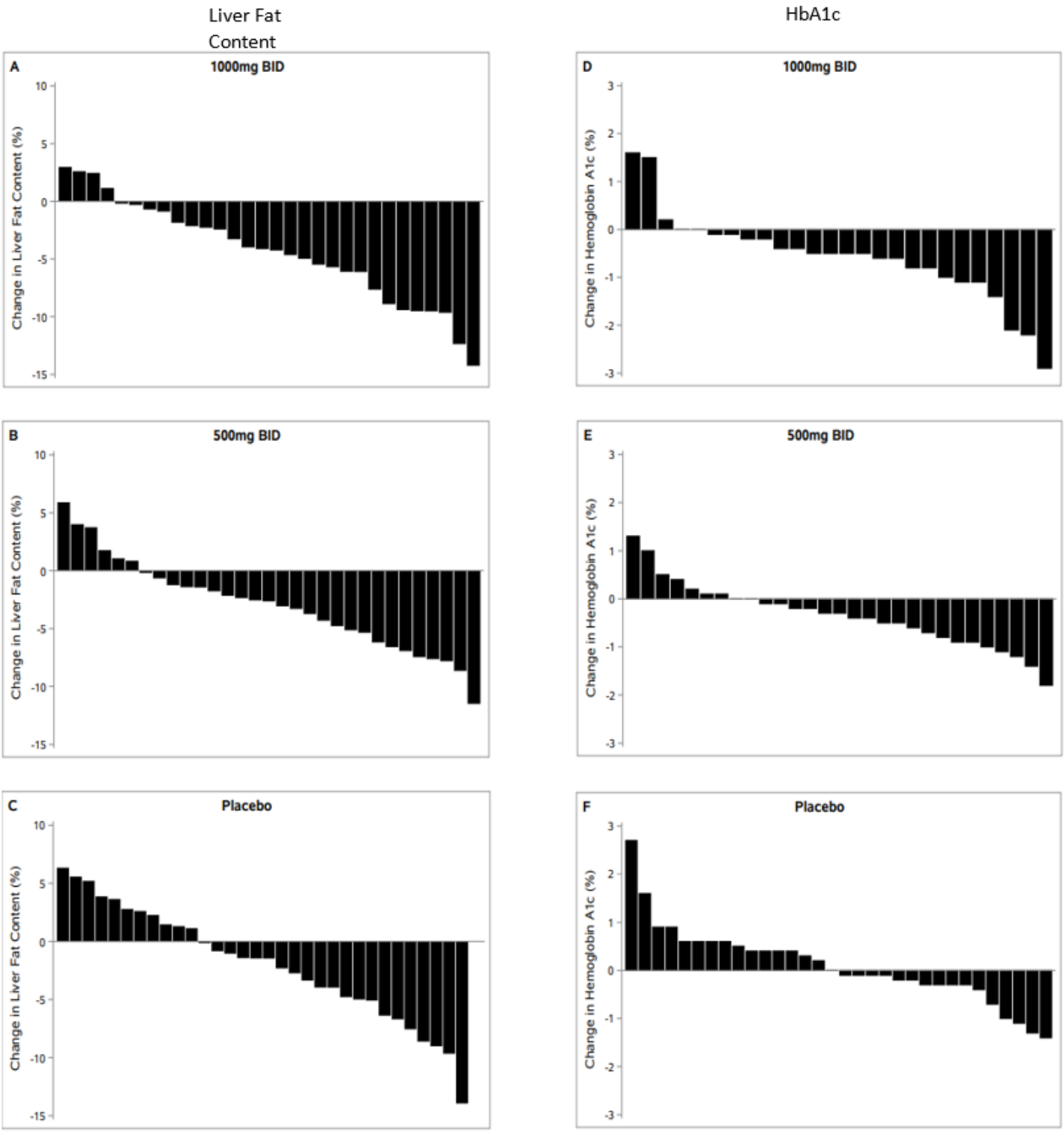

Figure 2

Waterfall plot of change in LFC and HbA1C among subjects receiving BUDCA 1000mg BID vs Placebo

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx