STUDY PROTOCOL & STATISTICAL ANALYSIS PLAN

Official title: Phase 2 Study of Obeticholic Acid for Lipodystrophy patients.

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Title: Phase 2 Study of Obeticholic Acid for Lipodystrophy patients.
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1. Introduction and Purpose:

Lipodystrophies are rare disorders characterized by selective loss of adipose tissue and predisposition to insulin resistance and its metabolic complications (1, 2). Hepatic steatosis is a common complication in patients with partial and generalized lipodystrophies (1,3,4). Advanced cirrhosis resulting in portal hypertension, hepatic encephalopathy, and hepatocellular carcinoma resulting in death (4, 5) or requiring hepatic transplantation (6) have be reported in patients with lipodystrophies possibly due to long-standing hepatic steatosis. Despite aggressive management of diabetes and hyperlipidemia, hepatic steatosis and its complications present a therapeutic challenge in many patients. A recent report of hepatic pathology in 50 patients with various types of lipodystrophies by Safar Zadeh *et al*. from the NIH, NIDDK (5), revealed mild to moderate perisinusoidal and periportal fibrosis (Fibrosis score 1A-1C) in 24%, periportal and perisinusoidal fibrosis (Score 2) in 20%, bridging fibrosis (Score 3) in 26% and cirrhosis (Score 4) in 16%. In some patients, especially those with generalized lipodystrophies, hepatic fibrosis and cirrhosis has been reported as early as during infancy (7) and early childhood (8, 9). In the NIH study (5), 9 out of 10 patients with congenital generalized lipodystrophy (CGL), type 2 (an autosomal recessive disorder), age 8-18 years, had bridging fibrosis or cirrhosis. These data point to the increased prevalence, early onset and severity of nonalcoholic hepatic steatosis and steatohepatitis and its complications in patients with lipodystrophies. Due to this large disease burden, it is important to assess the efficacy and safety of novel therapies for hepatic steatosis in patients with lipodystrophies.

There are, however, no systematic studies evaluating various therapeutic interventions for reducing hepatic steatosis in patients with lipodystrophies. We and others reported efficacy and safety of recombinant leptin therapy in improving hepatic steatosis in severely hypoleptinemic patients with generalized lipodystrophies (5, 10-16). Recently, metreleptin was approved by the FDA for treating metabolic complications in patients with generalized lipodystrophy. However, the reduction of liver volume or hepatic triglyceride (TG) concentrations in response to metreleptin therapy in hypoleptinemic patients with familial partial lipodystrophy of the Dunnigan variety (FPLD), an autosomal dominant disorder due to heterozygous missense mutations in lamin A/C (*LMNA*) gene, has been variable (17, 18). Furthermore, many patients with FPLD are not hypoleptinemic and some may even have normal or high serum leptin levels (see preliminary data). Therefore, there remains a need to develop other therapies for treatment of hepatic steatosis especially for patients with FPLD, many of whom are also predisposed to develop hepatic fibrosis (5).

A variety of drugs have been investigated in nonlipodystrophic patients with non-alcoholic hepatic steatosis and steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD). Most of these patients have obesity and/or type 2 diabetes as a concomitant condition. Previously, ursodeoxycholic acid (UDCA) (19-25), insulin sensitizers (metformin and thiazolidinediones) (26-30), fibrates (gemfibrozil and fenofibrate) (20, 31), vitamin E (30, 32), betaine (33), and probucol (34) have been tried in studies in non-lipodystrophic subjects with NASH or NAFLD. The trials with fibrates have reported inconsistent results in patients with NAFLD (20, 31). As compared to other agents, UDCA has been studied more extensively (19-25). However, a large multicenter trial showed that UDCA is not effective in reducing hepatic steatosis (35). Thus far, only vitamin E has been shown to improve histology in patients with hepatic steatosis (30). However, these results have not yet been replicated and concerns related to an increase in mortality with vitamin E (36) have limited its use.

Recent data support the activation of the farnesoid X receptor (FXR, NR1H4), a nuclear hormone receptor regulated by bile acids, for treatment of NASH and NAFLD (37). FXR activates transcription of several genes particularly the atypical nuclear receptor small heterodimer partner (SHP, NR0B2) and thus can influence triglyceride metabolism within hepatocytes (38). FXR-deficient mice exhibit a hepatic phenotype similar to NASH patients with significant hepatic TG accumulation, hepatic inflammation and injury and development of hepatocellular carcinoma (39, 40).

Both cholic acid (CA) and chenodeoxycholic acid (CDCA) are ligands for FXR, however, UDCA which is the 7 hydroxy β-epimer of CDCA, does not activate FXR (38, 41). Obeticholic acid (OCA) is a first-in-class selective FXR agonist which has approximately 100 fold greater FXR-agonistic activity in the nanomolar range, as compared to CDCA (42, 43). Interestingly, a recent study showed that giving 0.5% CA for 3 weeks reduced hepatic triglyceride content by over 50% in KK-A^{y} mice (a model for diet- induced hypertriglyceridemia) fed chow or high fat diet (44). Furthermore, WAY-362450, another potent, selective and orally active synthetic FXR agonist has been shown to protect against NASH in mice fed a methionine and choline deficient diet (45). This hepatoprotection by WAY-362450 is abolished in FXR-deficient mice, demonstrating the requirement for functional FXR (45).

In apolipoprotein E-deficient mice, OCA resulted in reduced expression of SREBP1c, the master transcription factor that regulates TG synthesis, and reduced hepatic TG and cholesterol content and ameliorated hyperlipidemia (46). OCA treatment further reversed insulin resistance and NAFLD in Zucker fa/fa rats (47). FXR activation alters the hepatic expression of many genes involved in lipid metabolism. While ApoC-I, ApoC-II, Apo-CIV, ApoE, FAS, PPARα are upregulated; ANGPTL3, ApoA-1, ApoC-III, SREBP-1c are downregulated. Increased expression of ApoC-II, a lipoprotein lipase (LPL) activator (48), and reduced expression of angiopoietin-like protein 3 (ANGPTL3) which inhibits LPL activity (44), may increase LPL activity upon FXR activation. All these changes may lead to reduction of hepatic and serum TG upon FXR activation.

In a double-blind, placebo-controlled trial in 64 subjects with Type 2 diabetes and NAFLD, OCA 25 or 50 mg once daily for 6 wk, significantly improved peripheral glucose disposal rates in euglycemic, glucose clamp study as well as alanine aminotransferase and gamma glutamyl transpeptidase levels (49). However, hepatic insulin sensitivity or steatosis was not directly assessed in this trial.

Preliminary data from the Farnesoid X Receptor Ligand Obeticholic Acid in Nonalcoholic Steatohepatitis Treatment (FLINT) trial reveal that OCA significantly improved nonalcoholic steatohepatitis as determined by liver biopsy in patients with NAFLD and the trial has been halted in January 2014 (http://www.interceptpharma.com/nash.php). Even though patients with lipodystrophies were not excluded from the FLINT trial, the likelihood of including enough lipodystrophy patients among 283 participants to provide informative data is very small given the estimated population prevalence of lipodystrophies to be < 1 in a million (1, 2). In a similar trial, Pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis (PIVENS), 247 patients with NASH with a mean ± SD triceps skinfold thickness of 33 ± 13 mm and body fat of 39 ± 9% were recruited. In comparison, our adult patients with FPLD have triceps skinfold thickness of 5.8 ± 2.1 mm (n=59) and body fat of 21.5 ± 5.3% (n=44). Patients with generalized lipodystrophy have much lower body fat than those with FPLD. Thus, there is extremely low likelihood of including lipodystrophy patients in trials of patients with NASH/NAFLD, who are usually obese. Furthermore, there is high likelihood of missing the diagnosis of lipodystrophy, if the investigators were not asked to specifically ascertain it.

It therefore appears that FXR modulation offers interesting therapeutic possibilities in treating hepatic steatosis (37). This study is primarily designed to study efficacy of OCA, a strong FXR ligand, in reducing hepatic triglyceride levels in patients with hepatic steatosis and FPLD. If proven to be effective, it may reduce morbidity and mortality as a result of sequelae of hepatic steatosis in patients with lipodystrophies.

2. Background:

This study is aimed at developing a novel intervention for therapy of severe hepatic steatosis in patients with FPLD. The intervention, OCA, is a strong FXR activator and there is emerging evidence of the role of FXR agonists in improving hepatic steatosis from the data obtained in experimental animals and in obese patients with NASH or NAFLD. Thus, this will be the first proof-of-concept study to ascertain the role of OCA in improving hepatic steatosis in patients with FPLD. We will use the state of the art technology of measuring hepatic fat content by proton magnetic resonance spectroscopy. We will also measure plasma FGF19 levels as marker of FXR activation and assess hepatic insulin sensitivity using the robust two-step, euglycemic, hyperinsulinemic glucose clamp technique along with infusion of stable isotope of glucose for measuring glucose turnover.

We are aware of the preliminary data from the FLINT trial of OCA for NASH treatment. However, the mechanisms of hepatic steatosis in patients with lipodystrophies are quite different than those in obese

patients with NASH who usually have high circulating levels of free fatty acids and marked upregulation of sterol regulatory element binding protein-1c (SREBP-1c) along with increased *de novo* lipogenesis (50, 51). On the other hand, in a mouse model of CGL, type 1 due to Agpat2 deficiency, *Mgat1* was markedly upregulated in the steatotic liver indicating that hepatic triglyceride synthesis was occurring through an alternative pathway; and a marked increase in hepatic *de novo* lipogenesis occurred without any upregulation of SREBP-1c (52). Similar observations were made in the steatotic livers of CGL, type 2 mice with seipin ablation where the expression of SREBP1c did not change despite increased expression of lipogenic genes such as fatty acid synthase (53). Furthermore, in both the models of CGL, serum levels of free fatty acids were not elevated or were in fact lower than those in the wild type mice (52-54). The transgenic mouse model of FPLD overexpressing the human p.R482Q *LMNA* mutation also develops hepatic steatosis without any increase in basal lipolysis (55). However, the investigators did not study the molecular changes underlying hepatic steatosis in the FPLD mouse model.

Preliminary Studies: The PI has been involved in studying fatty liver disease in patients with lipodystrophies for many years. For the last few years, we have been evaluating patients with various types of lipodystrophy to characterize their physical and biochemical features. We have noted hepatomegaly and abnormal liver function tests in many of these patients and have also performed ^{1}H Magnetic Resonance Spectroscopy in some of them to measure intrahepatic lipid content. Data from some representative adult patients with FPLD are shown in Fig. 1. Hepatic fat content ranged from 0.5-61%. Although traditionally, hepatic steatosis is defined as hepatic triglyceride content exceeding 5.0%, studies by Drs. Szczepaniak and Browning (co-investigator) in a large population based sample found the 95th percentile of hepatic triglyceride concentration in 345 subjects from the Dallas Heart Study without any risk factors for hepatic steatosis was 5.56% (56). Even considering a higher cut-off value (>5.6%) to define hepatic steatosis, it appears that 74% of the FPLD patients had hepatic steatosis. Thus, these data reveal high prevalence of hepatic steatosis in patients with FPLD (1, 57).

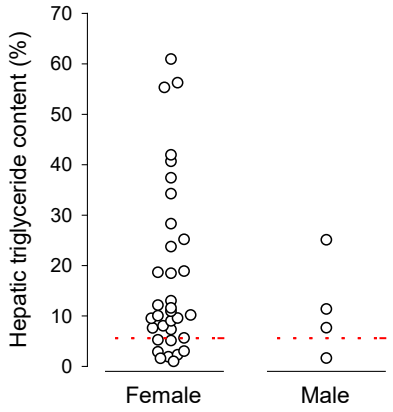

Fig. 1 Hepatic triglyceride content in adult patients with FPLD. Each symbol represents data from one patient. The interrupted line represents 5.6% liver fat. Thus, both males and females have increased prevalence of hepatic steatosis.

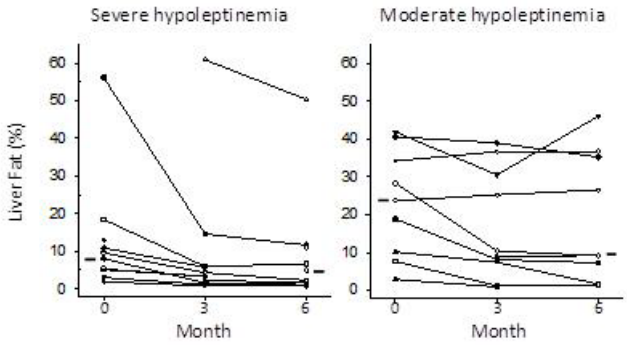

Fig. 2 Reduction of hepatic triglyceride concentrations measured using proton MRS in severely hypoleptinemic (serum leptin < 4 ng/mL) and moderately hypoleptinemic (serum leptin 4-7 ng/mL) female patients with FPLD in response to leptin therapy.

We have also reported marked reduction in hepatic triglyceride content with recombinant leptin therapy in patients with generalized lipodystrophy and severe hypoleptinemia (10, 11). In an open-label trial of human recombinant leptin in three patients with generalized lipodystrophy, 8 – 10 months of therapy resulted in a

mean decline in intrahepatic lipid content by about 80% (11). Compared to baseline, the intrahepatic lipid content decreased in the three patients from 14.9% to 1.2%, 5.7% to 2.2% and 11.1% to 1.5%, respectively.

In another recent study from our group (18) in 24 women with FPLD, although overall, metreleptin replacement therapy resulted in significant lowering of hepatic TG levels from a median baseline value of 10.2% to 5.9% - 8.2%, after 3 months and 6 months, respectively ($p<0.0001$). However, the response was quite heterogeneous and especially many female patients with moderate hypoleptinemia (serum leptin 4-7 ng/mL) did not respond to metreleptin (Fig. 2). Due to heterogeneity of response to metreleptin in our study as well as in previous studies (17), FDA did not approve this therapy for partial lipodystrophies. Furthermore, many FPLD patients do not have severe hypoleptinemia (serum leptin <4 ng/mL in females and <1.5 ng/mL in males), which can justify leptin replacement therapy (Fig. 3). In fact, their serum leptin levels may be normal or even high.

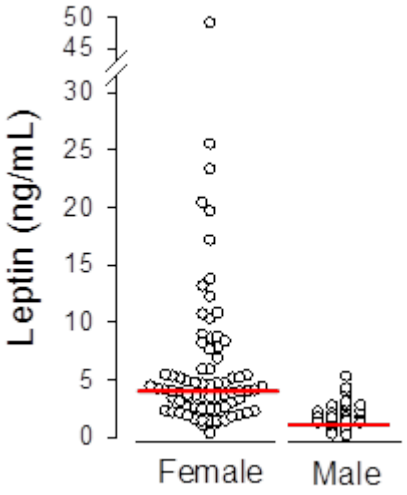

Figure 3: Serum leptin concentrations in patients with FPLD. The horizontal red line indicates serum leptin concentrations of 4 ng/mL in females, and 1.5 ng/mL in males, which correspond to 7th percentile of population. As shown, many FPLD patients have normal or even high levels of serum leptin. These normoleptinemic FPLD are not candidates for leptin replacement therapy.

Most recently, we evaluated the efficacy and safety of cholic acid for reducing hepatic steatosis in patients with lipodystrophies using a randomized, double-blind, placebo-controlled, crossover design. Eighteen patients with genetic or autoimmune lipodystrophies, and elevated hepatic TG content participated. Cholic acid (15 mg/kg/day) was compared with placebo for a period of 6 months each. Compared to placebo, cholic acid did not reduce hepatic TG content [median (interquartile range) 14.8% (9.4-19.0%) vs. 15.9% (10.5-26.5%), respectively; p = 0.42] (Fig. 4). Cholic acid therapy also did not change AST, ALT or GGT levels. Two patients developed diarrhea and excessive flatus while taking CA and these symptoms resolved after reducing the dose of cholic acid. It is likely that failure to reduce hepatic TG content with cholic acid may be due to relatively weak agonistic activity of cholic acid for FXR. Therefore, in the current protocol, we want to study more potent FXR agonist, obeticholic acid, which is ~100 times more potent than CDCA. OCA may prove to be efficacious in treating hepatic steatosis.

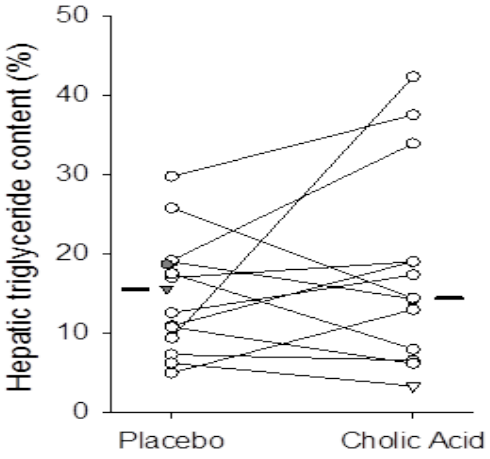

Fig. 4 Hepatic triglyceride content during the cholic acid study. Connected open circles represent the 12 subjects who completed both the six month study periods. For three subjects with incomplete data, filled symbols represent subjects with placebo data only; inverted triangles represent 3 month data. Median values for each phase are shown as horizontal bars.

3. Concise Summary of Project:

This study will be a randomized, double-blind, placebo-controlled, cross-over trial (Fig. 5). Patients who are considered eligible for the study will undergo screening evaluation to determine their eligibility for the trial. For those who are found to be eligible, during the baseline period, they will continue their usual diet and other lifestyle measures without changing any medications for 1 month in order to establish a baseline state. Three blood samples will be obtained during this period at the Clinical and Translational Research Center. Following the baseline period, the patients will receive obeticholic acid (OCA) or an identical placebo in the dose of 25 mg/day for a period of 4 months and then will receive the other treatment (OCA or placebo) for 4 months. There will be a wash-out period of 4 months in-between the two study periods. The dose of OCA is chosen based upon several considerations: a. In a previous study of patients with type 2 diabetes mellitus and hepatic steatosis, this dose resulted in significant improvement in insulin-mediated glucose disposal and reduction in ALT and GGT (49). b. This dose is generally well-tolerated with negligible side effects. If there are intolerable side effects which are considered probably or possibly related to OCA/placebo, the dose of OCA/placebo will be reduced to 25 mg every other day. If the side effects persist despite reduction of the dose, the medication will be stopped but the subject will continue to participate and complete the trial (as per intent to treat protocol).

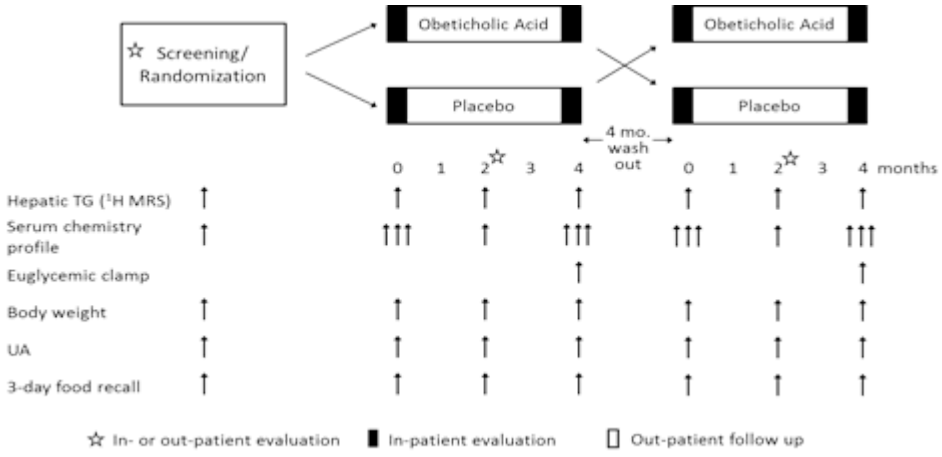

Fig. 5 : Study design and time points for determination of variables. Wash out period will be for 4 months.

Patients will be educated to maintain their usual physical activities and diet during the study. The subjects will be admitted to the Clinical and Translational Research Center for the baseline evaluations (at the beginning of the two study periods), and at the end of four months during each study period. In some subjects, unable to visit CTRC at the end of 2 months, we will provide kits to them for mailing the blood and urine samples using courier service. We have done such collections successfully in our previous trials of leptin therapy in patients with lipodystrophies.

Justification for the choice of design*:* We considered both the randomized, parallel design and the cross-over design. In view of the rare prevalence of FPLD, we decided to use the cross-over design. This allows us to achieve more power with fewer patients compared to the parallel design. We do not expect carry over effects of OCA on hepatic triglyceride levels given that there will be a four month wash-out period between the two study periods and an additional 4 month time before determining the end point variable during the second study period. We will attempt to keep the drop-outs to as minimum as possible to achieve maximum out of this design. Our choice of study design was guided by Lagakos's editorial entitled, "Clinical Trials and Rare Diseases" (58).

Primary and Secondary Endpoint Variables: The primary end-point variable will be the reduction in the liver TG content on ^{1}H MRS. Reductions in the levels of serum alanine and aspartate aminotransferases, gamma-glutamyl transpeptidase, triglycerides, insulin and suppression of hepatic glucose output during the low-dose and high-dose insulin infusions during the euglycemic clamp study (measure of hepatic insulin sensitivity) will

be the secondary end-points of the study. Complete blood counts and serum chemistries will be performed for safety considerations. As an evidence of FXR activation, fasting serum FGF19 levels will be measured. We will also measure liver size using magnetic resonance imaging. These additional tests will be performed at baseline and after 2 and 4 months of therapy.

Lundasen et al. (59) reported 250% increase in serum FGF19 levels after 2-3 wk of feeding 15 mg/kg/d of chenodeoxycholic acid, an FXR ligand. Meyer-Gerspach et al. (60) have further shown a dose dependent increase in plasma FGF19 levels 60 min after intraduodenal infusion of chenodeoxycholic acid. Recently, Mudaliar et al. (49) reported marked 92% and 223% increase in plasma FGF19 levels with 25 mg and 50 mg of OCA administration, respectively; compared to only 8% increase with placebo (Fig. 6). Thus, there is strong evidence supporting the use of plasma FGF19 levels as markers of FXR activation.

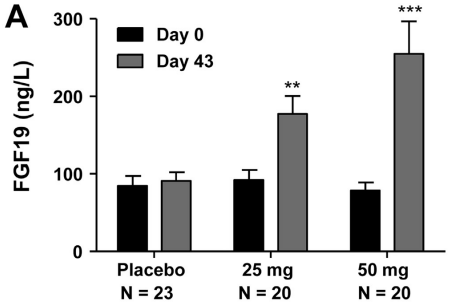

Fig. 6 Increased plasma FGF19 levels in patients with diabetes and NAFLD after treatment with OCA. Data are presented as mean ± SEM. **P < .01, ***P < .001. Statistical significance is based on comparisons of absolute change from baseline between the OCA treatment groups and placebo. From Mudaliar et al. (49)

Patients:

We are currently following a large number of patients with FPLD harboring heterozygous missense *LMNA* mutations including 128 affected adult women and 40 affected adult men. Of these, 50 women and 30 men are nondiabetic and the diabetes status of 20 FPLD patients was unclear. We will inform the eligible subjects of the availability of the study and from amongst these patients, a total of 20 patients who satisfy the following inclusion and exclusion criteria will be enrolled. They will be instructed not to change their medications during the study without consulting the investigators.

Based on analysis of our database of FPLD patients with *LMNA* mutations, diabetes as an exclusion criteria posed a challenge since >50% of female FPLD patients have diabetes. Therefore, we have revised our exclusion criteria and will consider enrolling patients with diet-controlled diabetes or those on metformin . We will also enroll FPL patients with disease-causing variants in *PPARG* and other FPL genes. We are in the process of identifying additional potential subjects from our large Lipodystrophy database who will be willing to take part in the study. We have identified 55 females and 35 males with *LMNA* variants and 4 females with *PPARG* variants with FPLD who may be eligible to take part in the study.

4. Study Procedures:

Demographic Characteristics, Health History, and Physical Examination:

The participants will be asked to complete questionnaires on demographic characteristics and health history at baseline. A detailed history will be obtained and physical examination will be conducted on each subject during the baseline period and during the last week of each study period. During these periods, they will also be asked to complete a 3-day food record and questionnaires to assess quality of life and drug side effects (see appendix). Height and body mass will be measured by standard procedures.

^{1}H Magnetic Resonance Spectroscopy (MRS): ^{1}H MRS studies will be performed at least 4 hours post prandially with the patients lying prone as described previously (61). Briefly, image-guided, proton-localized, MRS and high resolution T1-weighted imaging will be performed on a 1.5 T Gyroscan Intera whole body system (Philips Medical Systems, Best, The Netherlands) with the following parameters: repetition time (TR) of 5 s, spin echo time (TE) of 25 ms for liver, and 1024 data points over 1000 kHz spectral width. Volumes of interest (voxel) will be centered on the right lobe of the liver (30 mm^3) taking care to avoid vascular structures. Spectra will be processed and resonances quantified using a standard analysis package (NUTS; ACORNNMR, Fremont, CA). The hepatic TG concentration will be expressed as percentage of the intensity of the water resonance peak (61) **(Fig. 7).**

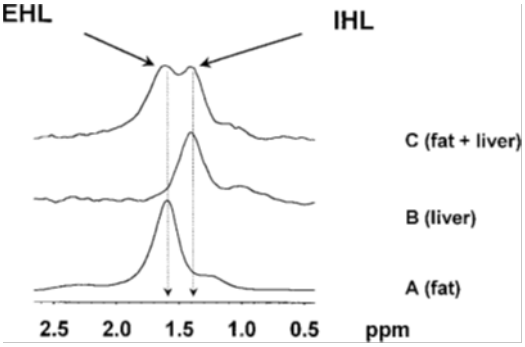

Fig. 7 ^{1}H MR spectra of the abdomen of a human subject with steatosis. Voxel was sequentially placed over subcutaneous juxtahepatic fat (*A*), liver (*B*), or both (*C*). Chemical shifts were measured relative to tissue water at 4.80 ppm. Note that a single methylene peak originates from adipose fat and is located at 1.6 ppm, whereas the intracellular fat within liver appears at 1.4 ppm. From Szczepaniak et al. (61).

Liver size: Prior to performing the ^{1}H MRS, sagittal, coronal and axial slices are obtained routinely through the liver to select a spectroscopic volume of interest avoiding major blood vessels, intrahepatic bile ducts and lateral margin of the liver. We will use the axial slices obtained on T-1 weighted images of 10 mm thickness with a 10 mm gap in-between. The volume of the liver at each slice will be mapped manually using a track ball on the computer screen and summed up for each slice and in-between gap to determine the liver volume. We have previously used similar program to measure subcutaneous and intra-abdominal adipose tissue volume (62, 63).

Serum chemistry, lipoproteins, insulin, and FGF19: Blood will be obtained after overnight (12 h) fast daily for chemistry profile (SMA-25), lipoprotein, insulin and FGF19 levels while the patients are admitted to the CTRC. Plasma insulin concentrations will be determined by radioimmunoassay using kits (Millipore, Billerica, MA). Plasma FGF19 levels will be measured by an ELISA kit (Phoenix Pharmaceuticals Inc., Burlingame, CA). Serum chemistry profile including the aminotransferases, gamma-glutamyl transpeptidase, lipids and lipoproteins, glucose and electrolytes etc. will be measured by a commercial laboratory (Quest Diagnostics).

Three-Day Food Record: Dietary intake will be assessed by 3-day food record (two weekdays and one weekend day), a valid and reliable method (64). The measure will be taken at baseline and each of the follow-up visits. The participants will be instructed on how to record in detail all the food and drink consumed in the 3-day food record booklet provided. The booklet will be available in both English and Spanish (see Appendix). The participants will also be provided a two-dimensional visual chart of food portions to aid them in estimating portion sizes. Any questionable input or incomplete food entries recorded will be promptly addressed when the subjects return their food records. The food records will be analyzed for nutrient content using the University of Minnesota Nutrient Data System (NDS) for research. The NDS database contains more than 18,000 foods, 8,000 brand name products, and many ethnic foods. The database is continuously updated to reflect newly published or provisional data provided by the United States Department of Agriculture, new analytic data from the scientific literature, and current food composition data for new and reformulated commercial products. The database is also put through rigorous quality control (65). The NDS has the capability of calculating daily totals

and amounts by food groups for 120 nutrients and nutrient ratios. It can also calculate gram weight for individual foods.

Oral Glucose Tolerance Test (OGTT): A 3 hour OGTT will be performed after 10-12 h overnight fast. An intravenous line will be placed for blood drawing. On the day of fasting, patient will have a fasting venous blood sample collected and then will be Instructed to drink the glucose solution (75 g of glucose in 200 ml of water) over a maximum of 15 minutes (ideally within 5 minutes). Time will be noted. Blood tests will be done at half-hour intervals for three hours to check patient's glucose and insulin response. A total of 45 ml blood or 3 tablespoons (for adults) will be withdrawn during this test. For oral glucose tolerance tests, the volumes are adjusted for weight, and the sample frequency and duration are decreased.

Mutational Analysis of *LMNA* gene: To confirm the diagnosis of FPLD, direct sequencing of the entire coding region and the surrounding intron-exon boundaries of the *LMNA* gene will be conducted in the probands from each pedigree followed by sequencing of the disease-causing variant in the rest of the family members. Primers that amplify each exon of *LMNA* from genomic DNA templates have been designed from published sequence information (69).

Monitoring of potential side effects: Biochemical testing and routine urinalysis will be done every 6 weeks, during the periods when patients are on study drug or placebo. Telephone contact will be initiated with subjects every 2 weeks to assess for symptoms such as new or worsening pruritus, abdominal pain, new or worsening fatigue, anorexia, nausea, rash, vomiting, diarrhea, jaundice (yellow skin or sclera, high amber colored urine and pale stools) and vague neuropsychiatric symptoms. For patients outside of Dallas, blood tests will be arranged every 6 weeks and if there are any abnormalities, they will be asked to either visit us in person (for those living in the Dallas area) or see their primary care physician for physical examination (for those from outside Dallas). We will communicate with their physicians about the lab data and physical examination findings.

Drug will be discontinued in subjects immediately who develop liver decompensation events (e.g., variceal bleed, hepatic encephalopathy, ascites, etc.) and will not restart the drug even if the events are resolved.

Patients will be asked to report following symptoms as it will trigger immediate evaluation of the patient for hepatic toxicity: new or worsening pruritus, abdominal pain, new or worsening fatigue, anorexia, nausea, rash, vomiting or diarrhea. Even vague neuropsychiatric symptoms have been associated with acute liver failure. If prompt evaluation is not possible, the investigational drug will be immediately discontinued.

Patients will be asked to withhold drug during intercurrent illness, such as gastroenteritis resulting in dehydration, or other reasons for dehydration, and that patients should be reevaluated with both biochemical testing and physical exam prior to restarting the drug.

Physical exam and liver biochemistries including ALT, AST, total bilirubin, and ALP will be performed at each clinic visit and at any unscheduled visit triggered by patients reporting symptoms. If elevations in ALT, AST, total bilirubin (TB), and ALP levels trigger reevaluation, then platelet count, INR, albumin, direct bilirubin, electrolytes and creatinine will be included in repeat testing.

We will use the same Liver Laboratory Criteria for Monitoring of Suspected Hepatic Injury or Decompensation as submitted recently by Intercept Pharmaceuticals (Table 1) (Protocol 747-303 Safety Amendment).

Table 1: Liver Laboratory Criteria for Monitoring for Suspected Hepatic Injury or Decompensation

Criteria Resulting in Immediate Interruption of Investigational Product Upon First Observation		
Baseline	**Upper Laboratory Threshold**	**Frequency of Monitoring (Repeat Testing)**
Conj. bilirubin ≤ULN	≥1.5x ULN	Not applicable
	If Total Bili rise >=0.5 mg/dL, reassess conjugated if >ULN	
Creatinine	>ULN AND increase by 20%	
Criteria Resulting in Repeat Testing		
Baseline	**Upper Laboratory Threshold**	**Frequency of Monitoring (Repeat Testing)**
Key Liver Parameters for Hepatic Injury		
ALT ≤ULN	≥3x ULN	2-3 days
ALT >ULN and <2x ULN	≥3x baseline or incremental increase from baseline >250 U/L	2-3 days
ALT ≥ 2x ULN	≥2x baseline or incremental increase from baseline >250 U/L	
AST ≤ULN	≥3x ULN	2-3 days
AST >ULN and <2x ULN	≥3x baseline or incremental increase from baseline >250 U/L	2-3 days
AST ≥ 2x ULN	≥2x baseline or incremental increase from baseline >250 U/L	
ALP ≤ULN	≥1.5x ULN	7 days
ALP >ULN	≥1.5x baseline	7 days
Total bilirubin ≤ULN[a]	≥1.5x baseline AND >ULN	2-3 days
Total bilirubin >ULN[a]	≥1.5x baseline	2-3 days
INR≤ULN[b]	≥30% increase from baseline	2-3 days
INR>ULN[b]	>1.5 AND ≥10% increase from baseline	2-3 days
GGT ≤ULN	≥3x ULN	2-3 days
GGT >ULN	≥3x baseline	2-3 days
Other Parameters Reflective of Overall Hepatic Health		
Albumin	<3.2 g/dL	2-3 days
Platelets	<100x10^3/mm^3 AND ≥10% decrease from baseline	2-3 days
Electrolytes[c]	Sodium <130	2-3 days

[a] Diagnosis of Gilbert's Syndrome should be established during baseline. Conjugated bilirubin to be sole determinant in such subjects.
[b] Does not apply in subjects on anti-coagulants
[c] Sodium will be measured as an assessment of liver failure (hyponatremia).

Study Medication – Obeticholic Acid

The chemical name of OCA is 3α,7α-dihydroxy-6α-ethyl-5βcholan-24-oic acid. It is also referred to as 6α-ethylchenodeoxycholic acid, or 6-ECDCA. The international nonproprietary name (INN) is obeticholic acid. The structure of this molecule is provided in Figure 9.

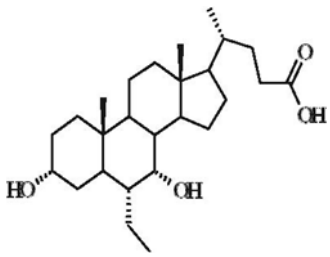

Fig. 9 Chemical structure of obeticholic acid

The drug and placebo will be made available by Intercept Pharmaceuticals, San Diego, CA (see letter of support). An IND (number 63,307) has been filed by Intercept Pharmaceuticals and our IND will cross reference to this IND.

Non Clinical Studies: OCA has been evaluated in a variety of animal models of acute and chronic liver injury. The preclinical results demonstrate that OCA has broad hepatoprotective effects including:

- Regulation of bile synthesis and flow to improve cholestasis
- Inhibition of hepatic and systemic inflammatory responses
- Action as a potent antifibrotic agent to prevent and reverse fibrosis
- Ability to reverse cirrhosis and portal hypertension

Such hepatoprotective effects are largely attributable to FXR activation, which is not seen with UDCA. In addition, other preclinical metabolic data demonstrate that OCA has the capacity to normalize fasting glycemia and insulin sensitivity in a rabbit model of metabolic syndrome. Studies in FXR knockout mice show that FXR is involved in the regulation of insulin signaling pathways and appears to have a beneficial role in decreasing insulin resistance (IR) and gluconeogenesis, as well as in regulating triglyceride, free fatty acid, and lipid levels (40, 70). This adds to the rationale for clinical testing of OCA in subjects with chronic liver disease where IR is a major risk factor for progression to cirrhosis; e.g., nonalcoholic fatty liver disease (NAFLD) and its more clinically significant subtype nonalcoholic steatohepatitis (NASH) (37).

Studies in cell-free assays, primary cultures of human and mouse hepatocytes, and human hepatocyte cell lines have established that OCA is a potent FXR agonist with an EC50 of 85 to 100 nM, approximately 100-fold more potent than the endogenous FXR agonist CDCA it is derived from (42, 43). Importantly, glyco- and tauro-conjugated OCA are as potent and selective FXR agonists as free OCA. OCA is highly selective for FXR. The compound does not bind other nuclear receptors and, with the exception of weakly activating the dedicated bile acid receptor TGR5 (EC50 = 20 μM), it does not activate any G-protein coupled receptors (GPCRs) screened (Investigator Brochure 2012). In addition, OCA does not block the human ether-à-go-go-related gene (hERG) potassium channel (Investigator Brochure 2012).

Nonclinical safety**:** Pharmacology studies have evaluated the neuropharmacologic, pulmonary, and gastrointestinal (GI) effects of OCA in rats. These studies showed no adverse effects due to OCA. OCA has also been evaluated for cardiovascular in vitro and in vivo effects. There were no clinically relevant effects reported on human ether-à-go-go related gene (hERG) channels. There were no cardiovascular effects observed in a dog in vivo study. Clinical trials have, to date, not shown any effects on cardiac conduction and, in particular, QTc prolongation effects.

Nonclinical toxicology studies: Administration of OCA to mice, rats, and dogs in toxicology studies as single doses and with daily dosing for up to 6 and 9 months, respectively, resulted primarily in adverse effects on the liver and GI tract. Bile acids are detergents which explain their well-recognized emulsificant properties in the

gut. In general, the toxicology findings with CDCA-derived OCA are consistent with tissue (primarily hepatic) exposure to detergents at high concentrations.

OCA was not genotoxic in a battery of 3 genotoxicity studies. The drug demonstrated no embryo-fetal toxicity in rats and rabbits, nor adverse effects on either fertility in male or female rats, or early embryonic development to implantation in female rats.

Currently, rodent carcinogenicity studies are ongoing; the in-life portion of these studies will be completed soon. Genotoxicity studies are also ongoing with the OCA taurine and glycine conjugates. In addition, a pre- and post-natal developmental toxicity study in rats is planned to complete the nonclinical safety testing with OCA.

Pharmacology: PK profiles for OCA and its metabolites (glyco-6-ECDCA and tauro 6-ECDCA) have been evaluated in 2 phase 1 healthy volunteer clinical trials following single doses of 50 to 500 mg and repeated dosing of 25 to 250 mg for up to 12 consecutive days. The glyco-6-ECDCA and tauro-6-ECDCA metabolites of OCA are known to be pharmacologically active, and due to the likelihood of enterohepatic recycling and reconversion to OCA, their plasma PK profiles were of interest.

Overall, the PK of OCA shows the profile expected of a bile acid. There is rapid absorption, followed by extensive metabolism to the glyco- and tauro-conjugates. These occur at significantly higher concentrations than the parent drug. There are numerous peak and trough plasma levels seen consistent with the expected extensive enterohepatic circulation of a bile acid. Plasma concentrations dramatically increase shortly after food intake, consistent with gall bladder emptying into the duodenum, as expected.

Pharmacokinetics: Following oral administration, OCA appeared to be rapidly absorbed with the first measurable plasma OCA concentration occurring at 0.25 to 0.5 hour post dose at the 2 higher dose levels. The initial peak plasma OCA concentrations occurred, on average, around 2 hours post dose. OCA was rapidly eliminated from plasma, and was generally not measurable by about 6 hours after administration of the 2 higher doses. Plasma t1/2 of OCA ranged from approximately 1 to 2 hours at the higher dose levels where it could be measured.

OCA has been administered to healthy volunteers in 2 phase 1 trials: a single dose trial (747-101) and a trial involving daily dosing for 12 days (747-102). Both trials demonstrated that OCA is rapidly absorbed, extensively metabolized via conjugation to 6-glyco-ECDCA and 6-tauro-ECDCA, and eliminated presumably in feces. The plasma profiles of both OCA and its conjugated metabolites (glyco-6-ECDCA and tauro-6-ECDCA) demonstrated multiple peaking in the elimination phase (consistent with extensive enterohepatic recirculation). Plasma concentrations of glyco-6-ECDCA and tauro-6-ECDCA were considerably higher than the parent drug (OCA) following administration of repeat doses.

The overall low urinary recovery for OCA in its parent and conjugated forms, as well as the plasma profiles of OCA and its conjugates, suggests that there is biliary excretion coupled with extensive enterohepatic recirculation that eventually leads to fecal elimination.

OCA and its glyco- and tauro-conjugates achieve an apparent steady-state concentration after approximately 1 week of once daily dosing. There is only mild accumulation of OCA in plasma following repeat doses and plasma concentrations of the parent drug increase in a dose proportionate manner over the dose range of 25 to 250 mg. There is significant accumulation of glyco-6-ECDCA and tauro-6-ECDCA concentrations in plasma following repeat dosing for 12 days.

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Trial 747-203 – Type 2 Diabetes Mellitus and NAFLD: This double blind, placebo controlled, trial evaluated the effects of OCA on insulin sensitivity by means of a 2-stage euglycemic insulin clamp **(49)**. Subjects with Type 2 diabetes mellitus and NAFLD, diagnosed by elevated aminotransferases (20%), imaging (84%) and/or histology (3%), were randomized to receive placebo, OCA 25 mg, or 50 mg once daily for 6 weeks. Sixty-four (64) subjects were enrolled (placebo: n = 23; OCA 25 mg: n = 20, 50 mg: n = 21). Forty-four (44) subjects had successful pre and post treatment insulin clamps and were analyzed for efficacy. Glucose disposal rate (GDR) was determined (pre and post treatment) after steady state was achieved with low and high dose insulin infusions rates (60 and 120 mU/m^2 body surface area/min).

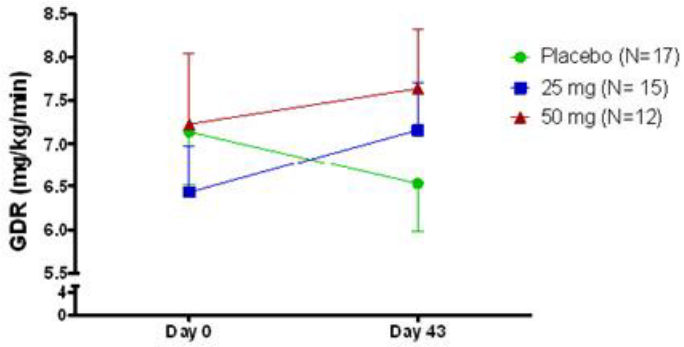


Fig. 10 Glucose disposal rates at low dose insulin infusion. Combined doses versus placebo p =0.048.

Glucose infusion rates showed a significant improvement at the 25 mg dose, compared with placebo. While the improvement at the 50 mg dose did not attain statistical significance on its own, when both OCA doses

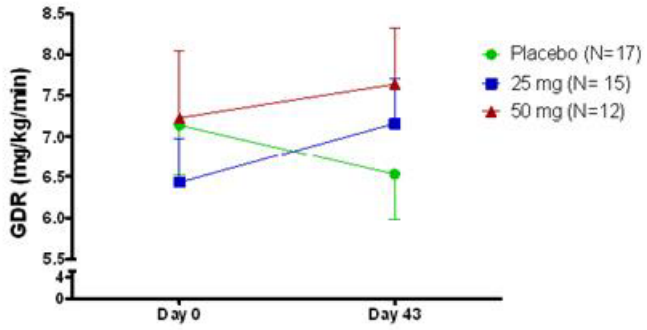


Fig. 11 Glucose disposal rates at high dose insulin infusion. Combined doses versus placebo p =0.022.

In conclusion, the improvements in glucose infusion rates after both insulin infusion levels implies that OCA has a beneficial effect on glucose disposal in both the liver (low dose) and peripherally (high dose, when hepatic glucose uptake is “switched off”). These data are consistent with an improvement in glucose disposal.

Increases in mean LDL and mean total cholesterol (TC) and decreases in mean HDL and mean triglycerides (TG) were observed at end of treatment/early termination compared to baseline in both dose groups (25 and 50 mg) which appeared to be greater than that observed in the placebo group. Serum ALT (25 mg) and GGT (25 and 50 mg) decreased by approximately 25% and 50% ($p < 0.001$), respectively.

Toxicology: In the ascending single dose trial (747-101) testing a dose range of 50 to 500 mg, only 2 mild AEs, upper abdominal pain and nasopharyngitis, were observed in 2 different subjects from a total of 24 subjects. No SAEs, deaths, or trial withdrawals were reported.

In the multiple dose escalation trial (747-102), daily doses of OCA for 12 days were safe and well tolerated in these healthy volunteers at doses up to 100 mg/day. The most commonly occurring AE was pruritus, which was only reported in subjects receiving the highest dose (250 mg) of OCA. One (1) subject in the OCA 250 mg group was withdrawn from the trial due to rash that was possibly related to study medication; the subject had also complained of pruritus. No SAEs were reported, and no deaths occurred during the trial. Overall, no clinically significant mean changes from baseline in vital sign measurements, electrocardiogram (ECG) results, physical examination findings, and laboratory values were noted with the exception of the liver transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Only at the dose of 250 mg, OCA daily was associated with transient increases in aminotransferase liver enzymes in 6 of 8 subjects; and 4 subjects (50%) developed mild pruritus. No changes of clinical note were observed at daily doses of 50 and 100 mg. Most importantly, all SAEs have occurred in trials of patients with primary biliary cirrhosis (PBC).

Trial 747-203 was a US multi-center clinical trial of OCA in subjects with Type 2 diabetes mellitus and NAFLD (49). In this trial, 64 subjects were randomized to receive placebo or OCA at 25 mg or 50 mg once daily for 6 weeks. The drug was well tolerated and the AEs were not clearly different to placebo although 24% of the subjects in the 50 mg group reported constipation (versus 0% in the placebo group).

5. Criteria for Inclusion of Subjects:

1. 1. Patients with familial partial lipodystrophy with heterozygous disease-causing missense mutation in lamin A/C (*LMNA)*, *PPARG,* or other known FPL genes.

2. Hepatic steatosis (>5.6% hepatic triglyceride content) as demonstrated by 1H magnetic resonance spectroscopy.
3. Age 18-70 years.
4. Alcohol intake of less than 20 g per day in females and 30 g per day in males.
5. Participants and their partners with whom they are having sex, must use medically-acceptable birth control (contraceptives) during the study. Medically-acceptable methods of contraception include: (1) surgical sterilization, such as hysterectomy, tubal ligation or vasectomy. (2) approved hormonal contraceptives, such as birth control pills, patch or ring; Depo-Provera, Implanon. (3) barrier methods, such as condom, cervical cap or diaphragm used with a spermicide. (4) an intrauterine device (IUD).
6. Patients with well-controlled diabetes on diet and /or metformin therapy with HbA1c <7.0%.

6. Criteria for Exclusion of Subjects:

1.Laboratory or other histologic findings highly suggestive of liver disease due to causes other than non-alcoholic steatohepatitis, such as chronic viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, biliary obstruction or genetic liver diseases such as Wilson’s disease, hemochromatosis or alpha-1-antitrypsin deficiency. Patients who have any evidence of cirrhosis will be excluded and

patients who progress to cirrhosis during the trial will also be discontinued. The definition of “no evidence of cirrhosis” will be: normal total and direct bilirubin (0.2-1.2 mg/dL & ≤0.2 mg/dL, respectively), normal platelet count (140- 400 thousand/µL), and normal prothrombin time (9-11.5 sec) /INR (0.9-1.1) (unless patient is on anticoagulants). In addition, patients with known or prior clinical evidence of cirrhosis on physical examination (such as ascites, splenomegaly) or noninvasive testing (esophageal varices), will be excluded.
2. Treatment with drugs associated with steatohepatitis, e.g., corticosteroids, high dose estrogens, methotrexate, amiodarone, tamoxifen, valproic acid, sulfasalazine, or oxacillin for more than 2 weeks in the 6 months prior to the study.
3. Decompensated liver disease as evidenced by clinical features of hepatic failure (variceal bleeding, ascites, hepatic encephalopathy etc.) and laboratory investigations (prolonged prothrombin time with INR > 1.3, hypoalbuminemia with serum albumin less than 3.0 g/dL, or presence of esophageal varices etc.)
4. Evidence of hepatocellular carcinoma: alpha-fetoprotein levels greater than 200 ng/ml and/or liver mass on imaging study suggestive of liver cancer.
5. Use of drugs which can potentially decrease hepatic steatosis during previous 3 months; ursodeoxycholic acid, thiazolidinediones, high-dose vitamin E, betaine, acetylcysteine and choline.
6.Significant systemic or major illnesses other than liver disease, such as congestive heart failure, cerebrovascular disease, respiratory failure, renal failure (serum creatinine >2 mg/dL), acute pancreatitis, organ transplantation, serious psychiatric disease, and malignancy, that could interfere with the trial and adequate follow up.
7. Acute medical illnesses precluding participation in the studies.
8. Known HIV-infected patient.
9. Current substance abuse.
10. Pregnant or lactating woman.
11. Hematocrit of less than 30%.
12. History of weight loss during past 3 months.
13. Patients on bile acid binding resins, cholestyramine, colestipol or colesevelam.
14. Hypersensitivity or intolerance to OCA or any components of its formulation.
15. Failure to give informed consent

16 .Previous clinical diagnosis of diabetes mellitus in patients taking insulin , GLP-1 analogues, SGLT2 inhibitors, DDP-4 inhibitors or sulfonylureas or hemoglobin A1c ≥ 7.0 %._____

7. Sources of Research Material:
Research material will consist of questionnaires, blood and urine specimens, physical examination, and 3-day food records obtained from the subjects according to the protocol.

8. Recruitment Methods and Consenting Process:

Subjects will be recruited from referring physicians, clinics and medical schools across the U.S. and Canada. Specifically, we will recruit from other researchers in lipodystrophy and gastrointestinal/liver specialists. Previously, patients with lipodystrophies have participated in our clinical trials from Canada, Portugal, Germany and Kazakhstan. All subjects will be informed of the nature and purpose of the research by the Investigators or Research Coordinator and will give informed consent as required by the UT Southwestern Institutional Review Board. Investigators will obtain informed consent after explaining the study and the subjects having read..Consent will be obtained prior to any study procedures being performed. A copy of the consent form will be given to the subjects and the original consent will be maintained in the subject’s research records, which will be maintained in the General Clinical Research Center.

9. Potential Risks:
All subjects will undergo a history, physical examination, liver MRS, quality of life questionnaire, a

questionnaire on gastrointestinal symptoms, subjects will be asked to undergo a glucose clamp study for which there is a minimal risk of psychological or physical discomfort from fasting and an intravenous saline locks, the risk of hypoglycemia, the inconvenience of time spent and the unlikely risk for bruising, fainting, or infection. Subjects will also have phlebotomy for DNA and genotyping for LMNA, and repeated phlebotomy for which there are the minimal risks of physical and psychological discomfort, the requirement of fasting, and the unlikely risk for bruising, fainting or infection.
The potential risks and discomforts of the evaluation methods being used are: *Magnetic Resonance Spectroscopy (MRS) scans*: requires holding still in a noisy scanner, no radiation risk. *3-day food recalls*: no risk, the inconvenience of the brief time spent. *Quality of life and gastrointestinal symptoms questionnaires*: no risk, the inconvenience of the brief time spent Phlebotomy and line placement for OGTT carries the rare to occasional minimal risk of discomfort, hematoma, infection, fainting and/or vasovagal response. Oral glucose tolerance test carries minimal, occasional risk for nausea and/or vomiting.
Subjects will be asked to undergo phlebotomy for DNA and genotyping for *LMNA*, for which there is the risk of phlebotomy, ,fasting, and the minimal risk of bruising, fainting, infection or hypoglycemia. Subjects will also undergo repeated
routine fasting phlebotomy for which there is a minimal risk of psychological or physical discomfort, the discomfort of fasting, the inconvenience of time spent and the unlikely risk for bruising, fainting or infection.

The potential risks of Obeticholic acid therapy: Obeticholic acid at the proposed dose of 25 mg per day appears safe and well tolerated. Approximately 385 subjects have been exposed to Obeticholic acid in various trials. Serious adverse events, possibly or probably related to Obeticholic Acid such as, chest pain, jaundice, GI Bleed, flare of primary biliary cirrhosis (PBC) were noted only in PBC trials. The most common non-serious adverse event with OCA therapy in PBC patients and across other trials is pruritus. In a multiple dose escalation trial in healthy volunteers OCA was safe and well tolerated at doses up to 100 mg/day without pruritus. Only at 250 mg/day dose, pruritus and transient LFT elevations were noted. Of note, the incidence and severity of pruritus in the type 2 diabetes NAFLD trial at OCA doses of 25 or 50 mg/day was similar in the treated and placebo groups. Additional adverse events associated with OCA include: diarrhea, constipation, abdominal pain, stomach discomfort, nausea, abdominal distension, excoriation, arthralgia, peripheral edema, sinusitis, pain in extremity, back pain, myalgia, hemorrhoids, muscle spasms, cough, gastroesophageal reflux disease, pharyngolaryngeal pain, fatigue, headache, upper respiratory infection, insomnia, pallor, chest pain, decreased hemoglobin, anxiety, headache, dizziness, excoriation, depressed mood, dysmenorrhea, rash, nasopharyngitis, dry eye, vomiting, pyrexia, epistaxis, oropharyngeal pain, maculopapular rash, palpitations, urinary tract infection and sinusitis. Elevations in the liver enzymes (ALT, AST) were reported only at the dose of 250 mg/d in healthy volunteers.

10. Subject Safety and Data Monitoring:
We propose the creation of an internal Data and Safety Monitoring Committee composed of experts in statistics, lipid and liver metabolism. Identified for the necessary expertise are individuals who are not associated with this trial and not collaborators of the principal investigator. 1. William Lee, M.D., Professor of Internal Medicine, Director of the Center for Liver Diseases. 2. Alan Elliott, Ph.D., Faculty Associate, Department of Clinical Sciences. 3. Scott Grundy, M.D., Ph.D. Professor of Internal Medicine 4. Fredrick Dunn, M.D., Professor of Internal Medicine.

The Committee will be asked to: a) Review the research protocol, informed consent documents and the plans for data and safety monitoring. b) Evaluate the progress of the study including periodic assessments of data quality and timeliness, recruitment, retention, and risk versus benefit. c) Report on the safety and scientific progress of the trial. d) Make recommendations to the PI, and if needed to the IRB, NIH, CTRC and FDA concerning continuation, termination or modifications of the trial based on the observed beneficial or adverse effects of the interventions under study. e) Conduct or review the interim analysis with regards to safety in accordance with stopping rules, which should be defined in advance of the data analysis. f) Ensure data integrity. g) Ensure the confidentiality of the data and results of monitoring. h) Maintain study integrity by commenting on any problems with study conduct, enrollment, and statistics or data collection.

The PI will hold the primary oversight responsibility for this trial and as such he will be responsible for surveying the medical literature for scientific or therapeutic developments that may impact the safety of participants or the ethics of the study. He will insure that: subjects are fully informed of the study requirements throughout the trial, insure that study subjects receive a study calendar upon enrollment, are updated on any new information relevant to their continued participation or change in the risk versus benefit ratio of the interventions. The PI will be responsible for reporting adverse events to the IRB, NIH, CTRC and FDA in accordance with IRB policies and time frames. The PI is responsible for submitting continuing review reports to the IRB, NIH and FDA.

11. Procedures to Maintain Confidentiality:

Confidentiality will be maintained by utilizing study codes to identify subjects, all files will be kept locked and all information on computers will be password protected. Access to research data is restricted to key personnel directly involved with the study who have been trained in the protection of human subjects and signed statements assuring their compliance with University policies protecting the privacy of research subjects.

12. Potential Benefits:

The potential benefit of obeticholic acid therapy is an improvement in hepatic steatosis as demonstrated by ^{1}H MRS of the liver, as well as possible improvement in raised levels of hepatic enzymes such as transaminases and improved insulin sensitivity. Reduction in hypertriglyceridemia will be shown through improved fasting serum triglyceride levels. The results of this study may help define a safe and effective treatment for non-alcoholic hepatic steatosis in people with lipodystrophy.

13. Biostatistics:

Power and sample size: The primary endpoint variable is the difference in hepatic triglyceride concentration between OCA and Placebo after four months of each treatment. Sample size estimates are based on our preliminary data with a geometric mean hepatic triglyceride concentration of 16% in 29 patients with FPLD who have hepatic triglyceride concentration >5.6% and a corresponding coefficient of variation of 25% based on log transformed data. We expect a reduction in hepatic fat of at least 25% (i.e., reduction from 16% to 12% liver fat with OCA) and calculate, for an alpha of 0.05 and power of >0.90, we will need 16 patients for the study. Allowing for a 20% drop-out rate, a total of 20 patients will be required. The drop-out rate is estimated from our recent studies in patients with FPL (18, 71).

Randomization: The sequence of OCA and placebo treatment will be determined by blocked randomization.

Statistical analysis*:* Descriptive statistics and 95% confidence intervals will be used to summarize responses to OCA and Placebo treatment and treatment differences of this crossover study. For hepatic triglyceride concentration, mixed linear repeated measures models will be constructed to compare OCA versus Placebo after four treatment months and to evaluate treatment sequence effects. Secondary endpoint variables will be FGF19 levels, hepatic insulin sensitivity measurements from the euglycemic, hyperinsulinemic glucose clamp, serum triglycerides, insulin, and aminotransferase levels and will be analyzed with similar mixed linear repeated measures models. Two month treatment response comparisons, gender subgroup interactions, per-protocol hepatic triglyceride concentration responses will be assessed as secondary analyses. The primary and secondary endpoints, which are likely to be skewed, will be log transformed prior to analysis, as appropriate. Nonparametric tests will be implemented, if necessary, to satisfy analysis assumptions. Exploratory analyses: Delta responses (OCA-Placebo) will be computed for primary and secondary variables and the association among the delta variables will be evaluated with Spearman correlation coefficients. A two-sided p value of <0.05 will be considered statistically significant. No interim analysis is planned because of the relatively short study duration and limited sample size. SAS (SAS Institute,

Cary, NC) statistical software will be used; particularly SAS PROC MIXED will be used for repeated measures models. The data will be analyzed according to intention-to-treat principles; our mixed model analysis approach can accommodate certain types of missing data. If a subject is unable to complete or tolerate the intervention in either blinded treatment period, we will continue follow-up if possible or the post-treatment and other final measurements may be made prior to 4 months to minimize missing outcome data.

Criteria for Discontinuing Drug/Placebo: Patients will be carefully recruited and screened to maximize retention. A proactive retention plan will be in place for study personnel to stay in close contact with study subjects. We will continue to follow patients and encourage patients to return for follow up evaluation regardless of adherence to protocol. The study drug/placebo will be discontinued as necessary, for safety reasons particularly if the patients report serious adverse reactions. Patients developing serious acute medical conditions, which in the opinion of the investigators will interfere with subjects continued participation, will be withdrawn from the drug/placebo as well as women who become pregnant.

Study Stopping Criteria:

- For individual subject: In the event of a single subject CTCAE Grade 3 (severe AE) or higher adverse event, regardless of whether it is attributed to OCA unless it is caused by an accident that could not reasonably be attributable to the drug.
- For all subjects: In the event of two or more subjects experiencing a CTCAE Grade 3 or higher adverse event, regardless of whether it is attributed to OCA unless it is caused by an accident that could not reasonably be attributable to the drug.

Potential Problems and Alternate Strategies: The accrual of adequate number of subjects and retention of study patients is considered to be a potential problem. We intend to address this problem by taking the following steps. a. Direct Referrals to the P.I.: In the past, several patients have been referred to the P.I. for diagnosis, clinical evaluation and management. Many colleagues and patients have contacted the P.I. after reading our publications. We continue to ascertain new pedigrees with FPLD. We have ascertained and molecularly confirmed 5 new pedigrees with FPLD in the last year. Currently, approximately 20 new pedigrees with FPL are awaiting to undergo *LMNA* genotyping. Many of these pedigrees have multiple (>5 affected subjects). We will also extend the current 79 FPLD pedigrees to find more eligible subjects. b. Contact authors of published papers: In the past, we have successfully recruited several new patients and their pedigrees by directly corresponding with the senior authors of papers or abstracts. We will continue these efforts in the future. c. Referrals through organizations for rare diseases: We have received referrals from the National Organization of Rare Disorders (NORD), U.S., and from the Children Living with Inherited Metabolic Diseases (CLIMB), U.K., in the past and expect to ascertain more patients in the future. d. WebSite: In addition, a website (www.lipodystrophy.info) is maintained by the PI for recruitment of patients for research studies. e. Advertisement on NIH Clinical Trials Website: Our studies will be posted at the website. f. Advertisement on Lipodystrophy United and Lipodystrophy Connect: Lipodystrophy United is a lipodystrophy patient support group based in the US and Lipodystrophy Connect is a new registry of patients with lipodystrophy (see letters of support).

We get free travel vouchers (>20/year) from Southwest airlines to provide medical transportation for our patients with lipodystrophies and plan to use those for patients living in the US. We will also utilize Angel Flight for free patient transportation to Dallas. For international patients, we will ask them to pay for their travel. We have previously used this model to successfully complete the trials in lipodystrophy patients (11, 18, 71).

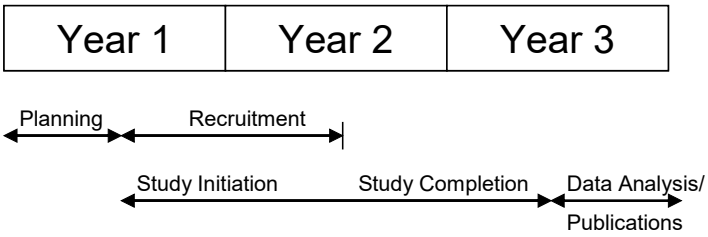
Another problem is related to patient compliance. Top priority will be placed on follow-up visits. Participants will be reminded by phone and in writing of their upcoming follow-up appointments. Missed visits will be rescheduled. We do not expect drop-outs due to side effects of obeticholic acid, as in previous studies it was reported to be well-tolerated.

We are fully aware of the editorial by Clark and Brancati (72) entitled “Negative trials in nonalcoholic steatohepatitis: why they happen and what they teach us”. We have taken care to avoid the pitfalls that they indicated which may result in negative trials. For example, we are avoiding to use low dose of obeticholic acid and have selected to administer 25 mg/day which was also used in the FLINT trial. Our study design which is a cross-over design and also includes a baseline period

approximately one month after the initial screening to determine eligibility will avoid regression to the mean. We have selected four month duration of treatment which is long enough to detect effects of obeticholic acid therapy on hepatic steatosis.

Benchmarks for success: In this pilot trial, reduction of hepatic fat by 25% will be considered a successful outcome for the study. In addition, among the secondary end point variables, we will consider 25% reduction in any of the aminotransferases and gamma glutamyl transpeptidase as successful outcome. Lastly, a 25% reduction in serum triglycerides will also be considered a successful outcome.

Timeline:



The timeline is shown in Fig 12. The budget is requested accordingly for 3 years.

Fig. 12 Timeline for accomplishment of the goals of the project. After an initial planning period of 6 months, we will initiate the study protocol.

Data Collection

The quality of the data will be ensured by extensive training of the staff and periodic reviews for all procedures. Database design and management will be overseen by the PI and study team and facilitated using REDCap (Research Electronic Data Capture), an easy to use web-based solution that is designed exclusively to support data capture for research studies. REDCap is a secure system backed up offsite nightly and hosted in a secure environment maintained by UT Southwestern Information Resources. The REDCap system includes an automatic audit trail of all activity and any study personnel who are granted log-in access to our REDCap database project will be approved by the principal investigator. REDCap has a longitudinal module for capturing the repeated measurements that are central to this crossover study design, provides a built-in project calendar, a scheduling module, a randomization module, and reporting tools. In RedCAP, customized forms for the treatment periods will be built by constructing a codebook which reinforces good data practices by requiring the codebook BEFORE data entry. We anticipate entering study data directly into REDCap but data from existing sources outside of REDCap can be imported easily once the data is checked for problems before final import. Data management activities will also have standard operating procedures (SOPs) for data entry that will be reviewed by the PI. Our database manager will supervise timely data entry, tracking, and cleaning of research data and oversee development of site specific data collection instruments for building and maintaining the local REDCap database and oversee quality assurance. Study specific case report forms (CRFs) will be developed that can be used by specific personnel to enter data; REDCap provides data validation at time of entry. Study form development consultation is available through the Division of Biostatistics for optimizing the data collection instruments.

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