Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN)

The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial

IND # 110354

Final Protocol

Confidential

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Design synopsis

Title: The **F**arnesoid X Receptor (FXR) **Li**gand Obeticholic Acid in **N**ASH **T**reatment (FLINT) Trial

Sponsor: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Population: Participants at least 18 years of age with a diagnosis of NASH

Objective: To determine whether 72 weeks of treatment with obeticholic acid compared to treatment with placebo improves nonalcoholic fatty liver disease (NAFLD) as determined from hepatic histology

Treatment groups:

Group 1: Obeticholic acid (25 mg q.d.)
Group 2: Placebo

Type of trial:

Multicenter, double-masked, placebo-controlled, parallel treatment groups with a 72-week histological change as the primary outcome. There will be a planned interim analysis by the trial Data and Safety Monitoring Board (DSMB) after 65 weeks of enrollment, but before any end-of-trial liver biopsies are performed. The interim analysis will commence approximately 65 weeks after the first enrollment using 24 week data from an estimated 25-40% of FLINT trial patients. The interim analysis will focus on (1) emergent safety issues, if any, and (2) differences in the mean changes of serum alanine aminotransferase (ALT) by treatment group that are of sufficient magnitude and direction to warrant the continuation of enrollment and treatment of patients toward the planned sample size and planned follow-up liver biopsies.

Inclusion criteria:

- Age 18 years or older at initial screening interview
- Histological evidence of definite or borderline NASH on a liver biopsy obtained no more than 90 days prior to randomization and a NAFLD activity score (NAS) of 4 or greater with a score of at least 1 in each component of the NAS (steatosis scored 0-3, lobular inflammation scored 0-3, ballooning scored 0-2).

Exclusion criteria:

- Significant alcohol consumption inability to reliably quantify alcohol intake
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, other known hepatotoxins) for more than 2 weeks in the past year prior to randomizationPrior or planned bariatric surgery
- Uncontrolled diabetes (HbA1c 9.5% or higher within 60 days prior to enrollment)
- Presence of cirrhosis on liver biopsy
- A platelet count below 100,000 /mm3
- Clinical evidence of hepatic decompensation (serum albumin < 3.2 g/dL, INR >1.3, direct

bilirubin >1.3 mg/dL, history of esophageal varices, ascites, or hepatic encephalopathy)
- Evidence of other forms of chronic liver disease
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Serum creatinine of 2.0 mg/dL or greater
- Inability to safely obtain a liver biopsy
- History of biliary diversion
- Known positivity for Human Immunodeficiency Virus infection
- Active, serious medical disease with likely life expectancy less than 5 years
- Active substance abuse including inhaled or injected drugs, in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in an IND trial in the 30 days prior to randomization
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Failure to give informed consent

Outcome measures

Primary:

Centrally scored histological improvement in NAFLD from baseline to the end of 72 weeks of treatment, where improvement is defined as:

(1) No worsening in fibrosis; and

(2) A decrease in NAFLD Activity Score (NAS) of at least 2 points

65 weeks from 1st patient randomized:

Change in serum ALT from baseline to 24 weeks

Adverse events and other safety measures through interim analysis

Secondary changes in the following from baseline to 72 weeks:

NASH diagnosis (from definite or indeterminate NASH to not-NASH)

Fibrosis score

Hepatocellular ballooning score

Each component score in the NAS

Change in serum aminotransferase and gamma-glutamyl transpeptidase (GGT) levels

Change in MRI-determined hepatic fat

Change in fasting markers of insulin resistance (HOMA, adipo-IR index)

Change in post-glucose parameters of insulin (2-hour glucose and fatty acids)

Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference)

Change in bile acid levels

Change in cytokeratin 18 (CK-18) fragment assay

Change in fibroblast growth factor (FGF-19) levels

Change in markers of hepatic apoptosis, inflammation, and fibrosis

Change in health related quality of life (HR-QoL) scores

Screening and enrollment

Up to 16 weeks screening prior to randomization
72-week treatment period
24-week post-treatment washout period
Length of recruitment: 78 weeks (18 months)
Expected enrollment rate: 35 patients each center (~2 patients per month at each center)

Number of clinical centers: 8

Visit schedule:

Screening period can last no more than 16 weeks (112 days) after registration
Randomization: final pre-treatment interview, dispensing of study drug
Follow-up visits: at 2 and 4 weeks and every 12 weeks after randomization throughout the 72-week treatment phase followed by a 96-week visit after 24 weeks of washout period

Randomization: Centrally administered randomization stratified by clinical center, diabetes status, and blocked by calendar time

Statistical analysis: All primary analyses will be on an "intention-to-treat" basis.
The primary analysis is an intention-to-treat analysis in which the proportions of subjects in the active-treatment group (obeticholic acid, 25 mg q.d.) with histological improvement in NAFLD, as defined by the primary outcome measure, is compared with the proportion of subjects in the placebo group in whom there is improvement. The comparison is made with the use of the Mantel–Haenszel chi-square test, stratified according to diabetes status and clinical center. Subjects who do not undergo an end-of-treatment biopsy are classified as not having had improvement.

Vanguard analysis

A formal vanguard analysis, marking the end of the vanguard phase of the FLINT trial, will be performed approximately 65 weeks after the first patient is randomized. The analysis will be focused on interim efficacy outcomes and safety information using data from the estimated 25-40% of patients who will have completed at least 24 weeks of follow-up by the time of the interim analysis. Our pre-specified interim criterion for efficacy for continuing the FLINT trial beyond the vanguard phase is based on serum ALT: the criterion for continuation will be considered met if the upper 95% confidence limit on the between-group mean difference in mean changes from baseline to the 24 week measure of ALT, comparing the obeticholic acid versus placebo groups, is at least a 20% net reduction in serum ALT from the overall baseline mean ALT. The analysis will also include detailed safety data, as required by the DSMB, to determine whether any emergent safety issues are present. This vanguard safety analysis will consist of, but is not limited to, counts of adverse events (AEs) and serious adverse events (SAEs) by treatment group with associated P-values based on Fisher's exact tests or other tests, as appropriate. The final decision as to whether to continue the FLINT trial beyond the vanguard phase will be made by the NIDDK program official for the NASH CRN trial , which will be informed by any recommendations from the FLINT DSMB, taking the interim criterion for efficacy, safety, and other relevant information that may arise into account.

Interim analysis

The DSMB will review one planned interim analysis of the primary histological outcome measure. O'Brien-Fleming statistical stopping guidelines for efficacy will be applied. This interim efficacy analysis will occur when approximately 50% (140 of the 280 patients) have completed both baseline and 72 week biopsies. Based on the Lan-DeMets method for group sequential trials using O'Brien-Fleming boundaries (Reboussin et al., 2000), the levels of significance for the interim and final analyses will be alpha=0.00305 and alpha=0.049, respectively.

Sample size considerations

Total of 280 patients in 2 groups of equal size (140 per group)
Primary comparison: Obeticholic acid group vs. Placebo group
Primary outcome measure: Histological improvement in NAFLD (defined above)
Error protection: Type I = 0.05 and Type II = 0.10 (90% power)
Missing data: 10% will not have 72 week biopsies and will be considered not improved
Minimum clinically important difference:
- 50% higher relative rate of improvement in the obeticholic acid group vs. the placebo group

Assumed response rates:
- Expected percent with improved NAFLD (defined above) in the placebo group: 39% (based on PIVENS data and 2 to 1 split for patients on vs not on vitamin E at start of trial)
- Expected percent with improved NAFLD in the obeticholic acid group: 58%

Safety monitoring: NIDDK appointed DSMB will monitor the data for safety and efficacy for outcomes such as hepatotoxicity, pregnancy, and any other outcomes or events identified as safety-related.

1. Hypothesis and principal objective

1.1 Primary hypothesis

Administration of the farnesoid X receptor (FXR) ligand obeticholic acid (OCA) for 72 weeks to subjects with biopsy evidence of nonalcoholic steatohepatitis (NASH) will result in improvement in their liver disease as measured by changes in the NAFLD activity score (NAS).

1.2 Principal objective

To evaluate whether treatment with obeticholic acid, 25 mg daily for 72 weeks compared to treatment with placebo, improves the severity of NAFLD as determined from hepatic histology.

2. Background and significance

2.1 Introduction

NASH has become the most common form of chronic liver disease in the developed world. It commonly occurs in the setting of obesity, insulin resistance, and a sedentary lifestyle, and it is often considered to be the liver manifestation of the metabolic syndrome. The primary form of treatment is lifestyle modification with changes focused on dietary and exercise habits. Unfortunately, achieving and maintaining these changes is difficult or impossible for many individuals. Pharmacologic treatments have been sought but none has proved universally efficacious. This may be related to the fact that the histopathological changes seen on liver biopsy currently described as NASH may be the result of multiple pathogenetic mechanisms acting in concert to varying degrees. Based on the prevalence and risk of progression of NASH to cirrhosis and cancer, the burden of significant disease is large and drug therapy to prevent or treat NASH is needed.

2.2 Definitions

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of greater than "normal" amounts of fat in the liver. The pathologists' definition is based on observed steatotic droplets (triglyceride) exceeding 5% of surface area. This definition evolved from older studies showing that the normal liver was 5% lipid. Properly performed, magnetic resonance imaging and spectroscopy can accurately quantify liver triglyceride content non-invasively. Based on a population study, an MR spectroscopy-determined 5.5% fat fraction has been suggested as a classification threshold for non-invasively differentiating individuals with NAFLD from those without fatty liver.[1] How well the histological and MR spectroscopy-based classification thresholds correlate with each other has not been determined.

Nonalcoholic steatohepatitis (NASH) is the name applied to a constellation of biopsy abnormalities occurring in the presence of NAFLD that typically includes hepatocyte ballooning with or without Mallory-Denk bodies, a mixed polymorphonuclear leukocyte and mononuclear inflammatory cell infiltrate in the lobules, chronic inflammation in the portal tracts and sometimes Rappaport zone 3 perisinusoidal fibrosis.[2]

A name for ***NAFLD that is not NASH*** has not been universally established. Terms such as nonalcoholic fatty liver (NAFL), simple steatosis, benign steatosis, bland steatosis, and isolated steatosis have been used, but each has limitations that preclude general acceptance.[3]

2.3 Significance of NASH

Prevalence

Estimates of the prevalence of NAFLD in adults range from 17% to 46%.[4-7] NASH has been found in 3-13% of adults, with its prevalence approaching 50% in patients with severe obesity and diabetes.[6, 8-10]

Progression to cirrhosis

Approximately 20-40% of patients with NASH have some degree of fibrosis on their initial diagnostic liver biopsy, and thus have the potential to progress to cirrhosis. There is scant longitudinal data on how many patients with NASH will go on to develop cirrhosis. However, NASH cirrhosis and cryptogenic cirrhosis (most of which represents prior NASH) account for 10-12% of liver transplants. This figure probably underrepresents the relative likelihood of NASH progressing to cirrhosis compared to other forms of liver disease because many patients with NASH cirrhosis are not candidates for liver transplantation because of concomitant cardiovascular disease or severe obesity. Liver disease is the third most common cause of death in patients with NASH, behind cardiovascular disease and cancer.[11]

Development of hepatocellular carcinoma

Hepatocellular carcinoma occurs in 2-4% of patients with NASH cirrhosis, roughly at the same rate as in patients with chronic hepatitis C.[12, 13] Occasionally, patients with NASH but without cirrhosis present with hepatocellular carcinoma as well.[14]

Comorbidities

NASH is often found in patients with features of the metabolic syndrome including centripetal obesity, hyperlipidemia, hypertension, and impaired fasting glucose or overt diabetes. Additional manifestations of insulin resistance such as polycystic ovarian syndrome and acanthosis nigricans are also found in association with NASH. Although some authors have suggested that the liver can contribute to the components of the metabolic syndrome, emerging data support the viewpoint that the liver is likely the target of lipotoxic injury in these settings and not a cause of the associated comorbidities.[15]

2.4 Pathogenesis of NASH

Multiple factors contribute to the development of NASH, and it is likely that the disease recognized clinically and pathologically is actually quite heterogeneous with respect to underlying mechanisms. A major contributor to hepatocellular injury in many if not most patients is likely the generation of fatty acid metabolites. This occurs when the supply of fatty acids to the liver through peripheral lipolysis and de novo lipogenesis exceeds the ability of the liver to either oxidize fat or convert it to triglyceride for secretion or, when secretion is impaired, short term storage in lipid droplets.[15, 16] In this paradigm, the accumulation of triglyceride is an adaptive protective mechanism rather than a contributor to injury. Additional contributing pathogenic factors include mitochondrial dysfunction, ATP depletion, oxidant stress, endoplasmic reticulum (ER) stress, and dysregulated adipokine and cytokine signaling. Since peripheral lipolysis is the major source of fatty acids that the liver must handle, adipose tissue inflammation, adipocyte handling of fatty acids, and insulin responsiveness are now recognized to be critically important in the pathogenesis of NASH.[17, 18]

Insulin resistance

Most patients with NASH have some degree of insulin resistance (IR), defined as an impaired response of tissues to physiological levels of insulin. IR is often measured by estimating insulin-mediated suppression of hepatic glucose production, and thus the most commonly used indices such as the homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) represent measures of hepatic insulin resistance.[19] Because insulin also suppresses adipocyte lipolysis, IR at the level of adipose tissue is likely more relevant to the pathogenesis of NASH than hepatic IR.[20] Adipose tissue IR (adipo-IR) can be estimated by multiplying the fasting insulin level times the fasting plasma free fatty acid level.

Mechanisms of hepatocellular injury

To the extent that mitochondrial dysfunction, adenosine triphosphate (ATP) depletion, ER stress, and oxidant stress play a role in hepatocellular injury in NASH, putative mechanisms have been proposed to tie these processes to subsequent cell death.[21, 22] Because the fatty acid metabolites responsible for initiating lipotoxic injury are not fully known, how they promote cell injury and death has yet to be resolved.[23] A major mechanism of hepatocyte death in NASH is apoptosis.[24]

Fibrosis and progression to cirrhosis

The canonical concept of liver fibrosis resulting from hepatocyte injury is based on studies showing that the production of cytokines and lipid peroxidation species from stressed or dying hepatocytes promote proliferation and activation of hepatic stellate cells. The balance of extracellular matrix deposition versus degradation is thus disrupted in favor of net accumulation of fibrosis. This pathway has been challenged by recent data. One set of data indicates that epithelial progenitor cells in the liver undergo epithelial-mesenchymal transition (EMT) when stressed.[25] The relative roles of EMT versus activation of existing stellate cells in progression of NASH fibrosis to cirrhosis have yet to be established. Another set of data shows that dying/apoptotic hepatocytes signal the progenitor cell compartment via the Hedgehog pathway; subsequent proliferation of these cells initiates the activation of hepatic stellate cells.[26]

2.5 Treatment of NASH

Lifestyle modification

Because obesity, poor dietary habits, and a sedentary lifestyle predispose to the development of NASH, the primary therapeutic intervention is to address these factors by promoting gradual and sustained weight reduction through a balanced, calorically restricted diet composed of healthy food choices coupled with increased physical activity.[27] Although the benefit of lifestyle modification has not been proven in large rigorous clinical trials, enough data have been accumulated from smaller studies to justify this approach.[28]

Drug therapy

Unfortunately, substantial barriers prevent success in achieving and sustaining lifestyle modifications to treat NASH and obesity. Thus pharmacological approaches have been evaluated. A number of thorough reviews have documented the results of the published drug studies for NASH.[29, 30] The NASH CRN recently published the results of the PIVENS trial; a large, multicenter, placebo controlled randomized trial that showed benefits of both pioglitazone and vitamin E in a subset of patients.[37] Further analyses are underway to determine if any pretreatment clinical or pathological findings predict who might respond to these therapies. The data regarding each of these interventions are reviewed in further detail below.

Thiazolidinediones

The thiazolidinediones (TZDs, glitazones) are a class of drugs developed to treat type 2 diabetes because of their insulin sensitizing effect in states of insulin resistance. Studies have shown that the benefits of TZDs are at least partly explained by their ability to improve insulin responsiveness in adipose tissue and reduce inappropriate peripheral lipolysis.[20, 31] As ligands for the nuclear transcription factor PPARγ, this class of drugs has multiple complex effects. Improvement in insulin signaling has been attributed to the ability of TZDs to induce adipocyte differentiation and also prevent the inhibitory effect of c-Jun N-terminal kinases (JNKs) on post-receptor insulin signaling. Pilot studies indicated that the TZDs rosiglitazone and pioglitazone might improve the histology of NASH. Placebo controlled trials have had somewhat mixed results with the French FLIRT trial showing improvement primarily in steatosis,[32, 33] and other trials showing improvement in inflammation as well.[34-36] The PIVENS trial demonstrated that pioglitazone treated patients did not reach a complex histological endpoint or demonstrate improved fibrosis, but there were significant improvements in steatosis, inflammation, and the presence of steatohepatitis.[37] Similar to findings in other trials of TZDs, the improvement in ALT occurred over 3-6 months and was not sustained when the drug was discontinued. Whether the histological improvement occurs in parallel with the ALT decrease is unknown since no trial has examined serial liver biopsies in

TZD treated patients. The primary side effect of using TZDs over the typical 1-2 year time course of most trials is significant weight gain in some subjects. Exacerbation of congestive heart failure, osteoporosis with distal limb fractures, and rare idiosyncratic hepatotoxicity are additional side effects known to occur with the use of TZDs but generally not observed in the relatively small NASH trials.

Vitamin E

Trials of antioxidant agents for the treatment of NASH have been undertaken because of the proposed role of oxidant stress in the pathogenesis of steatohepatitis.[38] Vitamin E (RRR-alpha-tocopherol) has been of particular interest. Pilot studies were inconclusive, but the PIVENS trial demonstrated that 43% of patients treated for two years met the complex histological endpoint compared to 19% in the placebo group ($P<0.01$).[37] Improvement in fibrosis was not observed. Similar to the pioglitazone-treated patients, the ALT improved over a time period of 3-6 months. Unlike treatment with pioglitazone, weight gain was not observed, but neither was any improvement in insulin sensitivity.

Other pharmacological agents

Other agents examined in placebo controlled trials include ursodeoxycholic acid and betaine. Neither proved superior to placebo despite encouraging results from prior pilot studies. Metformin has been evaluated in multiple small studies, but the results have been equivocal at best.[39]

2.6 The farnesoid X receptor (FXR) ligand obeticholic acid

Obeticholic acid (OCA) is 6α-ethyl chenodeoxycholic acid (6ECDCA), also known as INT-747; it is an ethyl substituted derivative of the natural bile acid chenodeoxycholic acid. It was discovered during screening of bile acid derivatives with high affinity for FXR. The EC_{50} of obeticholic acid for FXR is 0.1 μM, or about 100-fold higher than the most potent natural ligand, chenodeoxycholic acid. Obeticholic acid undergoes extensive conjugation to form glycine and taurine bile salts that have equally high affinity for FXR as the parent bile acid.

The farnesoid X receptor

FXR is a ligand activated nuclear receptor for which bile acids are the major known ligands. Chenodeoxycholic acid is the most potent natural FXR ligand (EC_{50} ~10 μM), whereas deoxycholic acid and lithocholic acid are weaker natural ligands; ursodeoxycholic does not activate FXR.[40] FXR is expressed in hepatocytes, biliary epithelium, small bowel enterocytes, renal tubular cells, adrenal glands, and to a lesser degree in adipocytes. The presence of alternative promoter regions and mRNA splicing lead to the expression of four FXR isoforms (FXRα1-4) which differ in their DNA binding affinity. Heterodimerization with the retinoid X receptor (RXR) facilitates binding to some FXR response elements, but FXR can also bind as a monomer. Cross-talk among nuclear receptors includes FXR regulation of pregnane X receptor (PXR) expression. As a sensor of intracellular bile acids, FXR activation decreases intracellular bile acid levels by multiple mechanisms. It causes down-regulation of bile acid synthesis and basolateral uptake (eg, Cyp7A1, Ntcp, Oatp) and up-regulation of bile acid secretion (eg, BSEP, MDR3, Mrp2/3/4, OSTα/β).[41] Additional target genes of FXR include small heterodimer partner (SHP) and fibroblast growth factor-19 (FGF-19). FXR mediated increases in SHP are responsible for many of the known effects of FXR.

Fibroblast growth factor 19

FGF-19 is a peptide hormone that regulates diverse metabolic effects through its interaction with specific membrane receptors in the liver and adipose tissue.[42, 43] FXR stimulated FGF-19 expression in small bowel enterocytes down-regulates bile acid synthesis in the liver, thus serving as a signaling mechanism between the gut and liver to maintain bile acid homeostasis. FGF-19 has been shown in preclinical models to improve adiposity, body weight, and insulin signaling with putative effects on metabolic syndrome.[44, 45]

FXR, insulin sensitivity, body weight, and NAFLD

The results of an exploratory Phase II study in patients with type 2 diabetes and NAFLD has been presented.[46] This double blind, placebo controlled study evaluated the effects of OCA (25 mg or 50 mg given once daily) on glucose disposal with the 2-step euglycemic clamp technique before and after six weeks of treatment. The 25 mg dose significantly improved insulin sensitivity with both insulin infusion steps (60 and120 mU x m^2 body surface area/min). The response seen at the 50 mg was not statistically significant, although when both doses combined were compared to placebo results they were also statistically significant suggesting that 50 mg is at the top of the dose response range. Serum ALT levels dropped in the 25 mg group but not the 50 mg group. There was a statistically significant decrease in body weight in the OCA treated patients. FGF-19 plasma levels were increased in the OCA treated patients in a dose related manner. Post-treatment OCA plasma concentrations were 33% and 41% of total plasma bile acid pool in the 25 and 50 mg groups respectively, and there was a significant reduction in the total plasma bile acids in the 25mg group. These data provide preclinical evidence that OCA is a potent FXR agonist in patients with NAFLD, and it is likely that at least some of its metabolic effects are mediated via induction of FGF-19. In addition, it has been suggested that FXR ligands may be effective for NASH based on earlier rodent data.[47] Interestingly, a small clinical trial in patients with NAFLD using the less potent FXR ligand chenodeoxycholic acid indicated that suppression of bile acid production in the liver is blunted, suggesting that a state of insulin resistance may be accompanied by a state of FGF-19 resistance.[48]

Most of the available data from rodent studies indicate that FXR may play an important role in regulating glucose and lipid metabolism.[40, 49-52] Early studies demonstrated that FXR null mice have muscle and adipose tissue insulin resistance, diminished adipose tissue and increased circulating free fatty acid levels and NAFLD.[53-55] Maintaining insulin sensitivity at the level of adipocytes is essential for preventing inappropriate release of fatty acids, an important contributory factor to muscle and hepatic triglyceride accumulation and the lipotoxic injury recognized in the liver as NASH.[16, 23] One study demonstrated that the FXR ligand GW4064 reduced circulating free fatty acids and improved insulin sensitivity in the db/db mouse[56] and the FXR ligand obeticholic acid (identified in this paper as INT-747) improved insulin signaling in the 3T3-L1 adipocyte cell line.[57] A more recent paper found that the FXR ligand obeticholic acid (identified as 6ECDCA) reversed insulin resistance and NAFLD in Zucker fa/fa rats.[58] In mice lacking both FXR and the LDL receptor (dual knockouts), many features of human NASH developed with steatosis, inflammation, and initiation of fibrosis, but the cause of this interaction requires further investigation.[59]

De novo lipogenesis is also a source of excessive fatty acids in the liver and FXR activation down-regulates the transcription factor SREBP-1c, a major regulator of lipogenesis; this was shown to occur both directly and through the interaction of FXR with SHP.[47, 60]

Lipotoxic liver injury can also be prevented by upregulating disposal of fatty acids in the liver. Rodents exhibit robust peroxisomal fatty acid oxidation which prevents NASH in animal models, but the role of PPARα stimulated peroxisomal fatty acid oxidation in humans has been less clear.[61] FXR has been shown to increase PPARα expression in humans,[62] but whether this might impact positively on the development of NASH has not been explored.

Thus studies in rodents suggest that FXR ligands could be useful to treat NASH through their effects on adipocyte insulin signaling and suppression of adipocyte lipolysis by insulin leading to decreased exposure of the liver to free fatty acids, decreased hepatic de novo lipogenesis, and possibly increased oxidative disposal of fatty acids.[63]

FXR interactions

The pleiotropic effects of FXR and its interactions with other nuclear receptors could lead to unanticipated outcomes.[50, 64, 65] In rodents, FXR activation down-regulates proinflammatory NFκB signaling, and conversely, FXR knockout mice are overly sensitive to endotoxin mediated injury,[66] a putative pathway of injury in NASH.[67]

FXR signaling can also increase PPARγ expression; whether this could play a role in sensitizing adipose tissue to the favorable effects of PPARγ ligands on adipose insulin signaling requires further investigation.[68]

An important recent study has shown that FXR is a target of post-translational acetylation that inhibits its function, and that the degree of FXR acetylation is increased in rodent models of obesity.[69] The deacetylase SIRT1 can counteract the acetylation and reactivate FXR, whereas the acetylase p300 has opposing effects.[69] FXR bound to DNA without ligand activation also facilitates histone deacetylase activity to repress gene expression, whereas ligand binding to FXR has the opposite effect and promotes histone acetylation, DNA decompaction, and gene expression.[65] These observations suggest that interactions between FXR ligands and the SIRT1 activators resveratrol and metformin may occur.

FXR effects on hepatocyte death and hepatic fibrogenesis

Apoptosis is a major cause of cell death in NASH. Whether interfering with this basic biological process is beneficial or potentially harmful in disease states is unknown, but FXR ligands have been shown to diminish hepatocyte apoptosis in rodent models and FXR knockout mice were shown to have increased levels of apoptosis.[70]

Limited rodent data and results from cell culture experiments suggest that FXR ligand might have a role in preventing inappropriate hepatic fibrogenesis.[47] FXR is expressed in rat[71] and human[72] stellate cells, and obeticholic acid prevented and reversed carbon tetrachloride induced liver fibrosis in rats.[71]

FXR-independent effects of bile acids relevant to NAFLD and insulin resistance

Bile acids also have FXR-independent effects on energy metabolism and thermogenesis.[63] Bile acids are ligands for a G-protein coupled receptor called Gpbar1 or TGR5, which when activated increases intracellular cyclic adenosine monophosphate (cAMP).[73, 74] The elevated cAMP activates the thyroid hormone deiodinase Dio2 that converts the inactive thyroid hormone T4 to active T3. In specific target tissues that express both the Gpbar1/TGR5 bile acid receptor and the enzyme Dio2, bile acids increase energy expenditure and thermogenesis through activation of thyroid hormone. The major target tissue for this in rodents is brown adipose tissue (BAT). In humans it is thought to be muscle, although with the recent recognition of significant BAT depots in humans, BAT could be a significant target in humans as well. OCA is a weak TGR5 agonist (with an EC50 of ~50μM), and hence it is likely that effects on body weight and energy expenditure are mainly mediated via FXR agonism. It is therefore uncertain what role this drug might have in directly regulating energy expenditure via TGR5 mechanisms.

FXR agonist effects and clinical safety

Clinical adverse events (AEs) were assessed in the pilot study of OCA in patients with NAFLD in which 23 patients received placebo, 20 received 25 mg daily, and 21 received 50 mg daily.[46] The frequency of AEs was similar in the 25 mg group compared to placebo, whereas constipation (24%) and headache (14%) were reported with the 50 mg dose, a dose not being evaluated in the FLINT trial.

2.7 The vanguard study design

Because the data supporting obeticholic acid as a treatment of NASH is limited to one pilot clinical trial, animal studies, and cell culture studies, the FLINT trial of obeticholic acid to treat NASH will use a "vanguard" trial design. A vanguard trial is designed to analyze predetermined interim data and based on

this analysis, either continuation of recruitment and participation of subjects for the planned duration or discontinuation of the trial will occur. In the case of the FLINT trial, this study design will allow evaluation of an interim surrogate endpoint (serum ALT) to determine if sufficient evidence is found that obeticholic acid is having a beneficial effect to justify continuing enrollment and performing end-of-treatment liver biopsies.

One example of the vanguard trial design is the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial.[75] The ACCORD trial recruited subjects over two non-contiguous periods. An initial vanguard phase was conducted to test the trial design and feasibility. The focus was not on response variables, but the ability of interventions to control glycemic control and blood pressure to achieve these goals in an initial subgroup. The ACCORD trial was terminated early on the recommendation of the DSMB because of adverse outcomes associated with intense glycemic control, but these were not identified in the vanguard phase.

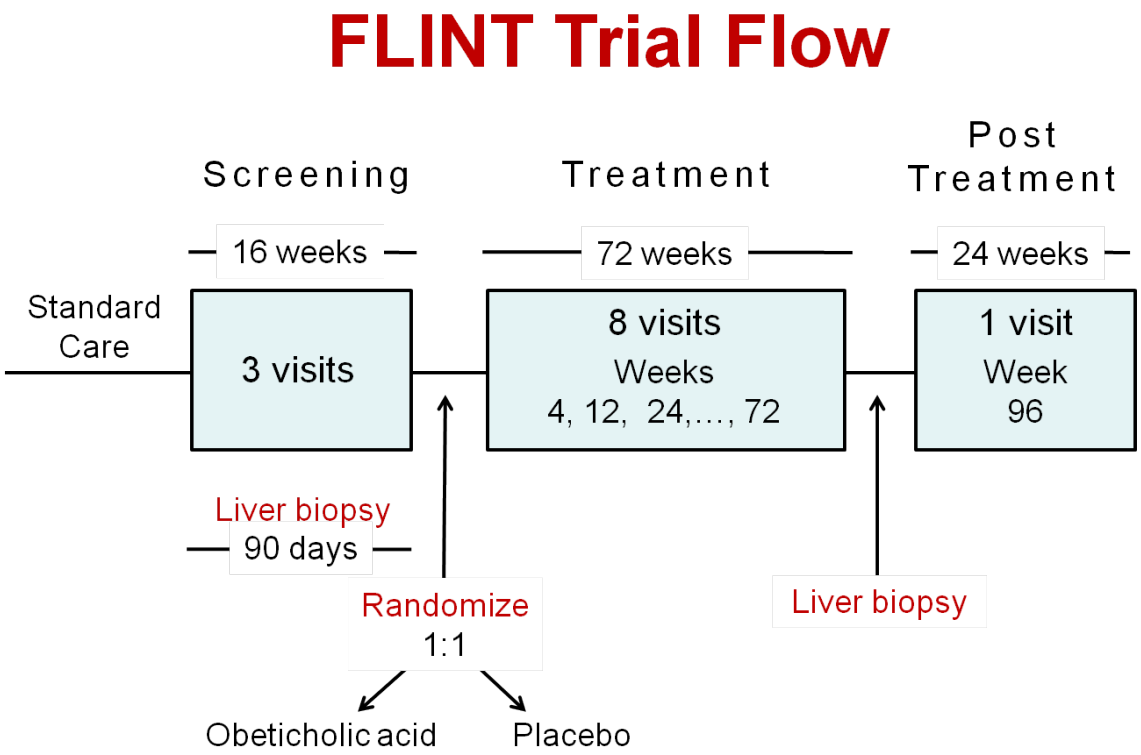
3. Study design

3.1 Design overview

The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trial is a multi-center, randomized, double-masked, placebo-controlled, phase 2b clinical trial of treatment with either obeticholic acid or placebo in patients with NASH. Screening for eligibility and collection of baseline data will span up to 16 weeks (112 days). Eligible patients will be randomized to receive either obeticholic acid (25 mg daily) or placebo daily for 72 weeks. The patients will return for safety visits (weeks 2 and 4) and follow-up visits every 12 weeks after randomization (weeks 12, 24, 36, 48, 60, 72), with a final follow-up visit 24 weeks after the end of treatment (week 96).

Given the limited efficacy and safety data in human studies in NASH, a vanguard analysis will be utilized to assess the interim safety and efficacy of obeticholic acid compared to placebo approximately 65 weeks after the first patient is randomized. The pre-specified interim criterion for continuing the FLINT trial beyond the vanguard phase is based on serum ALT and detailed safety data to determine whether any emergent safety issues are present. The interim analysis and decision to continue on treatment for the entire 72 weeks duration of the FLINT trial will be completed within an 8 week period. Following the 72 weeks of treatment phase, there will be a 24 week washout period to assess durability of effects, if any, and to ensure patient safety following the end of treatment.

The primary comparisons will be made using an intention-to-treat analysis of the change in the NAFLD Activity Score (NAS), as determined from standardized histologic scoring of liver biopsies taken at baseline and at week 72. Secondary outcome measures include change in (a) serum aminotransferase levels, (b) hepatic steatosis by MRI, (c) conventional anthropometric measurements, (d) insulin resistance by HOMA and adipo-IR index, (e) serum lipid levels, and (f) health related quality of life scores. This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements. Patients will be asked to participate in the NAFLD Adult Database 2 Study, an observational study with annual follow-up visits, after the trial has ended.



3.2 Treatment groups

Patients who have signed an informed consent statement and who meet the eligibility criteria will be randomly assigned to one of the two groups for 72 weeks of treatment:

Group 1: Obeticholic acid (25 mg daily as a capsule orally)
Group 2: Placebo (as an identical capsule as active drug)

The randomization scheme will assign patients in randomly permuted blocks of assignments stratified by presence of diabetes and clinical center; block size will be determined randomly. This scheme will ensure that the two groups will be balanced by calendar time of enrollment (to minimize secular effects), by clinic (to minimize clinic-specific effects of differences in patient populations and management), and by diabetes status.

The randomization plan will be prepared and administered centrally by the Data Coordinating Center (DCC) but will not require real time interaction with a DCC staff member. Requests for randomizations will be made by the clinics using a web-based application. An assignment will be issued only if the database shows that the patient is eligible, has signed the consent statement, and has had all required baseline data keyed into the database.

Drug administration

Obeticholic acid will be administered orally as a single capsule of 25 mg per day. An identical appearing placebo capsule will be taken daily by patients assigned to the placebo group. Patients will be instructed to take the medication at the same time each day to the extent that is practical, and they will be told not to take more than one dose in a day if they miss a dose.

Rationale for 25 mg daily dose of obeticholic acid

A recent small randomized-placebo-controlled study using obeticholic acid in patients with NAFLD based upon either elevated ALT, imaging, or histology who were treated with either obeticholic acid 25 mg orally daily or obeticholic acid 50 mg orally daily or placebo for 6 weeks showed that the 25 mg dose led to statistically significant improvement in serum ALT and GGT compared to placebo.[46] The patients receiving obeticholic acid at 50 mg orally daily did not have any improvement in ALT although GGT improved at this dose compared to placebo. Side effects were similar to placebo with the 25 mg dose, but constipation and headache were reported more commonly in patients receiving 50 mg of obeticholic acid daily. Given the improvement in ALT and GGT over 6 weeks of treatment and a better side-effect profile with obeticholic acid, the 25 mg per oral daily dose was chosen for this trial.

Rationale for a placebo group

In order to assess the efficacy of an agent in NASH, a placebo-arm is needed to determine its relative efficacy in improving NASH histology beyond that achieved with a placebo.[76] Currently, there are no FDA approved therapies for NASH. The recently completed PIVENS trial for NASH did not find either vitamin E or pioglitazone to be uniformly effective. The study demonstrated that 43% of vitamin E treated patients and 34% of pioglitazone treated patients met a pre-determined histological endpoint compared to 19% of placebo treated patients ($P = 0.001$ and $P = 0.04$ for each drug respectively).[37] Previous non-randomized and pilot studies have shown the efficacy of several agents such as ursodiol and betaine in the treatment of NASH, but follow-up randomized, placebo-controlled studies failed to show improvement in liver histology in patients with NASH beyond that observed in placebo groups.[77, 78] In order to have the highest quality of evidence to test our hypothesis, the FLINT trial utilizes a randomized, double-masked, placebo-controlled study design. As there is no proven pharmacologic therapy for NASH, using a placebo for comparative purposes is justified.

Rationale for duration between liver biopsy and randomization

Liver histology, especially steatosis, may change over time. A 90 day window provides for adequate time to screen eligible patients but minimizes the likelihood of changes in liver histology before the start of treatment.

Standard treatment recommendations

In addition to the study medication, patients will receive a set of recommendations about life-style modification (dietary modification, weight loss, exercise), use of prescription or non-prescription medicines or herbal remedies or dietary supplements, consumption of alcohol, and management of various co-morbid illnesses. These recommendations have been prepared by the NASH CRN Standard of Care Committee and are approved by the NASH CRN Steering Committee to be applied across all study sites. This will help ensure that the subjects in both groups receive the same standard of care treatment for NASH.

Rationale for 72 week treatment duration

An ideal duration of a treatment should be long enough to show meaningful improvement in liver histology if it is going to occur yet short enough so the positive findings can be reported as soon as possible. The most robust data available on treatment and placebo responses in subjects followed at the NASH CRN study sites are provided by the PIVENS trial in which subjects were treated for 96 weeks. A treatment duration of less than 48 weeks may not be long enough to allow measurable improvement in liver histology, which would lead to an increased likelihood of a type 2 error. A treatment period of 72 weeks was chosen as it is relatively close to the 96 week PIVENS treatment period, and thus reasonable extrapolations can be made regarding treatment and placebo effects with an acceptable balance between

type 1 and type 2 error rates. A 72 week study satisfies the following three needs: 1) Sufficient duration of treatment to show efficacy relative to placebo, 2) Time to allow for the interim vanguard analysis, and 3) Sufficient time to stop the study for futility or adverse side-effects before the first subject undergoes a follow-up liver biopsy.

3.3 Vanguard analysis

Rationale

In order to safeguard the interests of the patients against futility, unnecessary liver biopsies, and adverse events attributable to the study drug, the FLINT trial utilizes a vanguard study design. This is needed because there are no human data on the efficacy and safety of obeticholic acid as a treatment for NASH. The vanguard design is one that allows interim data analysis with a decision regarding trial design or continuation based on the results of this analysis.

Timing

The vanguard analysis will be performed 65 weeks after the enrollment of the first subject. We anticipate that approximately 25-40% of subjects will have completed 24 weeks of treatment with either placebo or obeticholic acid by this time.

Measures

ALT measurement at 24 week visit (window: 18-30 weeks after randomization).

Analysis

The interim ALT target will be considered met if the upper 95% confidence limit includes a 20% net reduction in serum ALT from baseline to 24 weeks comparing the obeticholic acid versus placebo groups.

Study continuation or discontinuation

After analysis of the interim vanguard data by the DCC, the results of the analysis will be presented and discussed during a meeting of the DSMB attended by the DSMB, DCC, and NIDDK. Shortly thereafter, the results of the analysis and the recommendations of the DSMB will be discussed on a conference call attended by the DCC, NIDDK staff, and industry partner. The final decision to continue or stop the study will be made by the NIDDK program official for the NASH CRN in consultation with the Data Coordinating Center, the DSMB, and the industry partner. This decision will be reached before any follow-up biopsies are obtained. The NASH CRN investigators will not be told the results of the analysis unless the decision has been made to discontinue the trial. If the NIDDK Program Official determines that the trial should be continued, only the decision to continue will be communicated; the investigators will not be told any details or results of the interim vanguard analysis. No study completion liver biopsies are to be performed until the decision has been made to continue the study.

Site notification of study termination

If the study is stopped based on the interim vanguard analysis or for safety reasons, clinical sites will be notified of study discontinuation before any follow-up biopsies are performed. The NIDDK will send a memorandum through the DCC to all the clinical sites to terminate the study.

Study termination plan

1) All subjects will be contacted by phone to stop the study medication within one week after notification from the DCC. A letter will also be sent to each subject instructing them to stop the study

medication. This process would be executed by each clinical site.

2) Each subject will return for a research clinic visit and be asked to bring back all the unused study medications. The number of capsules returned will be recorded and reported back to the DCC.
3) After study medication discontinuation, the subjects will be followed for another 12 weeks after the end of treatment for safety reasons. The DCC will notify the clinical sites regarding the treatment group allocation of individual subjects and each site PI or designee will inform subjects of their treatment group assignment after the 12 week post treatment termination visit and after the trial database is locked.

3.4 Primary endpoint

Liver histology improvement will be the basis of the primary end point. The definition of improvement after treatment as the primary outcome measure requires the following two conditions:

- No worsening of the fibrosis score and
- A decrease in NAS by 2 or more points

Justification for performing a liver biopsy

NASH is a clinico-pathologic entity and liver biopsy characteristics are required for diagnosis of NASH. Currently, there are no non-invasive markers to diagnose or grade NASH. Therefore, a liver biopsy is needed to document improvement in NASH. Several of the histologic features of NASH including ballooning degeneration and fibrosis have prognostic significance and predict liver-related deaths in patients with NAFLD. Therefore, documentation of improvement of the pathologic lesions of NASH in liver biopsy is needed to show efficacy in a clinical trial.

Rationale for using the Nonalcoholic Fatty Liver Disease Activity Score (NAS)

The primary outcome measure requires improvement in NASH activity after 72 weeks of treatment as determined by liver biopsies pre- and post-treatment. The measure is derived from changes from baseline to the end of treatment in the NASH CRN NAFLD activity score (NAS).[79] The NAS ranges from 0 to 8 (highest activity) and is calculated as the unweighted sum of three components of the standardized histologic feature scoring system for liver biopsies:

NAS = Steatosis score (0-3)
+ Lobular inflammation score (0-3)
+ Hepatocyte ballooning score (0-2)

The NAS provides a semi-quantitative tool to assess treatment response in a clinical trial. It has been validated, and this instrument is considered a gold standard in the field. It was utilized to assess the efficacy in the PIVENS and TONIC trials conducted by the NASH CRN. [37] This would also provide a tool to compare the findings across various treatment trials within and outside the NASH CRN. Therefore, we believe that the NAS provides the most objective and reproducible tool that is available to assess efficacy of treatment in NASH.

3.5 Secondary endpoints

Histology

- Proportion with a change from a histological diagnosis of definite NASH or indeterminate for NASH to not NASH at end of treatment
- Change in mean NAS

- Individual histological characteristics at end of treatment compared to baseline such as steatosis, lobular inflammation, portal chronic inflammation, ballooning, fibrosis score, and stage 1a vs 1b fibrosis

Laboratory

- Time course and end of treatment improvement in ALT, AST, GGT, total bilirubin, direct bilirubin, and alkaline phosphatase
- Time course and end of treatment improvement in CK-18 derived fragments in blood.[80]
- Time course and end of treatment free fatty acid levels
- Time course and end of treatment total cholesterol, LDL cholesterol, HDL cholesterol, and serum triglycerides
- Changes in hepatic and adipose insulin sensitivity as measured by HOMA-IR and adipo-IR index respectively
- Changes in fasting glucose and 2 hour glucose during glucose tolerance testing
- Changes in FGF-19 levels
- Changes in levels of bile acids and bile acid metabolites (e.g., 7alpha-hydroxy-4-cholesten-3-one[48])

Imaging

- Change in hepatic fat fraction determined by MRI

Symptoms and exam

- Time course and end of treatment Health-Related Quality of Life assessed by standardized and validated questionnaire
- Change in weight, body mass index, waist circumference, waist-hip ratio, and blood pressure

4. Patient selection

4.1 Recruitment

Approximately 280 patients will be recruited from the eight clinical centers of the NASH CRN (averaging 35 patients per center) over an 18 month period. Eligible patients will be identified and recruited at the participating clinical centers subject to the inclusion and exclusion criteria. Clinics will be expected to recruit sufficient overall numbers of minorities and women so that results can be generalized to these populations. Each clinic will develop a recruitment plan. These plans will vary from clinic to clinic depending on the available pools of patients and local recruitment resources and referral patterns.

4.2 Inclusion criteria

Patients must satisfy all of the following criteria to be eligible for enrollment:

- 18 years of age or older as of the initial screening interview and provision of consent
- Histologic evidence of definite or probable NASH based upon a liver biopsy obtained no more than 90 days prior to randomization and a NAFLD activity score (NAS) of 4 or greater with at least 1 in each component of the NAS score (steatosis scored 0-3, ballooning degeneration scored 0-2, and lobular inflammation scored 0-3).

4.3 Exclusion criteria

Patients who satisfy any of the following exclusion criteria will be ineligible for enrollment:

- Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening (significant alcohol consumption is defined as more than 20 g/day in females and more than 30 g/day in males, on average)
- Inability to reliably quantify alcohol consumption based upon local study physician judgment
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other known hepatotoxins) for more than 2 weeks in the year prior to randomization
- Prior or planned (during the study period) bariatric surgery (eg, gastroplasty, roux-en-Y gastric bypass)
- Uncontrolled diabetes defined as HbA1c 9.5% or higher within 60 days prior to enrollment
- Presence of cirrhosis on liver biopsy
- A platelet count below 100,000 /mm^3
- Clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities:
  - Serum albumin less than 3.2 g/dL
  - INR greater than 1.3
  - Direct bilirubin greater than 1.3 mg/dL
  - History of esophageal varices, ascites or hepatic encephalopathy
- Evidence of other forms of chronic liver disease:
  - Hepatitis B as defined by presence of hepatitis B surface antigen (HBsAg)
  - Hepatitis C as defined by presence of hepatitis C virus (HCV) RNA or positive hepatitis C antibody (anti-HCV)
  - Evidence of ongoing autoimmune liver disease as defined by compatible liver histology
  - Primary biliary cirrhosis as defined by the presence of at least 2 of these criteria
    (i) Biochemical evidence of cholestasis based mainly on alkaline phosphatase elevation
    (ii) Presence of anti-mitochondrial antibody (AMA)
    (iii) Histologic evidence of nonsuppurative destructive cholangitis and destruction of interlobular bile ducts[81]
  - Primary sclerosing cholangitis
  - Wilson's disease as defined by ceruloplasmin below the limits of normal and compatible liver histology
  - Alpha-1-antitrypsin(A1AT) deficiency as defined by diagnostic features in liver histology (confirmed by alpha-1 antitrypsin level less than normal; exclusion at the discretion of the study physician)
  - History of hemochromatosis or iron overload as defined by presence of 3+ or 4+ stainable iron on liver biopsy
  - Drug-induced liver disease as defined on the basis of typical exposure and history
  - Known bile duct obstruction
  - Suspected or proven liver cancer
  - Any other type of liver disease other than NASH
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Serum creatinine of 2.0 mg/dL or greater
- Inability to safely obtain a liver biopsy
- History of biliary diversion

- Known positivity for Human Immunodeficiency Virus (HIV) infection
- Active, serious medical disease with likely life expectancy less than 5 years
- Active substance abuse including inhaled or injection drugs in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in an IND trial in the 30 days before randomization
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Failure to give informed consent

5. Trial protocol

5.1 Visit schedule overview

The patient-related activities of the FLINT trial can be divided into 4 phases:

- Screening for eligibility for enrollment (up to 2 visits over a maximum of 16 weeks)
- Randomization to treatment (1 visit)
- Treatment phase (8 visits over 72 weeks)
- Post-treatment washout phase (1 visit at 96 weeks)

The visit and data collection schedule described below in detail is summarized in Appendix 9.2.

5.2 Screening and baseline data collection

Patients who appear to be eligible after chart review and have completed the standard of care tests and procedures for NASH or suspected NASH will be invited to undergo screening. Recording of screening data on NASH CRN forms may not start until the patient has signed the consent statement. Screening and baseline data collection procedures will include questionnaires, physical examination, complete blood count (CBC), measurement of fasting serum glucose and insulin, routine liver, lipid and metabolic tests, etiologic tests, and pregnancy testing. Prior therapy for NASH will be reviewed, and patients will be advised based upon inclusion and exclusion criteria relevant to the anti-NASH therapies. The initiation of treatment and use of vitamin E or thiazolidinediones (TZDs) is allowed.

Patient charts will be reviewed for historical information and previous liver biopsy findings. Patients suspected of having NASH but who have not had a liver biopsy previously will have a standard of care biopsy if clinically indicated to establish their diagnosis and guide their clinical management. If the biopsy shows NASH and meets the FLINT entry criteria, this biopsy will also be used for entry into the FLINT Trial.

Patients who have had a previous biopsy and a follow-up standard of care biopsy is not clinically indicated or whose previous liver biopsy does not meet the requirements for this trial may undergo a liver biopsy for the purpose of screening for the trial.

All participants who sign the consent statement will be registered in the trial database. Each participant who starts screening will be accounted for at the end of screening, as either screening success (enrolling in the trial) or a screening failure. A screening failure is defined as a participant who has 1) signed the consent form, but is found to be ineligible prior to randomization, 2) met medical eligibility criteria, but who refused enrollment in the trial, or 3) a liver biopsy that did not meet entry criteria.

Reasons for screening failure will be recorded in the trial database. Screening and baseline data collection will be conducted over two clinic visits usually completed on separate calendar days. The goal of the first screening visit is to obtain consent and record data regarding the trial's inclusion and exclusion criteria; the goal of the second screening visit is to complete collection of baseline data on patients who appear eligible. This separation of procedures between two visits is provided as a practical guideline. Screening procedures and data collection can be organized as appropriate at each clinical center. If a liver biopsy is required, it will be done after the patient has been found to be eligible with respect to other inclusion and exclusion criteria and prior to randomization. Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy. The procedures completed during screening are described below, and the screening period can last up to 16 weeks.

Screening visit 1

Determination of eligibility will be based mostly on chart review of standard of care tests and procedures that were completed before screening visit 1. The patient will sign the consent at screening visit 1 to obtain any tests and procedures needed to finalize eligibility after chart review and will undergo a history and physical examination to identify other abnormalities and contraindications for participation. Anthropometric assessments (body weight [kg], body height [cm], body mass index [BMI], waist circumference [cm], hip circumference [cm], and waist-to-hip ratio); vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature); examination for sclera icterus and pedal edema and auscultation of the heart and lungs; and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor, will be collected and recorded. History of prior liver biopsies and use of anti-NASH, antidiabetic, statin and fibrate medications in the 90 days prior to the biopsy will be obtained and recorded. The patient's HIV lab results will be only be recorded from chart review. Additional laboratory test results that need to be recorded from chart review or obtained as part of screening visit 1 include hepatitis B (HBsAg) and hepatitis C (anti-HCV), antinuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA), ceruloplasmin (if patient is less than 40 years old), A1AT concentration, iron, ferritin, and transferrin saturation, fasting glucose, insulin, CBC (hemoglobin (Hgb), white blood count (WBC), platelets, mean corpuscular volume, hematocrit), prothrombin time and INR, complete metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, blood urea nitrogen (BUN), creatinine, uric acid, albumin, total protein), GGT and hepatic panel (ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), and hemoglobin A1c (HbA1c). Frequency and amount of alcohol intake will be obtained (AUDIT). In addition, cardiovascular risk factors per Adult Treatment Panel III (ATP III)[82] guidelines will be assessed. Women of childbearing potential must have a negative pregnancy test to proceed to randomization.

Baseline liver biopsy

Patients who have not had a liver biopsy within 90 days of randomization or whose previous liver biopsy is not available for review or whose previous liver biopsy is of inadequate quality must have a liver biopsy no more than 90 days prior to randomization to be eligible to participate in the FLINT trial. The biopsy should be done once the patient has been found to be eligible with respect to all other criteria. Biopsy tissue should be of adequate size (1.5 cm or more in length and obtained with at least a 16 G instrument) and of adequate quality for interpretation. The patient must have histologic evidence of definite or probable NASH on the baseline liver biopsy and a NAS of 4 or greater with a score of at least 1 in each component of the NAS (steatosis scored 0-3, lobular inflammation scored 0-3, ballooning scored 0-2).

In the case of a biopsy done previously as standard of care, the NASH CRN study physician should check

if tissue blocks and/or additional slides can be obtained from the original biopsy. If the liver biopsy is completed as part of screening for this trial, the liver tissue will be prepared for light microscopy and stains will include hematoxylin and eosin, Masson's trichrome, and iron stain; additionally, a piece of liver tissue will be snap frozen and stored at -70 degrees C and set aside for banking.

Clinic staff should note that the date of the biopsy establishes a hard window for completion of screening for randomization – randomization must take place within 90 days of the date of biopsy. If the biopsy is more than 90 days old when the patient enters screening or if the patient has medications that need to be washed out in the opinion of the site PI, the biopsy will be too old by the time randomization occurs. This will require a new biopsy. Clinic staff will have to monitor completion of screening procedures and speed things up if the closing date on the 90 day biopsy window is getting close.

Baseline MRI

Patients who agree to have optional MRI studies as part of the FLINT trial and who have provided additional consent will undergo MRI assessment of liver fat at a point between screening and randomization in accordance with the plan as outlined below. The MRI exam must be performed no more than 90 days from the time of biopsy and done prior to randomization.

Screening visit 2

Patients will provide 20 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for FGF-19 and CK-18) at the NIDDK Biosample Repository. Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy. If patients have given additional consent, then they will provide 20 mL of blood for DNA banking at the NIDDK Genetics Repository. Patients will complete a health-related quality of life questionnaire (HRQOL, SF-36) and a life time drinking history questionnaire (Skinner). Laboratory tests for any remaining required screening labs from screening visit 1 will be performed at this time. A standard 75g oral glucose tolerance test (OGTT) with fasting serum glucose, and insulin measured at 0 and 2 hours will be administered to patients without a diagnosis of diabetes. Women of childbearing potential must have a negative pregnancy test to proceed to randomization. All patients will be given information on a healthy life style and diet appropriate for their weight and other factors.

The NASH CRN data system will include software to check patient eligibility based on keyed data forms. The eligibility check task may be run at any time, and there is no limit on the number of times it may be run. The output from the task will list the eligibility checks that the patient has failed and a summary finding that the patient is eligible or ineligible for the trial. Thus staff can use this task to identify the items that still need to be completed, keyed, or verified after data from screening visit 1 are keyed and again after data from screening visit 2 are keyed. The randomization visit should not take place until the eligibility check indicates that the patient is eligible except for the items that can be completed only at the randomization visit.

5.3 Randomization visit

The randomization visit is the visit at which randomization takes place and the patient is issued the study medication randomly assigned to the patient. Randomization is the act of generating the random study medication assignment and is the procedure which defines a patient's enrollment into the trial. Randomization can only occur after eligibility has been fully checked and all data collected at screening visits 1 and 2 have been keyed to the trial database. Since these processes take time, randomization cannot be done at screening visit 2, and since study medication needs to be issued to the patient, the randomization visit must be completed in person with the patient. Therefore, a visit separate from

screening visit 2 is necessary. Since this will be a visit on a different calendar day and medication will be started at this visit, good clinical practice requires that a few basic checks of the patient's well-being be completed at the randomization visit.

The procedures completed at the randomization visit are: pregnancy test for women of child bearing potential, verification that the patient is feeling well, affirmation of consent, and generation of the random treatment assignment. The generation process includes the same electronic check on eligibility that the staff may run prior to the randomization visit. The medication assignment will not be generated unless the check finds that the patient is eligible, and the clinic staff indicates that they want to randomize the patient. The random treatment assignment will consist of medication bottle numbers; these numbers will be unique and will be specific to the particular patient and visit they were generated for. They will correspond to numbered bottles of medications which have been sent to the clinical center's research pharmacy (or clinical coordinator if not using a pharmacy) by the NASH CRN Drug Distribution Center. The research pharmacy (or clinical coordinator) will issue the specific numbered bottles to the patient. Each patient's random treatment assignment will be generated for that specific patient and will not be transferable to another patient. Once the assignment has been generated, the patient should be issued the assigned study medication bottles in person and instructed about starting the drug and monitoring for adverse events.

At the randomization visit, patients will be instructed on how to take the study medication. They will be told to take one capsule daily at approximately the same time every day. If that specific time frame is missed, they can take their dose at a later time that same day. If a day's dose is completely missed, they will be instructed to resume dosing the next day at the scheduled time and to not double the dose to make up for a missed dose. They will also be instructed not to take their study medication on study visit days until after the visit has been completed.

The date of randomization is the time zero for reckoning all follow-up visits (ie, all follow-up visits are scheduled at specific times measured from the date of randomization). The randomization computer program will generate a personalized appointment schedule for the patient; this schedule will indicate the ideal date for each follow-up visit, as well as the time window around the ideal date during which the follow-up visit may be done and the data collected at the visit may be used in the trial.

5.4 Follow-up visits

Patients will return for follow-up visits at 2, 4, 12, 24, 36, 48, 60, 72, and 96 weeks after randomization. Each visit will have an interval of time surrounding the ideal date for the visit during which the visit may be done and the data included in the trial database. The ideal target date for a visit is the exact anniversary from randomization. Visit windows will be constructed to be contiguous, so that at any point in time, a follow-up visit window is open. The specific procedures to be completed at each of the follow-up visits are described below.

Week 2 and 4 visits

Follow-up medical history including review of medications, adverse events and study drug compliance, and interim alcohol consumption. These two visits may be conducted by telephone interview. Remind patient not to take study medication on the day of the week 12 visit until the visit has been completed.

Week 12 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol

consumption; focused physical examination, including height, weight, and vital signs (blood pressure, heart rate, respiratory rate, and body temperature). Draw blood for laboratory testing (hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), and fasting glucose and insulin). Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, bile acids, FGF-19 and CK-18) at the NIDDK Biosample Repository. Women of childbearing potential must have a negative pregnancy test to continue on study drug. Dispense study drug and review study drug adherence with patient. Remind patient not to take study medication on the day of the week 24 visit until the visit has been completed.

Week 24 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; HRQOL questionnaire; cardiovascular risk factors assessment; detailed physical examination, including height, weight, waist and hip measurements, vital signs (blood pressure, heart rate, respiratory rate, and body temperature), examination for sclera icterus and pedal edema and auscultation of the heart and lungs; and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor. Draw blood for laboratory testing (complete blood count (Hgb, WBC, platelet count, MCV, hematocrit), metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein), hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), fasting glucose, insulin, and HbA1c). Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, bile acids, FGF-19, and CK-18) at the NIDDK Biosample Repository. Women of childbearing potential must have a negative pregnancy test to continue on study drug. Dispense study drug and review study drug adherence with patient. Remind patient not to take study medication on the day of the week 36 visit until the visit has been completed.

Week 36 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; focused physical examination, including height, weight, and vital signs (blood pressure, heart rate, respiratory rate, and body temperature). Draw blood for laboratory testing (hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), and fasting glucose, and insulin. Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, FGF-19, and CK-18) at the NIDDK Biosample Repository. Women of childbearing potential must have a negative pregnancy test to continue on study drug. Dispense study drug and review study drug adherence with patient. Remind patient not to take study medication on the day of the week 48 visit until the visit has been completed.

Week 48 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; HRQOL questionnaire; cardiovascular risk factors assessment; detailed physical examination, including height, weight, waist and hip measurements, vital signs (blood pressure, heart rate, respiratory rate, and body temperature), examination for sclera icterus and pedal edema and auscultation of the heart and lungs; and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor. Draw blood for laboratory testing (complete blood count (Hgb, WBC, platelet count, MCV, hematocrit), metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein), hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), fasting glucose, insulin, and HbA1c. Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, bile acids,

FGF-19, and CK-18) at the NIDDK Biosample Repository. Women of childbearing potential must have a negative pregnancy test to continue on study drug. Dispense study drug and review study drug adherence with patient. Remind patient not to take study medication on the day of the week 60 visit until the visit has been completed.

Week 60 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; focused physical examination, including height, weight, and vital signs (blood pressure, heart rate, respiratory rate, and body temperature). Draw blood for laboratory testing (hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), and fasting glucose and insulin. Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, FGF-19, and CK-18) at the NIDDK Biosample Repository. Women of childbearing potential must have a negative pregnancy test to continue on study drug. Dispense study drug and review study drug adherence with patient. Remind patient not to take study medication on the day of the week 72 visit until the visit has been completed.

Week 72 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; HRQOL questionnaire; cardiovascular risk factors assessment; detailed physical examination, including height, weight, waist and hip measurements, vital signs (blood pressure, heart rate, respiratory rate, and body temperature), examination for sclera icterus and pedal edema and auscultation of the heart and lungs; and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor. Draw blood for laboratory testing (complete blood count (Hgb, WBC, platelet count, MCV, hematocrit), metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein), hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), fasting glucose, insulin, HbA1c, prothrombin time and INR. Draw 20 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, bile acids, FGF-19 and CK-18) at the NIDDK Biosample Repository. Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy. A pregnancy test (for women of child-bearing potential) should be obtained prior to the liver biopsy and MRI exam. Review study drug adherence with patient. Patient would undergo a repeat liver biopsy and an MRI of the liver for fat fraction while patient is still taking the study drug. In addition, patients without a diagnosis of diabetes will also complete a 2 hour OGTT with measurement of glucose and insulin.

Week 96 visit

Follow-up medical history including review of medications, adverse events, and interim alcohol consumption; HRQOL questionnaire; cardiovascular risk factors assessment; detailed physical examination, including height, weight, waist and hip measurements, vital signs (blood pressure, heart rate, respiratory rate, and body temperature), examination for sclera icterus and pedal edema and auscultation of the heart and lungs; and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor. Draw blood for laboratory testing (complete blood count (Hgb, WBC, platelet count, MCV, hematocrit), metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein), hepatic panel (total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), fasting glucose, insulin, and HbA1c. Draw 10 mL of blood for serum and 10 mL of blood for plasma banking (including measurements for free fatty acids, bile acids, FGF-19 and CK-18) at the NIDDK Biosample Repository. Closeout form will be obtained at this visit.

Patients electing to participate in the NAFLD Adult Database 2 observational study will be followed at subsequent yearly visits.

5.5 Standardized questionnaires

Alcohol Use Disorders Identification Test (AUDIT) is a 10-item questionnaire with a simple scoring scale that will be administered during screening visit 1. A 3- item interim alcohol consumption (AUDIT-C) measuring consumption since that patient's last visit will be obtained during follow-up visits every 12 weeks (12, 24, 36, 48, 60, and 72) and the post-treatment visit (week 96) as part of the follow-up medical history.

Skinner Lifetime Drinking History is a detailed questionnaire that will be administered during screening visit 2.

Health Related Quality of Life (HRQOL,SF-36 version 2) is a 36-item, self-report measure designed to assess the quality of life in patients that will be administered during screening visit 2 and follow-up visits (24, 48, 72, and 96).

5.6 Drug dispensing

Drug dispensing would be done at the following visits: at randomization visit, and then every 12 weeks including week 12, 24, 36, 48, and 60 visits.

5.7 MRI exam purpose and rationale

The purpose of the MRI exam is to quantify the hepatic proton density fat fraction non-invasively in participants of the FLINT trial. The fat fraction is the proportion of mobile protons in liver tissue attributable to fat and thus is a non-invasive MR-based biomarker of liver triglyceride concentration.

To quantify the fat fraction, the MRI exam will use a fast spoiled gradient recalled echo (FSPGR) sequence that uses a low flip angle to reduce T1 bias, acquires multiple echoes after a single excitation to measure and correct for T2*decay, and uses spectral modeling to address fat-water and fat-fat signal interference effects. Using MR spectroscopy as the reference standard, the proposed MRI technique measures hepatic fat fraction accurately in human subjects at 1.5T or 3T and across different vendors. The technique provides high within-examination and between-examination precision. Linearity is maintained across the entire relevant biological range from <1% to >40% hepatic fat fraction. The technique is robust to minor variations in acquisition parameters, including those that may be encountered during usage in a clinical trial.

The FSPGR sequence proposed for imaging-based hepatic fat fraction quantification can be implemented on any up-to-date clinical scanner and thus can be used at all NASH CRN clinical centers with access to such a scanner. Moreover, the technique is imaging based and covers the whole liver, thus providing information on both the quantity and distribution of hepatic fat fraction. These characteristics represent critical advantages over the alternative technique of single-voxel proton MR spectroscopy to measure hepatic fat fraction in a multicenter clinical trial. While MR spectroscopy is currently considered the most accurate noninvasive technique to quantify hepatic PDFF, [84, 85, 86, 87] it is not available at most NASH CRN clinical or satellite centers, and it is restricted in spatial coverage. The latter limitation may be is problematic for reliable assessment longitudinal changes in hepatic fat content,[88, 89] as is required by the FLINT trial.

Target population

The target population for the MRI exams will consist of eligible participants in the FLINT trial. Eligibility criteria for MRI examination are listed below:

- Inclusion: Willing and able to complete MRI examination procedures
- Exclusion:
  - A contraindication to MRI examinations
  - Extreme claustrophobia
  - Pregnant or trying to become pregnant
  - Weight or girth exceeds the scanner capabilities
  - Any condition or circumstance that, in the opinion of the site investigator, would interfere with completion of MR examinations

Safety screening
Clinical sites will screen subjects for MRI safety per institutional standards.

Exam scheduling
Subjects will undergo two MRI examinations. The first MRI exam will be performed after screening but prior to treatment randomization as contemporaneously as possible to, and no more than 90 days from the baseline biopsy. The second MRI exam will be performed as contemporaneously as possible to, and no more than 6 weeks before and no more than 12 weeks after the post-treatment biopsy. The goal will be to schedule the second MRI exam at the same or similar time of day as the first MRI exam. Clinical sites will determine the logistics of scheduling the MRI exam.

Pre-MRI exam instructions
Subjects will be instructed, if possible, to fast for 4 or more hours prior to the scheduled MRI exam but will be allowed to take necessary medications and small quantities of water.

Exam performance
Subjects will be positioned supine with a phased-array coil centered over the liver. After localizing sequences, an axial multi-echo-echo 2D FSPGR sequence will be performed through the liver. Imaging parameters will be selected as appropriate for 1.5T or 3T scanners. After completion of the exam, clinical sites will transfer MR images to the Radiology Reading Center (RRC). The RRC will be run through the Department of Radiology at University of California, San Diego.

Exam analysis
Image analysts at the RRC will review and analyze images. The analysts will place regions of interest in each hepatic segment. The fat fraction in each region of interest will be calculated using custom software developed by the RRC and validated in clinical patients and research subjects with liver fat fraction values ranging from <1% to >40%.[90, 91] The fat fraction in each region of interest will be recorded. The average fat fraction across segments will be calculated.

Data transmittal
The average hepatic fat fraction value calculated in each subject on each of the two MRI exams will be transmitted by the RRC to the Data Coordinating Center (DCC).

5.8 Liver biopsy
Liver biopsies will be performed 72 weeks after enrollment (window: 66-78 weeks) by the NASH CRN principal investigator designee. End-of-treatment liver biopsies will not be performed until the NIDDK program official has given approval to continue the FLINT trial based on safety and efficacy data obtained from the interim vanguard analysis.

Liver biopsies will generally be obtained from the right lobe of the liver using a 16G or larger biopsy

instrument. If the initial biopsy was obtained from the left lobe, then the 72 week biopsy must be obtained from the left lobe. Biopsies should be at least 1.5 cm in length.

5.9 Specimen repository

Specimens will be collected and stored at the NIDDK Biosample or Genetics Repositories for use as approved by the Steering Committee of the NASH CRN (see Appendix 9.3 for blood collection schedule). Specimens include serum, plasma, liver tissue, and DNA. The blood collected at screening and 12, 24, 36, 48, 60, 72, and 96 week visits will be centrifuged and divided into 0.5 mL aliquots of serum and plasma. Aliquots will be shipped to the NIDDK Biosample Repository and kept in a storage facility at -70 degrees C. When extra liver tissue is available, a portion of the liver biopsy specimen will be placed into 1 ml of RNA*later* Solution within 1 to 5 minutes after biopsy. To prepare the samples for storage at - 70 degrees C, the samples should incubate overnight at 4 degrees C to allow thorough penetration of the tissue, and then transferred to -70 degrees C until it is shipped to the NIDDK Biosample Repository on dry ice. If the patient has provided additional consent, blood will be collected during screening and shipped to the NIDDK Genetics Repository for extraction of DNA samples and stored at -20 degrees C.

5.10 Safety issues

Obeticholic acid

The latest version of the OCA Investigator's Brochure[83)] provides details about the safety profile of obeticholic acid obtained from clinical trials. This will be updated during the study as appropriate and in conformance with the appropriate FDA regulations. The investigators should be familiar with the safety profile of the drug.

To date the following AEs of note have been seen:

Normal Volunteers: Single doses up to 500 mg and multiple doses up to (and including) 250 mg (20 and 10x the dose used in this study, respectively) were administered. At the 100 mg daily dose, modest elevations in both ALT and AST were observed. At 250 mg, reversible elevations in ALT and AST (to a maximum of 5x ULN) were seen. Pruritus was experienced by half of the volunteers at 250 mg.

Primary Biliary Cirrhosis: In a 3 month, placebo controlled study in PBC patients evaluating 10 mg, 25 mg, and 50 mg of OCA pruritus was the only common AE clearly related to OCA use. The frequency of pruritus was higher at both the 25 mg and 50 mg doses and the severity of pruritus was worse in all 3 OCA treated groups (placebo group: 50%; OCA groups: 47-85%); 24% of the patients receiving 50 mg were discontinued from the study due to pruritus (which is the most common symptom in this cholestatic disease). Nausea (generally mild) was the only other AE seen in all three treatment groups more frequently than for placebo. Three patients at 50 mg had serious hepatic AEs (SAEs); two developed increases in bilirubin and liver enzymes which were considered likely related to OCA therapy. The other patient developed a bleed from previously existing esophageal varices. All of these patients recovered. The 50 mg dose is not considered a likely appropriate starting dose in cholestatic liver diseases. Subjects without severe pruritus at lower doses are likely to tolerate higher doses if titrated up.

Type 2 Diabetes Mellitus and NAFLD: A 6 week, placebo controlled study in patients with both diabetes and evidence of NAFLD was conducted with 25 and 50 mg doses. The drug was well tolerated. There were no AEs reported more frequently in more than 1 patient in the 25 mg group, except for palpitations (10%), compared to the placebo group. At 50 mg, only constipation (24%) and nausea (14%), both generally mild, were reported more frequently, compared to the placebo group. Constipation was not seen more frequently in the treated patients in the PBC study and was not expected as mild diarrhea is a common AE seen with CDCA administration. Further studies are needed to determine if constipation, or

other AEs, are associated with OCA therapy.

Placebo
Currently, there are no FDA approved therapies for NASH. Therefore, use of a placebo is justified for comparison of efficacy with obeticholic acid. Previous studies have shown that patients in NASH studies improve even on placebo,[76] and the response rate in the PIVENS trial was 19% in the placebo-arm. These improvements are related to several factors including Hawthorne effect leading to lifestyle changes, and sampling variability. If patients on placebo develop hypertension, or any other metabolic disease, they would receive standard of care treatment by their PCP. The risk of progression of NASH over a 96 week period is minimal based upon the PIVENS trial. Therefore, the placebo-arm is justifiably needed.

Management of adverse events

- During the trial if a patient develops side effects thought to be due to the study medication and requires cessation of the study medication, then the medication will be stopped for 4 weeks. If the side effects disappear, an attempt will be made to reintroduce the study medication after 4 weeks. If the symptoms reappear, the study medication will be stopped and the patient will no longer receive the study medication, but will continue to be followed in the trial according to the protocol, in keeping with the "intention-to-treat" paradigm.

MRI
MRI is a minimal risk procedure if standard precautions and practice are exercised. Standard precautions for MRI procedures include: claustrophobia, anxiety, discomfort from lying supine for 30 minutes, hearing loss, and heating of metal in the body. Patients will be screened by questionnaire and excluded when the presence of metal is known or suspected. Sources of metal include the presence of a cardiac pacemaker or defibrillator; metal fragments in eyes, skin, body; heart valve replacement, brain clips, venous umbrella; being a sheet-metal worker or welder; aneurysm surgery, intracranial bypass, renal, aortic clips; prosthetic devices such as middle ear, eye, joint or penile implants, joint replacements; hearing aid, neurostimulator, insulin pump; intrauterine device; shunts/stents, metal mesh/coil implants; metal plate/pin/screws/wires, or any other metal implants; permanent eyeliner, eyebrows.

Liver biopsy
Patients will have up to two liver biopsies for research purposes during their participation in this protocol. About 20% of people who have a liver biopsy have some degree of pain over the liver that may last a few minutes up to several hours. This occasionally requires pain medication and usually disappears completely within a day or two. A rare complication of liver biopsy is severe bleeding such that a blood transfusion or even radiological/surgical interventions are required to stop the bleeding (less than 1 in 1,000). Very rarely (less than 1 in 10,000 reported cases) death has occurred from bleeding after a biopsy. We intend to minimize the risks associated with liver biopsy (a) by requiring that each of the physicians who will obtain liver biopsies in the NASH CRN be very experienced in safely obtaining the liver biopsy specimens, (b) by not enrolling subjects with cirrhosis or subjects with coagulopathy, (c) by adhering to the good clinical practice in performing the liver biopsy, (d) by assuring that an attending hepatologist or radiologist directly supervises if a physician trainee is performing the procedure, and (e) by considering a transjugular liver biopsy in morbidly obese patients in whom a percutaneous, mid-axillary approach may not be feasible.

Patient privacy
It is the investigator's responsibility to conduct the protocol under the current version of the Declaration of Helsinki, Good Clinical Practice, and rules of local IRBs. The investigator must ensure that the patient's anonymity be maintained in their data submission to the Data Coordinating Center. Patients will be identified only by an identification code but not by their name, SSN, or hospital medical record number. Investigators will maintain a separate confidential enrollment log which matches identifying

codes with the patients' names and addresses (i.e., available only to local clinic staff). All study material will be maintained in strict confidence.

Specimen repository

It is anticipated that serum, plasma, DNA, and liver tissue from the participants will be stored for future studies related to NASH and possibly other liver/metabolic diseases. These samples will be stored at the NIDDK Biosample Repository and NIDDK Genetics Repository. The NASH CRN Steering Committee will develop specific guidelines addressing the issues such as (a) obtaining a separate informed consent, (b) storage, (c) transportation of the material, (d) who will have access to the material, and (e) what investigations will be conducted.

5.11 Adherence and retention

Two important goals of this protocol are to optimize adherence to the pharmacological regimen and to maximize the retention of participants in the study. Assessment of adherence to the assigned study medication will provide clinic staff a means to identify participants having problems with adherence. Adherence will be assessed by:

- Counts of capsules in the patient's returned medication bottles
- Conducting a brief, structured interview, in which the study coordinator will assist the patients to identify problems in taking the study medication and to estimate adherence to the prescribed medicine since their previous visit.

These assessments will guide the consideration of strategies to improve adherence. Resources will be provided to remove barriers to participation such as child or elder care, transportation, and parking expenses. These resources can be provided as cash, transportation vouchers, or parking passes. An honorarium may be paid to patients in recognition of their time and effort twice during the trial when scheduled visits and procedures are completed successfully. Certificates of appreciation may be given at enrollment and at conclusion as an incentive.

5.12 Management of concomitant conditions

Hypertension, hyperlipidemia and diabetes will be managed in conjunction with the patient's primary care physician according to the protocols described in the Standard of Care document prepared by the Standard of Care Committee of the NASH CRN. Pregnancy will be managed according to the guidelines described in section 5.15.

In the event of major dermatological reactions such as generalized urticaria, bullous rashes, exfoliative dermatitis, or Stevens-Johnson Syndrome, study medication will be discontinued immediately and not restarted. For local skin reactions, study medication may be discontinued if the skin reactions are potentially drug related. If the rashes clear, the study medication may be restarted.

If local skin reactions recur with restarting the study medication, study medication should be discontinued. In cases where the study medication has been discontinued, the study medication will be unmasked and the participant, investigator, and the primary care provider will be notified in order to prevent future exposures.

6. Adverse event monitoring and reporting

6.1 Food and Drug Administration

The FLINT trial will be conducted under an Investigational New Drug (IND # 110354) held by the NIDDK. The investigators will complete a Statement of Investigator (FDA Form 1572) and must obtain IRB approval per the Code of Federal Regulations before the initiation of the FLINT trial. The study will not begin until the IND is in effect. The safety data required to meet IND application regulations will be collected through adverse event reporting by the clinic investigators and will be provided by the Data Coordinating Center to the NIDDK for transmission to the FDA, Steering Committee, and DSMB.

6.2 Definitions[92]

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or life-threatening suspected adverse reaction. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

6.3 Adverse event reporting

The FLINT trial investigators and staff will monitor and report adverse events to ensure patient safety. There are two separate sets of government regulations that apply to unanticipated or adverse events in research studies: (1) 45 CFR Part 46, Subpart A; the “Common Rule”,[93] shared by 17 Departments and Agencies and (2) 21 CFR 312,[92)] the FDA regulation for adverse events. The Common Rule requires written procedures and policies for ensuring reporting of “unanticipated problems” involving risks to

participants to IRBs, appropriate institutional officials, and the Department or Agency Head. The FDA regulation requires notification of the FDA and participating investigators of any adverse event associated with the use of a test article that is "both serious and unexpected." Investigators should refer to the FDA guidance *Improving Human Subjects Protection*,[94] for adverse events reportable to their IRB. Since the definitions and reporting requirements for unanticipated events differ between the two sets of Federal regulations, the FLINT trial definitions and procedures for adverse events are designed to satisfy both sets of requirements.

Summary data on adverse events will be monitored by the DSMB quarterly and at its semiannual meetings or more frequently, as needed. These summaries will include analyses comparing rates of adverse events by treatment group, by clinic, or in other subgroups requested by the DSMB. Where applicable, signs and symptoms associated with the adverse event will be graded as to severity by the clinical site staff as mild, moderate, or severe using Version 3.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE).[95]

After each DSMB meeting, the NIDDK will issue a written summary of the review of the study data, including adverse events, for transmission to the IRBs at each of the study centers. Analyses or listings of adverse events will not be provided to the IRBs; however, adverse events involving unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants must be reported to local IRBs as soon as possible after they are discovered. Each participating center is responsible for ensuring that all local IRB requirements for reporting adverse events are met.

Reporting adverse events

Adverse events will be recorded on study data forms whether or not they are thought to be associated with the study or with the study drug. Adverse events may be discovered during regularly scheduled visits or through unscheduled patient contacts between visits. It is the responsibility of the clinical center principal investigator to determine if an adverse event is reportable to their local IRB. Events threatening the integrity of the study, or well-being of the participant should be reported to the Data Coordinating Center by completing and keying the Interim Event Report (IE) form. If the adverse events have a CTCAE Severity Grade of 3 (Severe),or higher, then a copy of the Interim Event Report (IE) should also be emailed or faxed to the Data Coordinating Center for review by the NASH CRN Safety Officer, Dr. Jeanne Clark, within 1 week. A summary of adverse events will be reported to the FDA as part of the IND Annual Report.

6.4 Reporting serious adverse events

Serious adverse events (SAE) must be reported upon discovery at the clinical center. This will involve completing an Interim Event Report (IE) data form describing the severity and details of the event. If the SAE is judged by the study physician to be unexpected and potentially caused by the FLINT study drug, then the investigator must also submit an SAE/IND Safety Report (SR) form, along a narrative summarizing the circumstances of the event, the current status of the patient, and a copy of the IRB submission to the Data Coordinating Center for review by the NASH CRN Safety Officer, Dr. Jeanne Clark and within two working days.Within three days, the DCC will notify the sponsor, NIDDK, and submit a preliminary report for further review of the material of the SAE. If deemed necessary, NIDDK will notify Intercept Pharmaceuticals within 1 week. NIDDK will inform DSMB members of the SAE per their charter.

Expedited IND Safety Report

If the NIDDK Program Official determines that the SAE requires an expedited IND Safety Report, then

the NIDDK program official or the NIDDK Regulatory Affairs Specialist will notify the FDA no more than 15 calendar days from the initial receipt of the SAE (no later than 7 days if the SAE is fatal or life threatening). Intercept Pharmaceuticals will receive a copy of the expedited IND Safety Report that is submitted to the FDA. . If the FDA determines that a change the investigator's brochure, IND or protocol is needed, the DCC will send a copy of the IND Safety Report to all clinical centers with instructions to forward the report to their IRB.

Follow-up IND Safety Report

Within one month of the initial expedited IND Safety Report, NIDDK will submit a Follow-up IND Safety Report to the FDA. The Data Coordinating Center will work the clinical center to obtain additional information and to report the details of the disposition of the SAE. The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form. Intercept Pharmaceuticals will receive a copy of the Follow-up IND Safety Report that is submitted to the FDA..

6.5 Procedures for unmasking treatment assignment

Treatment assignments are double masked throughout the study until all data collection for the trial has been completed and the database of trial data is officially "locked" for analysis (i.e., after completion of the 24 week post trial follow-up for all patients and entry of all data). Every effort will be made to maintain the masking throughout the study except in emergency situations. The code of specific pharmacological treatment will not be broken without the knowledge of the clinical center's principal investigator.

In unforeseen situations where the clinical center principal investigator considers unmasking is in the best interest of the participant's health and well-being, unmasking may be performed. In such circumstances, the NASH CRN Executive Committee will be notified within one working day. Unmasking of study medication may occur under the following conditions:

Severe allergic reaction (Stevens-Johnson Syndrome)*:* Study medication is stopped indefinitely. The patient, primary care provider (PCP), local principal investigator and pharmaceutical manufacturer may be unmasked.

Pregnancy during the study*:* Study medication will be stopped indefinitely, and the coded medication will be unmasked. The patient, PCP, and investigator may be notified of the assigned treatment and the associated risks of teratogenicity.

The Data and Safety Monitoring Board will review all instances of unmasking that occur.

7. Statistical design and analysis

7.1 Hypotheses and outcome measures

Primary Hypothesis

Obeticholic acid at 25 mg orally once daily for 72 weeks is better than placebo in improving liver histologic parameters as measured by the NAFLD activity score in patients with biopsy evidence of nonalcoholic steatohepatitis.

The ***primary outcome measure*** requires the following two conditions:

- No worsening of the fibrosis score
- A decrease in NAS by 2 or more points

Secondary Hypotheses

- Administration of the farnesoid X receptor ligand obeticholic acid for 72 weeks to subjects with biopsy evidence of nonalcoholic steatohepatitis will result in improvement in insulin sensitivity as measured by HOMA-IR and the adipo-IR index.

- Administration of the farnesoid X receptor ligand obeticholic acid for 72 weeks to subjects with biopsy evidence of nonalcoholic steatohepatitis will result in reduction in MRI-determined hepatic fraction.

- Administration of the farnesoid X receptor ligand obeticholic acid for 72 weeks to subjects with biopsy evidence of nonalcoholic steatohepatitis will result in a greater decrease in the proportion of patients with a biopsy diagnosis of definite or probable NASH compared to placebo

- Administration of the farnesoid X receptor ligand obeticholic acid for 72 weeks to subjects with biopsy evidence of nonalcoholic steatohepatitis will result in reduction in serum aminotransferase and gamma-glutamyl transpeptidase.

Secondary outcome measures

- Change in histological diagnosis from NASH or indeterminate for NASH to not-NASH
  - Whereas the primary endpoint is based on changes of specific features of NASH as summed in the NAS, it does not actually evaluate whether NASH resolves with treatment. Thus this essential secondary endpoint of determining whether NASH resolves with treatment will be a major outcome of the trial.
  - It will be anticipated that some subjects judged to have NASH on their locally read entry biopsy will not have NASH on central reading of deeper cuts from the same block.
  - For the purposes of this analysis, subjects with borderline NASH will be included with subjects with definite NASH.
- Mean changes in NAS
- Changes in individual histological characteristics:
  - Mean fibrosis score
  - Change from stage 1b to 1a fibrosis
  - Steatosis
  - Lobular inflammation
  - Portal chronic inflammation
  - Ballooning
  - Other specific features from the histologic scoring system
- Changes in serum aminotransferase levels:
  - Mean change in ALT, AST, and GGT
  - Normalization of ALT or AST using two absolute values to define normality (< 40 U/L and <30 U/L for men and <40 U/L and <19 U/L for women) and GGT (using local upper limits of normal)
  - Time-dependent decline in serum mean ALT, AST, and GGT
- Changes in serum CK-18 fragment assay

- Changes in bile acids
- Changes in FGF-19 levels
- Changes in free fatty acids and adipo-IR (fatty acids x insulin)
- Changes in MRI-determined variables in the subset of patients who meet eligibility requirements and undergo MRI evaluation
  - MRI-determined hepatic fat fraction
- Changes in HRQOL
- Changes in anthropometric measures

7.2 Statistical analysis

Primary hypothesis

Statistical analyses for the primary hypothesis will follow the intention-to-treat paradigm, which means that all randomized patients with baseline and 72 week liver biopsies will be included in the treatment group to which they were assigned. Any randomized patient who does not have the requisite biopsies will be accounted for and compared by assigned treatment group. Patients not able to be included in the intention-to-treat analyses will be compared to those who are included with respect to demographic and other characteristics.

Since the primary outcome measure, defined in sections 3.4 and 6.1, is a binary indicator of improvement in histologic activity score after 72 weeks of treatment compared to baseline and since the randomization is stratified by clinic and diabetes status, P-values will be derived from the Mantel-Haenszel $\chi 2$ test for stratified 2x2 tables. The proportion of patients improved in the group assigned to obeticholic acid will be compared to the group assigned to placebo and a two-tailed p-value of ≤ 0.05 will be considered statistically significant.

Stratification

Given the randomized design and adequate size planned for the FLINT trial, it is unlikely that confounding of the treatment groups by covariates related to the change in histologic activity score will occur. However, if confounding should occur, multivariate-adjusted logistic regression analyses with histologic improvement as the binary response and treatment group indicator and any suspected confounders as covariates will be carried out to determine the sensitivity of the primary P-value to confounding.

7.3 Missing data

The occurrence of missing data in this trial is expected to be low and equally distributed across the treatment groups. We estimate that careful selection of patients during the screening phase and the consent process should result in no more than 10% missing data from patients who drop out before completing the 72 week treatment period. In primary, intention-to-treat analyses, subjects with missing data will be considered unimproved on the primary outcome measure.

The proportions with missing data will be compared across treatment groups using $\chi 2$ tests. If the amount of missing data exceeds 10%, then a variety of sensitivity analyses will be carried out to compare to the primary analysis using all available non-missing data:

(1) compare pessimistic and optimistic imputations of the missing values,

(2) correct for missing data using multiple imputation with 10 replicated samples, and
(3) use mixed random effects logistic or linear regression models, depending on the type of outcome measure.

Sensitivity analyses (2) and (3) assume that the missing data are missing at random (MAR). It is possible that the missing data is not MAR and, therefore, non-ignorable. A few statistical methods are available when there are non-ignorable missing data patterns and these may be employed to assess sensitivity of the results to non-ignorable missing data if the level on the missing data exceeds 10%; however all such methods involve strong assumptions that cannot be verified from the available data.

7.4 Justification of sample size

A total of 280 patients in 2 groups of equal size (140 per group) will be included to compare the efficacy of obeticholic acid group vs. placebo group with the primary outcome measure of histological improvement in NAS (as defined in sections 3.4 and 6.1). A sample size of 280 patients with 1:1 randomization would provide 90% power to the study hypothesis to detect a significant difference between the two groups with a type I error rate of 0.05. This sample size takes into account that 10% of participants will not have 72 week biopsies and will be considered not improved based upon intention to treat analysis. The sample size estimation is based upon the assumption that the minimum clinically important difference, defined as 50% higher relative rate of improvement in the obeticholic acid group vs. the placebo group, will be expected with the assumed response rates in the placebo group in the placebo group of 39% (based on PIVENS data and 2 to 1 split for patients on vs not on vitamin E at start of trial) versus expected percent with improved NAFLD in the obeticholic acid group to be 58%. This estimate was derived using centrally-read histology data from the PIVENS trial in which 48% of the vitamin E-assigned group and 20% of the placebo-assigned group had the primary outcome similar to that defined for the FLINT trial. Weighting these data by the FLINT investigator's estimate that 2/3 of patients enrolled in the FLINT trial will be taking vitamin E, gives the 39% response rate. These sample size estimations are conservative and would have a minimal likelihood of a type 2 error.

7.5 Vanguard analysis

An unmasked vanguard analysis, using change in ALT (U/L) from baseline to the 24 week follow-up visit as the primary vanguard outcome, will be carried out for review by the DSMB in order to advise the NIDDK on whether the FLINT trial should continue. The vanguard design requires that the vanguard analysis and the decision to continue must be completed before any patient undergoes a 72 week end-of-study biopsy. This means that the vanguard analysis of ALT change must occur approximately 65 weeks after the first randomized patient is enrolled when between 70 (25% of total sample size) and 112 (40%) will have a 24 week ALT measure, but before the first patient (or any other patient) has undergone a 72 week liver biopsy.

The vanguard analysis statistical guideline indicating sufficient evidence that, compared to placebo, 24 weeks of treatment with obeticholic acid results in a greater reduction in ALT (BL – 24 weeks) will be considered met if the upper 95% confidence limit for the net percentage reduction in mean ALT (BL-24 weeks) in the obeticholic acid group vs. placebo group is greater than or equal to 20%. The net reduction and 95% upper confidence interval will be calculated from a multiple linear regression model with patient level changes in ALT (U/L) at baseline minus ALT (U/L) at 24 weeks as the response variable (y) and independent variables: obeticholic acid indicator (x1=1, if obeticholic acid, 0, if placebo) and baseline ALT (U/L) (x2): $E(yi)=\beta 0+ \beta 1x1+ \beta 2xi2+\varepsilon i$, i=1,2,…,n; n=number of patients in the vanguard analysis and εi are i.i.d. Gaussian$(0,\sigma 2)$.

The estimated between group (obeticholic acid vs. placebo) difference in mean change in ALT from BL

to 24 weeks is b2 with 95% CI: $b_2 \pm t_{n-3}\,\widehat{\sigma}\,(b_2)$ where t_{n-3} is the 97.5th percentile of the t-distribution with n-3 degrees of freedom and $\widehat{\sigma}\,(b_2)$ is the estimated standard error of b2 from the regression analysis. The estimated net reduction in ALT in the obeticholic acid group, expressed as percentage of the overall baseline mean ALT, $\overline{ALT}$, is 100×b2/($\overline{ALT}$) with upper 95% confidence limit: $100\times[b_2 + t_{n-3}\,\widehat{\sigma}\,(b_2)]$ / ($\overline{ALT}$). SAS PROC REG will be used to fit the regression model and calculate the upper 95% confidence limit to determine whether its value is a 20% or more net reduction in ALT.

The vanguard analysis will also include detailed safety data DSMB, to determine whether any emergent safety issues are present. This vanguard safety analysis will consist of, but is not limited to, counts of adverse events (AEs) and serious adverse events (SAEs) by treatment group with associated P-values based on Fisher's exact tests or other tests, as appropriate.

7.6 Interim analysis

The DSMB will review one planned interim analysis of the primary histological outcome measure. O'Brien-Fleming statistical stopping guidelines for efficacy will be applied. This interim efficacy analysis will occur when approximately 50% (140 of the 280 patients) have completed both baseline and 72 week biopsies. Based on the Lan-DeMets method for group sequential trials using O'Brien-Fleming boundaries,[96] the levels of significance for the interim and final analyses will be alpha=0.00305 and alpha=0.049, respectively.

Adverse events and other safety measures

The interim analysis will also include detailed safety data, as required by the NIDDK and DSMB, to determine whether any emergent safety issues are present. This interim safety analysis will consist of, but is not limited to, counts of adverse events (AEs) and serious adverse events (SAEs) by treatment group with associated P-values based on Fisher's exact tests or other tests, as appropriate. The final decision as to whether to continue the FLINT trial beyond the vanguard phase will be made by the NIDDK program official for the NASH CRN trial, which will be informed by any recommendations from the FLINT DSMB, taking the interim criterion for efficacy, safety, and other relevant information that may arise into account.

An ongoing assessment of adverse events and other safety measures by the Data and Safety Monitoring Board will determine whether the trial should be continued throughout the duration of the trial including at the interim vanguard analysis time point.

Other interim analyses

An independent Data and Safety Monitoring Board (DSMB), appointed by the NIDDK, will review the protocol for the FLINT trial and monitor the accumulated vanguard, interim, and safety data as the trial progresses to ensure patient safety and to review efficacy. The DSMB is a multidisciplinary group with a written charge provided by the NIDDK. The DSMB reports to the NIDDK, which will in turn, communicate DSMB recommendations to the investigators, as appropriate. The DSMB will hold a meeting to approve the protocol. After the trial commences, the DSMB will meet twice a year to review data and other issues. The DSMB may conduct more frequent meetings if necessary to fulfill its charge. It may also request additional safety reports on a more frequent basis. For example, all serious adverse events are reported to the DSMB for their consideration and recommendations as they occur.

Interim data on safety measures requested by the DSMB are reviewed at each of the scheduled semi-annual full meetings. Two additional written safety reports will be reviewed by the DSMB between scheduled full meetings. Serious adverse events will be reviewed by the DSMB as they occur with the option of a teleconference discussion if any DSMB member so requests.

The DSMB will review quarterly reports by masked treatment groups of incident hepatotoxicities, as well as counts of patients who required more frequent liver function testing due to rises in ALT levels of more than 1.5 times baseline ALT or beyond 300 U/L. The DSMB will also examine the trends in ALT or AST levels for each patient who experiences a rise in ALT.

The DSMB will review one planned interim vanguard analysis of the interim outcome measure, which includes ALT, not the primary efficacy outcome, which is liver histologic improvement. This interim efficacy analysis will occur 65 weeks after the first patient is randomized using serum ALT data obtained in each subject at the 24 week visit.

The DSMB also reviews the overall progress of the trial in terms of recruitment and data quality and makes a formal recommendation to the NIDDK at the end of each scheduled meeting as to whether the trial should continue unmodified, continue with protocol modifications, or be stopped

8. Human subject issues

8.1 Overview

The study protocol, questionnaires, and consent forms will be submitted to each participating center's IRB. Sites which recruit patients will submit their recruitment materials to their IRB prior to use. A site may not initiate any patient contact about the FLINT trial until the site has IRB approval for the trial. All study personnel must complete training in the Protection of Human Subjects per NIH guidelines. The proposed study anticipates recruiting a significant proportion of racial/ethnic minorities (African-Americans, Asian-Americans and Hispanics) as well as non-Hispanic white subjects.

8.2 Institutional Review Board (IRB) approvals

A site may not initiate patient screening and randomization activities in the FLINT trial until the site has IRB approval for the trial and the IND is in effect. Consent forms must have IRB approval. Sites must provide the DCC with a copy of the initial IRB approval notice and subsequent renewals as well as copies of the IRB approved consent statements.

8.3 Informed consent

A template consent will be prepared for the trial for screening to determine eligibility with an affirmation of consent for randomization in the trial. Individual sites may add material but may not delete material thought to be necessary for informed consent. Clinics may reformat and reword information to conform to their local requirements. The patient must sign the consent to be eligible for the trial. The consent form will describe the purpose of the trial, the procedures to be followed, and the risks and benefits of participation. Copies of the signed consent forms will be given to the patient, and this fact will be documented in the patient's study record.

8.4 Subject confidentiality

All laboratory specimens, study forms, reports, and other records that are part of the study data collection materials will be identified by coded number to maintain patient confidentiality. All records will be kept in a locked office. All electronic records of study data will be identified by coded number. Clinical

information will not be released without written permission of the patient, except as necessary for monitoring by the IRB. Consent procedures and forms, and the communication, transmission and storage of patient data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA).

8.5 Administration of study drug

An investigator may not administer an investigational new drug to human subjects until the IND goes into effect (30 days after IND receipt by FDA or sooner if notified). An investigational drug under IND may only be used by an investigator in compliance with 21 CFR Part 50 and 21 CFR Part 56.[97,98]

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10. Appendices

10.1 Participating centers

Clinical Centers

- Case Western Reserve University
  - Cleveland Clinic Foundation
- Duke University Medical Center
- Indiana University
- Saint Louis University
- University of California, San Diego
- University of California, San Francisco
- Virginia Mason Medical Center
- Virginia Commonwealth University

Radiology Reading Center

- Liver Imaging Group, University of California, San Diego

Data Coordinating Center

- The Johns Hopkins University Bloomberg School of Public Health

National Institutes of Health

- National Institute of Diabetes and Digestive and Kidney Diseases
- National Cancer Institute

10.2 Visit and data collection schedule

Assessment/Procedure	Screening Visits			Follow-up visits Weeks from randomization								
	S		RZ	2	4	12	24	36	48	60	72	96
Consent and reaffirmation	X	.	X	.	.	.	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.	.	.	.	.	.
Follow-up medical history (with AUDIT-C)	.	.	.	X	X	X	X	X	X	X	X	X
AUDIT, Skinner alcohol questionnaires	A	S	.	.	.	.	.	.	.	.	.	.
Review of concomitant drugs	X	.	X	X	X	X	X	X	X	X	X	X
Review for adverse effects	.	.	.	X	X	X	X	X	X	X	X	X
Drug dispensing	.	.	X	.	.	X	X	X	X	X	.	.
Review of study drug adherence	.	.	.	X	X	X	X	X	X	X	X	.
Physical exam	D	.	.	.	.	F	D	F	D	F	D	D
Cardiovascular risk factors assessment	X	.	.	.	.		X		X		X	X
MRI for hepatic fat	.	X	.	.	.	.	.	.	.	.	X	.
Liver biopsy	X	.	.	.	.	.	.	.	.	.	X	.
HR-QOL (SF-36v2)	.	X	.	.	.	.	X	.	X	.	X	X
Fasting lipid profile	X	.	.	.	.	X	X	X	X	X	X	X
Complete blood count	X	.	.	.	.	.	X	.	X	.	X	X
Metabolic panel	X	.	.	.	.	.	X	.	X	.	X	X
Hepatic panel and GGT	X	.	.	.	.	X	X	X	X	X	X	X
Fasting glucose and insulin	X	.	.	.	.	X	X	X	X	X	X	X
2-hour OGTT (glucose and insulin)	.	X	.	.	.	.	.	.	.	.	X	.
PT and INR	X	.	.	.	.	.	.	.	.	.	X	.
HbA1c	X	.	.	.	.	.	X	.	X	.	X	X
Pregnancy test (females)	X	.	X	.	.	X	X	X	X	X	X	.
Serum, plasma for banking (bile acids, free fatty acids, FGF-19, CK-18)	.	X	.	.	.	X	X	X	X	X	X	X
Etiologic tests	X											
Closeout form	.	.	.	.	.	.	.	.	.	.	.	X

Detailed (D) physical exam includes measurement of height, weight, waist, and hips; vital signs (temperature, heart rate, respiratory rate, and blood pressure); examination for scleral icterus and pedal edema and auscultation of the heart and lungs; general physical findings (hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting, or fetor); cardiovascular risk factors assessment
Focused (F) physical exam includes measurement of height and weight, and vital signs (temperature, heart rate, respiratory rate, and blood pressure).
Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein.
Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
CBC: hemoglobin, white blood cell count, platelet count, mean corpuscular volume (MCV), hematocrit
Oral Glucose Tolerance Test (OGTT): Fasting serum glucose and insulin; 75 gram oral glucose load and then repeat serum glucose and insulin after 2 hours. Will only be done in non-diabetic patients.
Etiologic tests: Hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV), ceruloplasmin (if less than 40 years old), α-1 antitrypsin level (A1AT), and autoantibody studies (ANA, ASMA, AMA), iron, ferritin, and transferrin saturation.
Fasting visits: S1, S2, 12, 24, 36, 48, 60, 72, and 96.
Safety visits: weeks 2 and 4 may be done by telephone interview.

10.3 Blood collection schedule

Assessment/Procedure	Screening Visits S		Follow-up visits Weeks from randomization 12	24	36	48	60	72	96	T
Fasting lipid profile	5	.	5	5	5	5	5	5	5	40
Complete blood count	5	.	.	5	.	5	.	5	5	25
Metabolic panel	5	.	.	5	.	5	.	5	5	25
Hepatic panel and GGT	5	.	5	5	5	5	5	5	5	40
Fasting glucose and insulin	5	.	5	5	5	5	5	5	5	40
2-hour OGTT (glucose and insulin)		20	.	.	.	.	.	20	.	40
PT and INR	5	.	.	.	.	.	.	5	.	10
HbA1c	5	.	.	5	.	5	.	5	5	25
Serum banking*	.	20	10	10	10	10	10	20	10	100
Plasma banking *	.	10	10	10	10	10	10	10	10	80
Other screening (etiologic testing if needed)	20	.	.	.	.	.	.	.	.	20
DNA banking	.	20	.	.	.	.	.	.	.	20
Total	**55**	**70**	**35**	**50**	**35**	**50**	**35**	**85**	**50**	**465**

Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein.
CBC: hemoglobin, white blood cell count, platelet count, mean corpuscular volume (MCV), hematocrit
Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
Oral Glucose Tolerance Test (OGTT): Fasting serum glucose and insulin; 75 gram oral glucose load and then repeat serum glucose and insulin after 2 hours. Will only be done in non-diabetic patients.
Other screening including etiologic tests: Hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV), ceruloplasmin (if < 40 years old), α-1 antitrypsin level (A1AT), and autoantibody studies (ANA, ASMA, AMA), iron, ferritin, and transferrin saturation.
Fasting visits: S1, S2, 12, 24, 36, 48, 60, 72, and 96.

*Measurements of bile acids, free fatty acids, FGF-19, and CK-18 will be derived from banked serum and/or plasma samples.

10.4 Glossary

6-ECDCA	6α-ethyl chenodeoxycholic acid
A1AT	alpha-1 antitrypsin
adipo-IR	adipose tissue insulin resistance
AE(s)	adverse event(s) or adverse experience(s)
ALT	alanine aminotransferase
AMA	antimitochondrial antibody
ANA	antinuclear antibody
ASMA	anti-smooth muscle antibody
AST	aspartate aminotransferase
ATP	adenosine-5'-triphosphate
BAT	brown adipose tissue
BMI	body mass index
BSEP	bile salt export pump
CBC	complete blood count
CYP	cytochrome P450
BUN	blood urea nitrogen
cAMP	cyclic adenosine monophosphate
CDCA	chenodeoxycholic acid
CK-18	keratin-18 (cytokeratin-18)
C_{max}	maximum plasma concentration
CRN	clinical research network
DCC	data coordinating center
db/db	reference for diabetes mouse model
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
EC_{50}	median effective concentration
ECG	electrocardiogram
EMT	epithelial-mesenchymal transition
ER	endoplasmic reticulum
FGF19	fibroblast growth factor 19
FLINT	Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment
FLIRT	fatty liver improvement with rosiglitazone therapy
FSPGR	fast spoiled gradient recalled echo
FXR	farnesoid X receptor
GGT	gamma-glutamyl transferase
GI	gastrointestinal
G-protein	guanine nucleotide-binding protein
GPBAR1	G protein-coupled bile acid receptor 1

HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
HRQOL	health-related quality of life
INR	international normalized ratio
JNK	c-Jun N-terminal kinase
LDL	low-density lipoprotein
MDR3	multidrug resistance 3 gene
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRP	multidrug resistance-associated protein
NAFL	nonalcoholic fatty liver
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD activity score
NASH	nonalcoholic steatohepatitis
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NTCP	Na+-taurocholate cotransporting polypeptide
OATP	organic anion transporting polypeptide
OGTT	oral glucose tolerance test
OCA	obeticholic acid
OST	organic solute transporter
PBC	primary biliary cirrhosis
PIVENS	Pioglitazone vs Vitamin E vs Placebo for Treatment of Non-diabetic patients with Nonalcoholic Steatohepatitis
PPARγ	peroxisome proliferator-activated receptors gamma
PXR	pregnane X receptor
q.d.	once daily
QUICKI	quantitative insulin-sensitivity check index
RXR	retinoid X receptor
RNA	ribonucleic acid
SAE	serious adverse event
SHP	small heterodimer partner
SIRT1	sirtuin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae)
SREBP1c	sterol regulatory element binding protein 1c
T3	triiodothyronine (thyroid hormone)
T4	thyroxine (thyroid hormone)
TZDs	Thiazolidinediones

10.5 Document History

Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial Protocol (09 December 2010)

Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial Protocol (07 July 2011)

- Removed anti-NASH medications as an exclusion criterion
- Removed ursodiol as an exclusion criterion
- Removed requirement of a 90 day washout of medications prior to liver biopsy
- Clarified the use of vitamin E and TZDs for the duration of the trial
- Clarified timing of liver biopsy prior to randomization
- Clarified timing between liver biopsy and MRI exams
- Revised liver biopsy tissue processing using RNA*later* Solution®
- Clarified that free fatty acids and bile acids will be derived from banked serum and plasma
- Clarified that OGTT will only be for non-diabetic patients
- Adjustments made to estimated blood draws

Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial Protocol (28 May 2013)

- Added an interim analysis of the primary outcome measure based on the NASH CRN Pathology Committee central read of 50% of participants with a post-treatment liver biopsy.
- Added the collection of cardiovascular risk factors to better identify patients at risk of cardiovascular disease according to Adult Treatment Panel III Guidelines
- Clarified and expanded procedures for adverse event monitoring and reporting