Clinical Research Protocol

Pilot Randomized Controlled Trial of Spironolactone in Women With Nonalcoholic Steatohepatitis (NASH)

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Approval:

_____ _____

PI or Sponsor Signature (Name and Title) *Date*

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the University of California, San Francisco Institutional Review Board (IRB) with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Title: Pilot Randomized Controlled Trial of Spironolactone in Women With Nonalcoholic Steatohepatitis (NASH)

Protocol Date: June 9, 2020

_____ _____

Investigator Signature *Date*

_____

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List of Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUDIT	Alcohol Use and Disorders Identification Test
CARDIA	Coronary Artery Risk Development in Young Adults
CFR	Code of Federal Regulations
CBC	Complete blood count
CFR	Code of Federal Regulations
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DHEAS	Dehydroepiandrosterone
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
eCRF	electronic case report form
EDC	Electronic Data Capture
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic model assessment for insulin resistance
HRPP	Human Research Protection Program
ICF	informed consent form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IND	Investigational New Drug
INR	International normalized ratio
LDL	low-density lipoprotein
LDL-R	low-density lipoprotein receptors
MeDRA	Medical Dictionary for Regulatory Activities
MRE	Magnetic Resonance Elastography
MRI	Magnetic Resonance Imaging
MRI-PDFF	Magnetic Resonance Imaging Proton Density Fat Fraction

NAFLD	Nonalcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic Steatohepatitis
NF	National Formulary and Drug Standards Laboratory
PI	Principal Investigator
PCOS	Polycystic Ovary Syndrome
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
SAE	serious adverse experience
SAP	Statistical Analysis Plan
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
UCSF	University of California San Francisco
U.S.	United States
USP	United States Pharmacopeial Convention, Incorporated

PROTOCOL SYNOPSIS

TITLE	Pilot Randomized Controlled Trial of Spironolactone in Women With Nonalcoholic Steatohepatitis (NASH)
SPONSOR	Monika Sarkar
FUNDING ORGANIZATION	NIDDK
NUMBER OF SITES	1
RATIONALE	Nonalcoholic steatohepatitis (NASH), or fat-related liver inflammation and scarring is projected to be the leading cause of cirrhosis in the United States (U.S.) within the next few years. Women are at disproportionate risk for NASH, with approximately 15 million U.S. women affected. There is an urgent need to understand risk factors for NASH and its progression in women, and sex hormones may provide a missing link. Our preliminary data support a detrimental role of androgens, or "male sex hormones" on fatty liver in women but no studies have evaluated whether androgens are associated with liver inflammation and/or scarring from fatty liver (aka NASH). To better understand the mechanism by which androgens might promote NASH and/or metabolic co-factors that contribute to NASH, we are conducting a pilot clinical trial to primarily assess the feasibility of using an androgen blocking medication, spironolactone, in women with NASH. Spironolactone was selected because it is has been commonly prescribed for decades with good safety profile and tolerability to treat symptoms of high androgens, like acne and hirsutism in women. Though primarily a feasibility-focused study, we also aim to explore the pathways by which blocking testosterone receptors might alter the biologic processes that promote NASH and its associated metabolic co-morbidities in women.
STUDY DESIGN	This is a single center, double-blind, placebo-controlled, randomized, (2:1) parallel group pilot clinical trial of spironolactone in women with biopsy-proven NASH receiving 6 to 12 months of spironolactone or placebo.
PRIMARY OBJECTIVES	To establish the feasibility and tolerability of the intervention and study outcomes measures.
SECONDARY OBJECTIVES	• To examine the effect of spironolactone on features of NASH on MRE and MRI-PDFF • To examine the effect of spironolactone on features of NASH on liver biopsy • To examine the effect of spironolactone on metabolic parameters that contribute to NASH progression • To examine the effect of spironolactone on sex hormone levels and androgen receptor expression in liver tissue

NUMBER OF SUBJECTS	30 total women: 20 randomized to spironolactone and 10 randomized to placebo
SUBJECT SELECTION CRITERIA	Key Inclusion Criteria: - Women with NASH identified on standard of care liver biopsy performed for clinical purposes. Key Exclusion Criteria: - Uncontrolled diabetes - Alcohol consumption >2 drinks per day for at least 3 consecutive months over the previous 5 years - Other chronic liver disease (i.e. hepatitis B virus, hepatitis C virus, autoimmune hepatitis) or cirrhosis from any cause - Recent or planned upcoming weight reduction surgery within five years of diagnosis of biopsy-confirmed NASH - HIV infection - Drugs associated with fatty liver (i.e. amiodarone, methotrexate, systemic glucocorticoids, tamoxifen, anabolic steroids, valproic acid) for more than 4 weeks prior to baseline or during study - Recent, current, or planned upcoming pregnancy or current perimenopausal status - Renal impairment (glomerular filtration rate <45 ml/min/1.73m or potassium levels > 5.0 mmol/L) - Androgen receptor antagonist use (i.e. flutamine, spironolactone or flutamide) for more than 3 months within one year prior to baseline
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Spironolactone 100mg. Product will be administered orally once daily for a length of 6 to 12 months.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Placebo. Product will be administered orally once daily for a length of 6 months.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 10 or 16 months **Screening:** up to one month **Treatment:** 6 to 12 months **Follow-up:** up to 3 months from end of treatment The total duration of the study is expected to be 4 years. Women will be enrolled and treated on a rolling basis, with a 30 month total recruitment period, 6 to 12 months of treatment per participant, and up to 3 months for the final subject follow-up post treatment.

CONCOMMITANT MEDICATIONS-PROHIBITED	- Androgen receptor antagonists (i.e. flutamine or flutamide) including current use of spironolactone - Epleronone as also blocks aldosterone receptor - Drugs associated with fatty liver (i.e. amiodarone, methotrexate, systemic glucocorticoids, tamoxifen, anabolic steroids, valproic acid)
EFFICACY EVALUATIONS	As a pilot trial this study is not powered to detect an effect of spironolactone on NASH progression in women. The primary goal of the study is rather to evaluate the feasibility and tolerability of the study intervention and outcome measures in women with NASH while also exploring the mechanisms by which blocking testosterone receptors may alter NASH severity or associated metabolic comorbidities leading to NASH. If the intervention is deemed to be feasible, and we detect a signal that spironolactone may alter these biologic processes, then these data would be used to support a larger trial aimed at assessing the efficacy of spironolactone or other related androgen modulating medication on the treatment of NASH in women.
PRIMARY ENDPOINTS	Feasibility endpoints: • Adherence and acceptability of spironolactone in women with NASH • Adherence and acceptability of study outcome measures (MRI and optional EOT biopsy) Determine the range of NASH on baseline biopsy to inform inclusion criteria for larger trials
SECONDARY ENDPOINTS	• Biochemical endpoints: serum lipids, HOMA-IR, liver enzymes • Radiologic endpoints: liver fat quantity, visceral fat volume, liver inflammation, liver scarring • Histologic endpoints: Change in NAFLD Activity Score (NAS) • Expression of androgen receptors in the liver and circulating hormone levels.
SAFETY EVALUATIONS	Spironolactone is an old, commonly used medication to treat symptoms of high androgens in women therefore has a longstanding favorable side effect profile. Change in clinical safety labs and in person review of potential side effects will be assessed at each study visit. Incidence of adverse events will be collected.

PLANNED INTERIM ANALYSES	The Data Safety Monitoring Board (DSMB) will review the progress of the study and perform an interim review of the safety data after the first 10 participants have completed 3 months of spironolactone, or at any time that a comprehensive review of safety is felt to be necessary. The committee will provide recommendations to Dr. Sarkar whether the nature, frequency, and severity of adverse effects associated with study treatment warrant the early termination of the study in the best interests of the participants, whether the study should continue as planned, or the study should continue with modifications. The DSMB may also provide recommendations as needed regarding study design, or changes to study conduct.
STATISTICS **Primary Analysis Plan**	The focus of the pilot trial will be feasibility testing and obtaining preliminary assessments of the effects of spironolactone. Exact 95% confidence intervals for rates of agreement to participate, meeting eligibility criteria, completing follow-up will be calculated using the binomial distribution. Between-group analyses will be performed according to the intention-to-treat principle. We will attempt to collect complete follow-up data on every randomized participant, regardless of adherence to treatment. As randomization in this small sample may not result in the perfect balance of relevant biological variables (e.g., age or weight between groups) we will perform analyses adjusting for any of these that are substantially imbalanced. A planned interim analysis will be conducted after the first 10 participants (25%) have completed 3 months of spironolactone. Interim results will provide useful preliminary safety and feasibility data.
Rationale for Number of Subjects	The sample size of 30 was determined based on budget and feasibility within a 4-year grant timeline. The sample size is also reasonable for pilot trial, and will allow us to estimate effects and feasibility with reasonable precision.

1 BACKGROUND

Nonalcoholic steatohepatitis (NASH), or fat-related liver inflammation and scarring is projected to become the leading cause of cirrhosis, primary liver cancer, and the leading indication for liver transplantation in the United States (U.S.) within the next few years.(1, 2) Women are at disproportionate risk for NASH, with approximately 15 million U.S. women affected, and therapeutic options in NASH are limited. There is an urgent need to understand risk factors for NASH and its progression in women, and our preliminary data support a detrimental role of androgens, or “male sex hormones” on this process in women.(3)

To better understand the mechanism by which androgens might promote NASH, we are conducting a pilot clinical trial using a competitive inhibitor of the testosterone receptor known as spironolactone. Spironolactone was chosen because it is well tolerated and has a favorable safety profile without the associated liver side effects of other androgen blocking medications. Moreover, it has been commonly prescribed for decades to treat symptoms of hyperandrogenism in women, such as hirsutism and acne. While this trial is a feasibility-focused study, we do aim to obtain preliminary data on pathways by which blocking testosterone receptors might alter the biologic processes that promote NASH in women, including measures of NASH severity on biopsy and comprehensive serologic and imaging measures of metabolic co-morbidities.

1.1 Overview of Clinical Studies

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting one third of the United States (U.S.) population.(4) NAFLD encompasses a spectrum of pathology including isolated steatosis in approximately 75% of individuals, with the remaining 25% having more serious fat-related liver inflammation and scarring, known as non-alcoholic steatohepatitis (NASH).(4) While isolated steatosis is more common in men, women have higher rates of NASH, with an estimated 15 million women in the U.S. affected.(5, 6) To prevent liver-related deaths due to NASH- associated cirrhosis and liver cancer- there is an urgent need to understand key risk factors for NASH in women and sex hormones may provide an important link.

Androgens, traditionally called “male sex hormones”, are produced in men and women. Data on androgens in women with NAFLD have largely focused on hyperandrogenic women with Polycystic Ovary Syndrome (PCOS).(7) Indeed half of these women have imaging-confirmed steatosis and liver enzyme elevation.(8, 9) In our prior work in the prospective Coronary Artery Risk Development in Young Adults (CARDIA) cohort we showed that higher testosterone levels in pre-menopausal women were associated with subsequent risk of NAFLD in midlife. Importantly this association was present even among women without hyperandrogenism suggesting that androgens may play an important role in the pathogenesis of NAFLD in a broader spectrum of women that extends beyond the well-recognized subgroup of women with PCOS.(3) Although the

mechanism linking androgens to NAFLD in women is not well-defined, we found that visceral adiposity was a significant mediator of this association, therefore may play an important role along the causal pathway from testosterone to NAFLD in women.

Consistent with our preliminary data, clinical studies have demonstrated increased visceral adiposity in post-menopausal women treated with synthetic testosterone.(10) Testosterone has been further shown to directly regulate lipid metabolism within both visceral and subcutaneous adipocytes in women.(11) Androgens have been shown to regulate cholesterol clearance in the liver.(12) In mouse models of NASH, spironolactone has also been shown to improve HOMA-IR and serum lipids, key metabolic factors that contribute to NASH in humans.(13) Taken together, these data suggest a complex interplay of sex hormones within the liver and need for investigation into their role in NAFLD-related injury in women.

2 STUDY RATIONALE

Women have disproportionate risk for NASH and androgens might promote NASH, or contribute to metabolic derangements that contribute to NASH. Spironolactone is a commonly used medication in women to treat high testosterone levels, and data in animal models and humans support a role of testosterone in promoting metabolic disease including fatty liver. This study will assess whether measures of NASH and associated metabolic co-morbidities that promote NASH may be altered from blocking testosterone receptors with spironolactone. The goal of this study is to assess the feasibility of the intervention and the selected study outcomes, as well as to determine whether biologic processes that lead to NASH are affected by testosterone blockade. If we detect a signal of these biologic effects, our data could be used to support a future, larger clinical trial aimed at evaluating the efficacy of an androgen-modulating therapy to treat NASH in women that incorporates the feasibility results identified in this pilot trial.

2.1 Risk / Benefit Assessment

The risks involved in this pilot clinical trial are reasonable in relation to the anticipated benefits to the participant and to society, including understanding the role of androgens in the pathway of NASH and associated metabolic conditions, which may provide preliminary feasibility and biologic data to support larger trials to develop hormone targeted therapy to treat NASH in women.
The specific risks and methods for mitigating these are as follows:

1) Privacy breach: Eligible participants will be initially contacted in person during clinic visits by the principal investigator or research assistant to ascertain interest in study participation. If participants are interested and come for a screening visit, they will be sent the informed consent form prior to the visit to allow ample time to review. At the study visit, the PI or study coordinator will read aloud the consent form with the

participant, describing the information we are seeking, procedures and indications, the voluntary nature of the study, the risks and benefits, and the ability to refuse or withdraw at any time without adverse consequences. The participant's understanding of the study and its requirements will be confirmed, and any questions they have will be addressed prior to signing consent. A copy of the consent will be retained for the participant and the participant's file, which is kept in a locked cabinet in a locked office.

There is a risk of loss of confidentiality for subjects in the study, despite maximal measures to minimize this risk. Maximal safeguards to maintain participants' confidentiality will be employed, including: 1) unique study identification numbers on all forms and specimens 2) maintaining identifiers in a password-protected database, and 3) restricting access to the password protected database and locked study documents to essential study personnel.

2) Risks of frequent phlebotomy: The risks of routine blood drawing are rare but can include pain, bleeding, bruising at the site, anemia, dizziness, and very rarely an infection at the site. Only trained phlebotomists will perform blood draws. Phlebotomy risks will be minimized by the use of trained personnel and application of pressure after the blood draw. Should any adverse event occur, medical attention by a licensed physician will be provided immediately.

3) Risks of liver biopsy: About 20% of persons having a liver biopsy have some degree of pain over the liver that may last a few minutes to several hours. This rarely requires pain medication and usually resolves within 1-2 days, with most people not needing to alter their daily activities or work schedule. A rare complication of liver biopsy is severe bleeding such that a blood transfusion or an operation is needed to stop the bleeding. These complications occur in less than one in 1,000 times. Very rarely (in less than one in 10,000 reported cases), death has occurred from bleeding after a liver biopsy. Other rare complications of liver biopsy include infection, and puncture of the gallbladder, lung or kidney. These complications occur in less than one in 1,000 times.

On the day of the biopsy, women will receive baseline CBC and coagulation tests to ensure a platelet count > 50,000 and INR < 1.5. Women with values that do not reach these parameters will not undergo biopsy due to increased bleeding risks, although this is not anticipated to be the case as women with cirrhosis that might have such hematologic abnormalities are excluded at baseline. Women with renal failure will also have already been excluded. To reduce bleeding risk, patients are instructed to hold aspirin and non-steroidal anti- inflammatory medications for 7 days prior to the procedure. Participants are observed in our procedural care unit for 2 hours after their liver biopsy, where vital signs are obtained every 15 minutes for one hour and then again 1 hour later for a total 2 hour observation period post biopsy. A complete blood count is performed at 1.5 hours after the biopsy and must be reviewed by the hepatologist prior to discharge home to ensure < 10% decline in hematocrit. If greater hematocrit decline, the patient will undergo additional evaluation including ultrasound to ensure no significant post procedural bleeding. Patients are instructed to limit physical activity, including straining or lifting over 10 pounds, for 48 hours and are provided with emergency contact information for the on-call hepatologist. If any injury occurs, medical attention by a licensed physician will be provided immediately.

4) Risks of Spironolactone: Spironolactone is an old, commonly prescribed blood

pressure medication and diuretic, and also commonly used for treating symptoms of high androgens including acne and hirsutism at a starting dose of 100mg daily. This medication has a longstanding history of favorable side effects profiles. The most likely side effect is increased urination. Other side effects with a dose of 100mg daily in the absence of renal failure (an exclusion criterion for the current study) are < 1%.

Potential side effects include breast tenderness, tiredness, headache, dizziness, lightheadedness, diarrhea, cramping, nausea, vomiting, fever, or confusion. There is a low risk of renal insufficiency, electrolyte disturbances, dizziness and/or dehydration. To minimize any harm, all participants will have baseline renal and electrolytes obtained, an in-person study visit within one month of treatment initiation and then every 3 months thereafter to obtain subjective information regarding medication tolerability, to obtain vital signs, as well as laboratory assessment of sodium, potassium, and creatinine levels. All values will be reviewed by the principal investigator and Data Safety Monitoring Board to determine whether the current dose may be maintained, decreased, or discontinued.

Spironolactone is categorized as Pregnancy Class C due to potential risk of feminization seen in animal studies of male fetuses, though no studies have implicated spironolactone in these birth defects in humans. Urine pregnancy testing will be performed every 4 weeks in all pre- menopausal women. Urine pregnancy testing is not required for women who are not sexually active with men. As sex organ development begins between 6-8 weeks of gestational age, spironolactone would be discontinued no later than week 4 of conception to avoid fetal risk if a woman were to become pregnant.

3 STUDY OBJECTIVES

3.1 Primary Objective

To establish the feasibility and tolerability of the intervention and study outcomes measures.

3.2 Secondary Objectives

- To examine the effect of spironolactone on features of NASH on MRE and MRI-PDFF
- To examine the effect of spironolactone on features of NASH on liver biopsy
- To examine the effect of spironolactone on metabolic parameters that contribute to NASH progression
- To examine the effect of spironolactone on sex hormone levels and androgen receptor expression in liver tissue

4 STUDY DESIGN

4.1 Study Overview

This is a single center, double-blind, placebo-controlled, randomized (2:1), parallel group pilot clinical trial. 30 women are targeted for enrollment. Each participant will be administered a single dose of spironolactone or placebo once daily for a total of 6 months. In person evaluations will take place at Month 1, 3, 6, and at the follow up visit within 3 months of end of treatment (up to Month 9). Participants may opt to extend the

study treatment period to 12 months. Participants who opt to extend will complete additional in person evaluations at Month 9 and Month 12. Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- Experimental treatment – spironolactone, 100 mg once daily
- Placebo or Comparator – one capsule, once daily

Total duration of study treatment will be 6 months or 12 months. Total duration of the study is expected to be ≤10 or ≤16 months (including up to 1 month for screening).

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoints

This is a pilot clinical trial that is largely feasibility focused. The primary goal is to establish if spironolactone is feasible in women with NASH, and if our study outcome measures are reasonable. The feasibility endpoints will include:

- Rates (and reasons) for the following: a) % women that decline/women contacted for study inclusion (i.e. need for a second liver biopsy, concern regarding randomization to placebo) b) % women enrolled/women screened (i.e. exclusion criteria too narrow), c) study dropout (i.e. medication side effects, too frequent study visits, and/or phlebotomy)
- Describe the range of NASH histology seen on biopsy in consideration of designing a future clinical trial.
- Radiologic endpoints: liver fat quantity, visceral fat volume, liver inflammation, liver scarring. These endpoints will be examined because they offer a non invasive way of assessing visceral fat, another metabolic factor that may be altered by testosterone blockade, as well as other non invasive measures of assessing liver fat and inflammation that could potentially allow for avoidance of liver biopsy in future studies.

5.2 Secondary Endpoints

- Biochemical endpoints: serum lipids, HOMA-IR, liver enzymes. These endpoints will be examined because existing data outside of NASH suggest that androgen blockade may alter these metabolic pathways. These metabolic pathways are also keys players in the development and progression of NASH, therefore understanding the biologic mechanism of androgen blockade in women with NASH could be used to support the need for a larger clinical trial aimed at evaluating such hormone modulation to treat NASH in women.
- Histologic endpoints- Change in the NAFLD activity score (NAS), which measures different components of NASH on liver biopsy. If we detect a signal that spironolactone may affect NASH histology, these data could also help support need for larger trial evaluating efficacy of androgen modulating therapy for treatment of NASH in women.
- Expression of androgen receptors in the liver and circulating hormone levels. It is not known whether spironolactone changes expression of androgen receptors in the liver, and this research question can help us to understand the mechanism of spironolactone effects on the liver. Likewise, data are conflicting on whether spironolactone changes circulating hormone levels, and this pathway will be studied by measuring hormone levels pre and post treatment.

5.3 Safety Evaluations

- In-person visits will be conducted at baseline, at one month (given highest risk of adverse events in first month of drug exposure) and every 3 months thereafter. Additionally women will be seen within 3 months after end of treatment to ensure resolution of any drug related side effects. In-person study visits will include a physical exam with vital signs, anthropomorphic measurements, assessment of adherence with pill counts, assessment of side effects, and performance of safety monitoring labs including electrolytes, renal function and urine pregnancy tests. Each visit will also include contraceptive counseling for pre-menopausal women because spironolactone has potential risk of feminization seen in animal studies of male fetuses, though no studies have implicated spironolactone in these birth defects in humans. Pregnancy testing will performed every month to ensure that if a participant were to become pregnant, spironolactone is discontinued within 4 weeks of conception. This will ensure no fetal risks as human sex organ development begins between 6-8 gestational weeks.
- There will be continuous safety surveillance of participants through the reporting of incidence of adverse events (AEs). AEs will be recorded in a systematic manner at every study visit and when otherwise volunteered by the participant.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of NASH who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Women 18 to 45 years of age at Baseline Visit.
2. Documentation of NASH diagnosis confirmed on baseline liver biopsy (performed as clinical care) prior to study enrollment.
3. Written informed consent (and assent when applicable) obtained from subject and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

1. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study
2. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data
3. Uncontrolled diabetes (HbA1c 9.5% or higher within 60 days prior to enrollment)
4. Routine alcohol consumption >7 drinks per week during the preceding 3 months prior to baseline liver biopsy.
5. Other forms of chronic liver disease including hepatitis B virus infection (hepatitis B surface antigen positive), chronic hepatitis C virus (HCV) infection (HCV Ab and HCV ribonucleic acid positive), autoimmune disorders (e.g., primary biliary cholangitis, primary sclerosing cholangitis, and autoimmune hepatitis), drug-induced hepatotoxicity, Wilson disease, iron overload, and alpha-1-antitryspin deficiency, based on medical history and/or centralized review of liver histology

6. Any upcoming weight reduction surgery (e.g., Roux-en-Y or gastric bypass) or weight reduction surgery within five years prior to diagnosis of biopsy-confirmed NASH.
7. HIV infection
8. Receipt of drugs associated with NAFLD (i.e. amiodarone, methotrexate, systemic glucocorticoids, tamoxifen, anabolic steroids, valproic acid) for more than 4 weeks prior to baseline biopsy
9. Perimenopausal status (defined as within 3 years of self-reported menopause) due to unstable hormonal levels during that time
10. Renal impairment defined as glomerular filtration rate <45 ml/min/1.73m or potassium levels > 5.0 mmol/L due to the diuretic effect of spironolactone
11. Participation in another clinical trial of an investigational drug or device
12. History of medication non adherence as noted upon chart review or patient report of difficulty with medication adherence
13. Androgen receptor antagonist use (i.e. flutamine, spironolactone or flutamide) for more than 3 months within one year prior to baseline biopsy.
14. Epleronone use as this is a diuretic that also blocks the aldosterone receptor and could compound side effects
15. Cirrhosis on baseline biopsy as this condition leads to altered sex hormone metabolism
16. Unstable dosing (i.e. dose increase, intermittent use, or initiation) of Vitamin E anytime during the 3 months prior to baseline biopsy
17. Significant weight loss (at least 10% decrease in body weight) over preceding 3 months prior to baseline biopsy
18. Contraindication to MRI scanning (e.g. presence of permanent pacemakers, implanted cardiac devices, etc.)
19. Unstable dosing (i.e. dose increase, intermittent use, or initiation) of diabetes medications or lipid lowering medications anytime during the 3 months prior to baseline biopsy

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for management of metabolic co-morbidities is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7.2 Prohibited Medications and Treatments

Flutamide, spironolactone, or flutamine (because also blocks androgen receptors) and eplerenone (because same diuretic effect as spironolactone and could increase renal side effects) are prohibited during the study and administration will be considered a protocol violation.

Drugs associated with NAFLD (i.e. amiodarone, methotrexate, systemic glucocorticoids, tamoxifen, anabolic steroids, valproic acid) taken for more than 4 weeks are not allowed between baseline and follow-up biopsies.

Any investigational drug throughout the study is prohibited.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

The UCSF pharmacy will generate a randomization table using random number generating software, assigning patients on a 2:1 basis to spironolactone or placebo in a blocked randomization scheme. Patients will be assigned to a treatment arm in the order to which they present for the first day of dosing. Patients assigned to a treatment arm who later drop out of the study will not be replaced in the randomization scheme.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments

Access to the randomization code will be strictly controlled.

A color and size-matched placebo capsule that looks identical to the spironolactone capsule will be used.

Packaging and labeling of test and control treatments will be identical to maintain the blind.

No active drug measurements will be obtained.

The study blind will be broken on completion of the clinical study, after all study endpoints have been ascertained by blinded study coordinators and after the study database has been locked. At that time, the investigators, research staff, and participants will be informed about their treatment group assignments.

During the study, the blind may be broken **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management. Spironolactone has a longstanding favorable side effect profile. Therefore the only condition that will definitively lead to participant unblinding is pregnancy. The UCSF investigational pharmacy would then be notified and responsible for unblinding.

8.3 Test and Control Formulation

Spironolactone and matching placebo will be prepared by a California state-licensed compounding pharmacy.

Spironolactone capsules will be prepared from USP grade powder at a dose of 100 mg.

Matching placebo capsules of the same color, mass, and appearance to the spironolactone capsules will be filled using microcrystalline cellulose powder.

8.4 Supply of Study Drug at the Site

The UCSF Investigational Drug Service (IDS) will receive the bulk supplies of spironolactone and matching placebo from the compounding pharmacy. Capsules will be stored, accounted for, assigned per the randomization schedule and packaged and labeled for dispensing to the subject by the IDS pharmacy staff once ordered by the investigator on a study order/prescription form.

Drug will be provided by the manufacturer to the UCSF Investigational Pharmacy every three months. Subjects who were randomized and not treated will be immediately replaced when an “earlier” slot becomes available due to the dropped subject.

8.4.1 Packaging and Labeling

The UCSF Investigational Drug Service (IDS) will receive the bulk supplies of spironolactone and matching placebo from the compounding pharmacy. Capsules will be stored, accounted for, assigned per the randomization schedule and packaged and labeled for dispensing to the subject by the IDS pharmacy staff once ordered by the investigator on a study order/prescription form.

The IDS generates labels in compliance with California State pharmacy regulations, e.g., bottles will bear the name and address of pharmacy, name of subject, date of dispensing, name of the prescriber, instructions for use, expiry date and name, strength and quantity of the material dispensed. Capsules will be labeled as “spironolactone or placebo” to maintain the blind.

8.4.2 Storage

Study drug will be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of the study drug storage exceeds or falls below this range, this will be reported to the Sponsor or designee and captured as a deviation. Subjects will be instructed to store the medication in original packaging (plastic bottles and protected from light) at room temperature according to the instructions outlined on the Drug Administration Instructions.

8.4.3 Dispensing

An order form/prescription will be sent to the IDS pharmacy. Drug will be selected, counted and labeled by the IDS staff based on the randomization schedule. Labeled bottles will be picked up from the pharmacy by the investigator’s staff and dispensed to the subjects by the research staff during a study visit. Participants will receive 3 month supplies.

8.5 Dosage Regimen

Participants will take either spironolactone or placebo by mouth once daily in the morning. Morning is preferred due to the diuretic effect of spironolactone, which may be less convenient at nighttime. Absorption is not affected by fasting state therefore participants may take this with or without food.

8.6 Administration Instructions

At the participant’s baseline visit she will receive the pill bottle and be shown the pills with instructions on how to take this.

8.7 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record.

8.8 Measures of Treatment Compliance

Subjects should be instructed to retain all bottles of investigational product, even if empty, and to return them to the Investigator at the indicated visits. The Investigator or designee should perform investigational product accountability (i.e., count of returned capsules) and, if applicable, follow-up with the subject to retrieve any investigational product bottles that have not been returned. If the Investigator has concerns about a subject's dosing compliance, she should discuss this with the subject, assess the reasons for noncompliance and the likelihood that the subject will remain noncompliant, and notify the Sponsor accordingly.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening and at all subsequent in person study visits and at early termination when applicable. Dose, route, unit, frequency of administration, indication for administration, and dates of medication will be captured.

9.1.2 Demographics

Demographic information (i.e. date of birth, race, ethnicity) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, alcohol use, and other pertinent information regarding underlying diseases will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a subinvestigator who is a physician or other licensed medical professional at Screening. An abbreviated physical examination tailored to the symptoms the subject reports will be done at all subsequent in person visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at all in person study visits.

9.1.6 Anthropomorphic measures

Height will be measured at Screening. Weight will be measured at all in person study visits. Waist circumference will be measured at all in person study visits.

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Blood Chemistry Profile

Blood will be obtained and sent to the site's clinical chemistry lab for determination of serum sodium, potassium, creatinine at in person study visits. Liver tests (aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, and direct bilirubin), hemoglobin A1c, glucose, insulin, and lipid panel will be performed at each visit.

9.2.2 Pregnancy Test

Women of childbearing potential are required to perform a pregnancy test every 4 weeks.

A urine pregnancy test will be obtained from participants of childbearing age prior to their participation in the study and at every in person study visit. Women who are not sexually active with men may decline the urine pregnancy test.

Home pregnancy test kits will be supplied at each visit to women of childbearing potential and these are to be performed as per the kit instructions every 4 weeks between study visits from Day 1/Baseline to End of Study/End of Treatment.

The site will call the participant every 4 weeks in between visits to record the result of the home pregnancy test. In the event of a positive result the patient must discontinue study drug immediately and report the result to the site as soon as possible.

9.2.3 Sex Hormone Measurements

A comprehensive panel of sex hormones (testosterone, androstendione, DHEAS, estrone, estradiol, follicle stimulating hormone, and sex hormone binding globulin) will be performed at baseline, Month 6, and Month 12 (if subject opts to extend the study period). These will be sent to the research laboratory of Dr.Toni Zeigler at the University of Wisconsin National Primate Laboratory.

9.2.4. Constitution of biobank

Samples of blood and liver tissue will be kept from patients who provide their written informed consent for the purpose of conducting future studies investigating the link between reproductive health in women and liver disease. An additional amount of blood (2 Tbsp) will be collected along with the main study lab samples at each visit. Approximately 1 cm of the 3 cm core of tissue obtained in the Month 6 (or Month 12)

End of Study/ End of Treatment Visit liver biopsy (optional procedure) will be stored in the biobank.

Specimens will be stored at the UCSF Clinical Research Services Moffitt site in a specimen bank. Specimens and certain non-identifying medical information may be shared with other scientists or companies not at UCSF.

9.3 Liver Biopsy

A core liver biopsy will be performed prior to Screening and at the End of Study/ End of Treatment Visit (either Month 6 or Month 12). The liver biopsy at the End of Study/End of Treatment is optional for the subject. A historical biopsy prior to the Day 1/Baseline Visit will be used as the screening biopsy. A full pathology report confirming diagnosis of NASH must be available.

The liver biopsy specimens will be collected in a sterile specimen cup containing 0.1% ethanol. The cup will be labeled and sent to UCSF Pathology. Pathology will mount tissue onto a formalin block and send to UCSF Histology for slide preparation and reading.

9.4 Magnetic Resonance Imaging

Liver stiffness assessments will be performed by MRE (shear wave 60 Hz) and MRI-PDFF at the Baseline/Day 1 Visit, Month 6, and Month 12 (if subject opts to extend to 12 months). The MRE and MRI-PDFF assessments will occur sequentially. The scans will be read by a radiologist in the UCSF Department of Radiology & Biomedical Imaging.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Screening)

- Review the study with the subject and obtain written informed consent and HIPAA authorization.
- Assign the subject a unique screening number.
- Record demographics data.
- Record medical history, in relation to inclusion/exclusion criteria.
- Record concomitant medications.
- Perform a complete physical examination.
- Perform and record vital signs (blood pressure, pulse), height, and weight.
- Measure waist circumference.
- Collect blood for clinical laboratory tests
- Collect urine sample and perform pregnancy test for women of childbearing potential.

10.2 Visit 2 (Day 1/Baseline)

- Record any Adverse Experiences.
- Concomitant medications review.

- Assess liver stiffness by MRE and MRI-PDFF
- Randomize to treatment group, dispense study drug, and provide instructions on how to take it.

10.3 Visits 3 – 4 (Months 1 and 3)

- Record any Adverse Experiences.
- Record changes to concomitant medications.
- Perform abbreviated physical examination.
- Perform and record vital signs (blood pressure, pulse) and weight.
- Measure waist circumference.
- Collect blood for clinical laboratory tests.
- Collect urine sample and perform pregnancy test for women of childbearing potential.
- Perform pill count for compliance assessment.
- Collect all unused study drug.
- Dispense study drug.

10.4 Visit 5 (Month 6 End of Study/Treatment)

- Record any Adverse Experiences.
- Record changes to concomitant medications.
- Perform abbreviated physical examination.
- Perform and record vital signs (blood pressure, pulse) and weight.
- Measure waist circumference
- Collect blood for clinical laboratory tests
- Collect urine sample and perform pregnancy test for women of childbearing potential.
- Obtain liver biopsy (optional for subject).
- Assess liver stiffness by MRE and MRI-PDFF
- Perform pill count for compliance assessment.
- Collect all unused study drug.

10.5 Visit 6 (Follow-Up)

- Record any Adverse Experiences.
- Record changes to concomitant medications.

10.6 Optional Visits (if subject opts to extend study period)

- Month 9 visit will include the same evaluations as listed for Visits 3 and 4
- Month 12 Visit will serve as the end of study/treatment visit (liver biopsy will be completed at Month 12 instead of Month 6 if subject wishes to extend study period).

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient-administered pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator or research assistant will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

11.1.1 AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

11.1.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF HRPP Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Investigational Product Adjustment & Interruption Criteria

Dosage for investigational product should be held constant throughout the course of the study. In the event that the subject experiences adverse experiences that the Investigator suspects are related to the investigational product, a dosage adjustment or interruption of investigational product may be implemented. Subjects may also be permanently discontinued from investigational product by the Investigator at any time for clinical safety

concerns (see section 12.3).

Subjects who are temporarily discontinued from investigational product are expected to continue in the study and should follow the regular visit schedule for the duration of the study. Rechallenge with investigational product may be initiated at the Investigator's discretion upon discussion with the medical monitor. Additional safety monitoring may be initiated.

11.4 Medical Monitoring

Dr. Marcelle Cedars should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: 415-353-3032
Email: marcelle.cedars@ucsf.edu

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Protocol violation requiring discontinuation of study treatment
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Lost to follow-up
- Positive pregnancy test

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for End of Treatment visit as soon as possible and be encouraged to complete all visit procedures.

12.2 Withdrawal of Subjects

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Refer to Section 10 for End of Treatment procedures.

12.3 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with Good Clinical Practice (GCP) guidelines.

The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 DATA SAFETY MONITORING

The Data Safety Monitoring Board (DSMB) will include one reproductive endocrinologist (Heather Huddleston, MD) and one hepatologist (Jennifer C.Lai, MD) who are UCSF faculty who are not directly involved in the study itself, but have expertise in use of spironolactone to treat elevated androgens in women, and expertise in liver disease respectively. This DSMB will review data relating to safety and efficacy, conduct and review interim analyses, and ensure the continued scientific validity and merit of the study. Further details regarding the timing and content of the interim reviews is included in the statistical section below.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients will be included in the feasibility analyses because a key feature of this study is to determine the proportion of eligible patients that agree to participate. Only those randomized participants will be included in the analyses of secondary outcomes, though analyses will be conducted and presented separately as intention to treat, per protocol, and as treated, as the information gleaned from these different analytic approaches provides different, clinically relevant information.

All eligible patients who are randomized into the study and receive at least one dose of the study drug (the Safety Population) will be included in the safety analysis.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by treatment group: race, ethnicity, age, body mass index, HOMA-IR, lipid levels, androgen levels, PCOS diagnosis, and menopausal status.

15.3 Analysis of Primary Endpoint

Exact 95% confidence intervals for rates of agreement to participate, meeting eligibility criteria, and completing follow-up will be calculated using the binomial distribution.

15.4 Analysis of Secondary Endpoints

Logistic regression will be used to assess the association of spironolactone with each dichotomous outcome, and ordinal logistic or linear regression used to evaluate the association of spironolactone with change in either ordered categories or continuous measures, where appropriate.

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system and MedDRA classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

15.5 Interim Analysis

Planned interim analyses will be conducted after 10 women (~33%) have completed three months of treatment. Interim results will provide useful preliminary safety and feasibility data.

15.6 Sample Size and Randomization

The sample size of 30 with 2:1 randomization was determined based on budget and feasibility within a 4-year grant timeline. The sample size is also reasonable for pilot trial, and will allow us to estimate effects and feasibility with reasonable precision. The 2:1 randomization was selected to increase the chance that women would receive the treatment, and therefore potentially increase study enrollment, while still allowing the investigator to obtain the necessary training in conducting a placebo-controlled trial in preparation for a larger trial in the future, and allowing for preliminary endpoint assessments in the treatment as compared to placebo groups.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a subject number and initials.

If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor.

Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRB is notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new

information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).

7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. Schedule of Events

	Screening	Baseline/ Day 1	Month 1	Month 3	Month 6 End of Study/ Treatment	Month 9*	Month 12* End of Study/ Treatment	Follow-Up within 3 months of EOS/EOT
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	
Obtain informed consent	X							
Review your medical history	X							
Review medications you are taking	X	X	X	X	X	X	X	X
Perform complete physical exam	X							
Perform physical exam depending on your symptoms			X	X	X	X	X	
Measure your vital signs (blood pressure, heart rate)	X		X	X	X	X	X	
Measure your weight and height	X[a]		X	X	X	X	X	
Measure your waist circumference	X				X		X	
Review your general health (side effects and illnesses)		X	X	X	X	X	X	X
Draw samples of your blood for to check your general health, liver health, cholesterol, glucose, and insulin levels	X		X	X	X	X	X	
Draw samples of your blood to check your sex hormone levels	X				X		X	
If you are a female who can have children, collect a sample of your urine for pregnancy	X[b]		X	X	X	X	X	
Collect a small liver sample (biopsy)	X[c]				X*		X*	
Scan your liver: MRI-PDFF and MRE		X			X		X	
Give you study drug and instructions on how to take it		X	X	X	X	X	X	
Review the amount of study drug you have taken and collect unused			X	X	X	X	X	

a Height only measured at Screening
b In addition, home pregnancy tests are to be performed by women who can have children every 4 weeks from Day 1 until End of Study/Treatment
c Performed prior to screening as part of your standard of care for NASH
*Optional visits/procedures

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